DEVELOPMENT, OPTIMIZATION AND IN VITRO CHARACTERIZATION OF HALOPERIDOL NANOCRYSTALS USING 3² FACTORIAL DESIGN

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ABSTRACT

Objective: The main aim of the present study was to improve the dissolution rate of Haloperidol nanocrystals and thereby increase their bioavailability. Haloperidol is a typical antipsychotic drug and it is used to treat schizophrenia as well as acute mania and mixed states associated with bipolar disorder. Haloperidol falls into the Biopharmaceutics Classification System (BCS-II) class of drugs (poorly soluble aqueous and highly permeable) and has poor bioavailability.

Methods: The present study involves the preparation and optimization of Haloperidol nanocrystals by the anti-solvent precipitation method using Poloxamer 407 and polyvinyl pyrrolidone K30 (PVP K30). The prepared nanocrystals were evaluated for various parameters like particle size, zeta potential, % drug content, % yield, surface morphology, drug-excipient compatibility studies (Fourier-transform infrared spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC)), and in vitro dissolution studies.

Results: Nine preparations were done and the best preparation amongst them was selected for further studies. F₇ preparation containing poloxamer407 and PVP K30 was selected as optimized preparation based on their evaluation parameters. 3² factorial design was used in the preparation. The particle size of the F₇ nanocrystals was 300±2.7 nm and the zeta potential-36.3±3.2 mV. The % yield was in the range of 63.62±0.3%-98.21±0.8 %. The drug content of various preparations was found to be in the range of 58.46±0.8%-93.54±0.5 %. In vitro dissolution studies showed the highest % drug release for F₇ (91.54±0.03%) in 10 h.

Conclusion: F₇ preparation was found to be having acceptable characteristics and thus selected as optimized preparation.

Keywords: Haloperidol, Antipsychotic drug, BCS-II class of drugs, 3² factorial design

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INTRODUCTION

Schizophrenia, characterized by a range of clinical manifestations, is influenced by a combination of genetic and environmental factors contributing to the initiation of symptoms. It ranks among the top 15 causes of disability, representing a multisystem disorder with a worldwide prevalence ranging from 0.33% to 0.75%. Additionally, individuals with schizophrenia face a heightened suicide risk, estimated at 4.9% [1].

A drug’s in vivo performance is mainly dependent on its solubility and permeability. The Biopharmaceutical classification system proposed by the US Food and Drug Administration differentiates the drugs on the basis of their solubility and permeability [2]. Approximately 40% of newly discovered drugs show poor solubility in water [3, 4]. The poor water solubility of drugs causes their poor bio-availability. BCS includes four classes each class of drugs having its own rate limiting factors for absorption. Poor solubility drugs create delivery problems means low oral bioavailability and absorption problems [5, 6].

Currently, approximately 40% of drugs in the development stage exhibit poor solubility despite the fact that as much as 90% of compounds produced directly through synthesis also share this characteristic. Among these, 20% fall into class 4, while 70% belong to class 2 of the Biopharmaceutics Classification System (BCS). However, in many cases, they cannot solve the bioavailability problem [7, 6]. For example, micronization of poorly soluble drugs has been applied for many years to improve the dissolution velocity of poorly soluble drugs. But, reducing the drugs to micron size does not increase the saturation solubility of the drug, especially in case of BCS class II drugs that have very low saturation solubility [9-12].

This can be overcome by the preparation of drug Nanocrystals which is basically a nanosizing method, utilized to enhance the oral bioavailability of poorly water-soluble drugs. Nanocrystal dispersions contain dispersion media (Aqueous solutions or nonaqueous media, water), active drug substances, and surface-active agents or polymers required for stabilization [13-15]. If necessary, other substances such as salts and sugars, buffers can be added. Drug Nanocrystals were prepared by using two techniques [16].

The smart Crystal® technology developed by PharmaSol (owned by Abbott Lab since 2007) is composed of a variety of combative techniques. Spray-drying, precipitation, lyophilization, and pearl milling are applied as pretreatment followed by high-pressure homogenization as the main treatment. By applying this technology, ultra-small nanocrystals with particle sizes below 100 nm can be produced [17, 18].

Haloperidol is an antipsychotic drug used in the treatment of schizophrenia and acute mania. Haloperidol falls into the BCS-II class of drugs (poorly soluble aqueous and highly permeable) and has poor bioavailability. The main objective of the present research is to enhance the bioavailability and to achieve control release. Nanocrystals were prepared and improve the dissolution rate of Haloperidol nanocrystals and, thereby increasing the bioavailability.

MATERIALS AND METHODS

Haloperidol was gifted from Vamsi Labs Ltd., India. Poloxamer 407 was obtained from Sigma Laboratories, India. PVP K30 was obtained...
from Himedia Laboratories, India. Ethanol and Potassium dihydrogen phosphate was obtained from Merk Ltd, Mumbai, India. Sodium hydroxide was procured from Poly chem. Laboratories, Mumbai, India.

Preparation of haloperidol nanocrystals

Haloperidol nanocrystals were prepared by using an anti-solvent precipitation technique. 50 mg of drug was dissolved in 1 ml of ethanol (50 mg/ml) to form the organic phase. Poloxamer 407 (Surfactant) and PVP K30 (Polymer) were dissolved in 10 ml water to form the aqueous phase. Then the aqueous phase was rapidly stirred at 1200 rpm for 5 min at room temperature and 1 ml of the organic phase was injected into the aqueous phase under rapid stirring (1200 rpm) for 5 min. The mixed phases were sonicated at 80 W for 15 min. 1.0 ml of water was added and sonicated again for 5 min. The resultant Haloperidol nanosuspensions were lyophilized into dry powder [19].

Optimization of variables using full factorial design

A 3²-randomized full factorial design was used in the present study by utilizing the design expert® software. In this design, two independent factors were evaluated, each at three levels and experimental trials were performed for all nine possible combinations. Different ratios of Polymer and Drug (PVPk30: Haloperidol) (X1) and different ratios of Surfactant and Drug (Poloxamer407: Haloperidol) (X2) were chosen as independent variables in 3² full factorial design [20]. Particle size, Zeta potential, and cumulative % drug release were taken as dependent variables (table 1, 2 and 3).

Table 1: Design of 3² factorial method

<table>
<thead>
<tr>
<th>S. No</th>
<th>Coded values</th>
<th>Ratios of polymer: drug (PVPK30: haloperidol) (X1)</th>
<th>Ratios of surfactant: drug (Polaxomer407: haloperidol) (X2)</th>
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<tbody>
<tr>
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<td>-1</td>
<td>50:1</td>
<td>10:1</td>
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<tr>
<td>2</td>
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</tr>
<tr>
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<td>1</td>
<td>150:1</td>
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Table 2: Drug: polymer: surfactant ratios used

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<td>-1 (50:1)</td>
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<td>9</td>
<td>F9</td>
<td>1 (150:1)</td>
<td>1 (30:1)</td>
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Table 3: Composition of various preparations

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<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
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<td>PVPK30(g)</td>
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<td>2.5</td>
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</tr>
<tr>
<td>3</td>
<td>Poloxamer 407(g)</td>
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<td>0.5</td>
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Characterization studies of haloperidol nanocrystals

Particle size distribution

The particle size of the nanocrystals was measured by Horiba scientific instruments (particle size analyzer). Samples were prepared by diluting nanocrystals with enough water. The average particle size was determined by a Laser diffractometer [21].

Zeta potential

The zeta potential was measured by using Laser Doppler micro electrophoresis (Horiba scientific). 1-2 ml of sample was taken in an electrode cuvette and scanned for the zeta potential by using zeta sizer [22].

Drug content

Haloperidol content was determined by dissolving an accurately weighed (10 mg) quantity of Haloperidol nanocrystals in methanol. The solution volume was made up to 10 ml with buffer pH 7.4. The solutions were filtered, and samples were measured spectrophotometrically at 260 nm [22, 23].

Drug content (%) = \( \frac{\text{Weight of drug in nanocrystal}}{\text{Weight of nanocrystal}} \times 100 \)

% Production yield

The production yield can be determined by calculating initial weight of raw materials and final weight of Nanocrystals [24].

\[ \text{Production yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100 \]

Scanning electron microscopy (SEM)

The morphology and surface of nanocrystals were observed using SEM(5-3700). The samples of freeze-dried nanocrystals were dispersed on a glass slide and kept under a vacuum. The samples were coated with a gold/palladium layer using a sputter coat unit [25].

Drug-excipients compatibility studies

FT-IR spectroscopy

This study was performed to find the compatibility between the drug (Haloperidol), polymer (PVP K30), and surfactant (Poloxamer 407). Sample and KBr were taken in the ratio of 1:100 in a mortar and triturated. A small amount of triturate was taken into a pellet maker and was compressed at 10 kg/cm² to form a transparent pellet using a hydraulic press. The pellet was kept in a sample holder and scanned from 4000 cm⁻¹ to 400 cm⁻¹ in an FT-IR.
spectrophotometer. The possible interaction between Haloperidol PVP K30 and Poloxamer 407 was assessed by comparing FT-IR spectra of the pure drug (Haloperidol), polymer (PVP K30) and surfactant (poloxamer 407) [26, 27].

**Differential scanning calorimetry (DSC)**

The differential scanning calorimetry thermograms were recorded using differential calorimetry (DSC) (Mettler Toledo, Japan). Indium standards were used to calibrate the DSC temperature and enthalpy scale. Approximately 2-5 mg of each sample was heated in a pierced aluminum pan from 300 °C to 300 °C at a heating rate of 100 °C/min under a stream of nitrogen at a flow rate of 50 ml/min. Thermal data analyses of the DSC thermogram were conducted using STARe software [26].

**In vitro drug dissolution studies**

*In vitro* drug dissolution studies were performed by using USP Type 2 dissolution apparatus (Electrolab, India) using the basket method at the rotation speed of 75 rpm. The dissolution was carried out in acidic media pH 7.4. The volume and temperature of the dissolution medium were 900 ml and 37±0.2 °C, respectively. The samples were withdrawn every hour till 10 h and were filtered through Whatman's filter paper. The filtered samples were analyzed by determining the absorbance at 260 nm using a UV–visible spectrophotometer. The mean result of triplicate measurements and the standard deviation is reported [29].

**RESULTS AND DISCUSSION**

Nine preparations of Haloperidol nanocrystals were prepared by varying concentrations of Poloxamer 407 and PVP K30. These formulations were evaluated for drug content, % yield, and particle size by zeta sizer were determined for all the prepared nanocrystals. From this, the best preparation was selected and analyzed for surface morphology by SEM, compatibility studies by DSC and FT-IR and % drug release were performed.

**Characterization of haloperidol nanocrystals**

**Drug content**

The drug content in each preparation was determined by weighing 10 mg of prepared Haloperidol nanocrystals dissolved in a minimum quantity of methanol and the solution was made up to 10 ml with buffer pH 7.4. The solutions were filtered through Whatman’s filter paper and absorbances of the resultant solution were measured spectrophotometrically at 260 nm using methanol as a blank. The drug content of all preparations was shown in following fig. 1. The drug content of all formulations from F1 to F9 was calculated and the yield was a range of 58.46±0.8%–93.54±0.5%. F7 was found to have maximum % drug content (93.54±0.5%). The % drug content of the prepared Nanocrystals of Haloperidol using anti-solvent precipitation method within the range of 96-98%.

**Percentage yield**

The prepared Nanocrystals were collected and accurately weighed. The % yield of all preparations from F1 to F9 were calculated and the yield was found to be in the range of the % yield (63.6±0.3%-98.21±0.8 %) of prepared Haloperidol nanocrystals are depicted in fig. 2.

**3^2 Factorial design (FD) [30]**

The response surface plots were generated based on the results to find the effect of different factors on the performance of Haloperidol nanocrystals and to select the optimized formulation (F7) using 3^2 factorial designs of the experiment.

The response surface plot and contour plot, which indicate the effect of Haloperidol: Poloxamer 407 and haloperidol: PVP K30 ratio, Particle size of Haloperidol nanocrystals is shown in fig. 3. The response surface plot and contour plot, which indicates the effect of Haloperidol:Poloxamer 407 and haloperidol: PVP K30 ratio, Drug content of Haloperidol nanocrystals is shown in fig. 4.

**Fig. 1: % drug content of haloperidol nanocrystals, values are expressed are mean±SD, n=3**

**Fig. 2: % yield of nanocrystal formulations (F1-F9), values are expressed are mean±SD, n=3**
Fig. 3: Two-dimensional contour plot and three-dimensional response surface plots for particle size

Fig. 4: Two-dimensional contour plot and three-dimensional response surface plots for drug content

The response surface plot and contour plot, which indicates the effect of Haloperidol: Poloxamer 407 and Haloperidol: PVP K30 ratio, Entrapment efficiency of Haloperidol nanocrystals is shown in fig. 5.

Fig. 5: Two-dimensional contour plot and three-dimensional response surface plots for entrapment efficiency

Fig. 6: Particle size of F7
Particle size analysis by zeta sizer

The mean particle size of optimized formulation (F7) are shown in the following fig. 6.

The present study particle size of all nine preparations F7 has shown acceptable particle size (300.2 nm). The particle size was determined for all the prepared Haloperidol nanocrystals (F1-F9). It was found to be 64.5 nm to 510.5 nm. The particle size of Haloperidol Nanocrystals was prepared by media milling technique in the range of 238.2 nm.

Zeta potential

Zeta potential higher than +30 mV indicates the stability of nanoparticulate systems. The Zeta potential of various ratios of Haloperidol nanocrystals was determined by Zeta sizer and optimized formulation (F7) results were shown in fig. 7. In the present study, zeta potential was obtained for preparation F7, which is considered as optimal for good stability (-36.3 mV). Bhujbal NS et al. aimed to develop and evaluate BSA-loaded nanocrystals containing paclitaxel using the desolvation technique. Various characteristics such as particle size, zeta potential, entrapment efficiency, and drug release, were assessed. A factorial design was used to investigate the formulation variables’ impact. Nanocrystals were successfully prepared with a particle size of 251.3 nm, ZP values between −21.1 and −27.7 mV, high drug content (96.04%), and entrapment efficiency (92.05%) [30].

FTIR spectroscopy

The IR spectra of pure drug formulated nanocrystals were studied using a potassium bromide pellet. Spectral measurements were done using a thermo electron FTIR spectrometer at wavelengths 4000 cm⁻¹ to 400 cm⁻¹. FTIR spectroscopy of pure Haloperidol, Haloperidol nanocrystals, Poloxamer 407 and PVP k30 are shown in fig 8.

FTIR spectra of pure Haloperidol demonstrated the characteristic absorption peaks at 2377 cm⁻¹ for Thiol stretching, at 1743 cm⁻¹ for Ketone (R=O) stretching, 1743 cm⁻¹ for amide (C-N-H) stretching, 1599 cm⁻¹ for alkene (C=C) stretching, 1494 cm⁻¹ for aromatic (C-H)
stretching, 1357 cm\(^{-1}\) for amine (NH\(_2\)) stretching, 844 cm\(^{-1}\) for alkyl halide (C-Cl) stretching. The absorption peaks with Haloperidol Nanocrystals at 1432 cm\(^{-1}\) for aromatic (C-H) stretching, 1287 cm\(^{-1}\) for amine (NH\(_2\)) stretching, 844 cm\(^{-1}\) for alkyl halide (C-Cl) stretching, 1680 cm\(^{-1}\) for ketone (RC=O). Further peaks of Poloxamer 407, at 2586 cm\(^{-1}\) for thiol stretching, 844 cm\(^{-1}\) for alkyl halide (C-Cl) stretching, 1468 cm\(^{-1}\) for alkene (C=C) stretching, 1349 cm\(^{-1}\) for amine (NH\(_2\)) stretching. Further peaks of PVP K30 at 844 cm\(^{-1}\) for alkyl halide (C-Cl) stretching, 1649 cm\(^{-1}\) for alkene (C=C) stretching, 1287 cm\(^{-1}\) for amine (NH\(_2\)) stretching, 1427 cm\(^{-1}\) for aromatic (C-H) stretching.

**DSC (Differential scanning calorimetry)**

In order to further confirm the physical state, drug-excipient compatibility DSC was performed. DSC thermogram of Haloperidol nanocrystals is shown in the following fig. 9.

![DSC thermogram of formulation F7](image)

**Particle shape and morphology**

The shape and morphology were examined using Scanning electron microscopy (SEM). The SEM images of the optimized formulation are shown in the fig. 10. Dina Louis et al. formulated nanocrystals of atorvastatin calcium, a drug with poor water solubility and bioavailability, using high-pressure homogenization. The nanoparticles showed a significant change in particle size compared to the untreated drug with the exception of formulation 11, which did not significantly differ from the drug. The particle size ranged from 231 nm to 970 nm [31].

**In vitro drug dissolution**

The in vitro drug dissolution of Haloperidol nanocrystals was studied. The samples were withdrawn at definite time intervals and were analyzed for drug concentration by UV-Visible spectrometer at 260 nm. In vitro dissolution data of pure drug (Haloperidol) and various Haloperidol nanocrystals are shown in fig. 11 [30]. In the preceding research, researchers Aya ME et al., (2022) formulated and evaluated fast-dissolving tablets of haloperidol solid dispersion. The optimized formulation from their study exhibited a peak drug release of 85.00±0.08% at 60 min. However, the current formulation, specifically F7, surpassed this result, demonstrating a sustained release of 91.25±0.06%. This enhanced performance is attributed to the presence of nanocrystals in the formulation [33].

Arvind S et al. focused on addressing the issue of poor solubility in drug compounds by formulating nanocrystals using an anti-solvent precipitation method with varying stabilizer concentrations. Physicochemical evaluations, including visual appearance, DSC, SEM, XRD, solubility studies, particle size distribution, and zeta potential were conducted. In vitro studies compared nanocrystals to drug powder using dissolution apparatus. Results indicated that the tablet formulation of RVT and PSNC-3 (optimized formulation) showed a drug release of approximately 12% and 32%, respectively after 15 min, and 41% and 83% after 60 min. Similarly, the capsule formulation of RVT and PSNC-3 exhibited a drug release of about 10% and 40% respectively after 15 min, and 41% and 83%, respectively after 60 min.

![SEM image with A. Mag = 3.00KX and B. Mag = 2.00KX](image)
Fig. 11: *In vitro* drug release data in % (F1-F9), values are expressed are mean±SD, n=3

Fig. 12: % *In vitro* drug dissolution of pure drug and optimized preparation F7

Fig. 11 shows *in vitro* drug release of Haloperidol nanocrystals (F1-F9). The % drug release from different Haloperidol nanocrystals formulations was found to be 65.62±0.09 to 91.54±0.03 %. F1 shows the (65.62±0.09%) lowest % drug release and F7 shows the (91.54±0.03%) highest % drug dissolution. Hence F7 was considered as optimized preparation. The % drug release of Haloperidol pure drug was found to be 40.58±0.08 % and % drug release of Haloperidol nanocrystals was found to be 86.32±0.05 % (fig. 12).

CONCLUSION

The present study was to prepare, characterize, and optimize the Haloperidol nanocrystals. Haloperidol is a typical antipsychotic drug. Haloperidol nanocrystals were prepared by using an anti-solvent precipitation technique. A total of nine different concentrations of nanocrystals (F1 TO F9) were prepared by taking different ratios of polymer (PVP K30) and surfactant (Poloxamer 407). Amongst the preparations, F7 was found to have desirable characteristics and was considered an optimized formulation. The %drug release found to F7 i.e., 91.54±0.03 %. The particle size of F7 was found to be 300.2 nm and the zeta potential was about -36.3 mV, which is acceptable. Further evaluation tests of the F7, such as SEM, FTIR, and DSC were carried out. DSC thermogram of pure Haloperidol showed a sharp melting endothermic peak at 60.91 °C. The thermogram of Haloperidol nanocrystals containing Poloxamer 407, PVP K30 showed a typical endothermic peak with an onset of 44.92 °C, peak at 48.92 °C and end set at 62.03 °C. To improve the process variables were identified and optimized 3² factorial design at two stages. Based on the preliminary studies, the formulation variables selected were polymer-drug ratio and surfactant-drug ratio. And process variables selected were particle size, drug content, and entrapment efficiency it shows response surface plots.

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AUTHORS CONTRIBUTIONS

Conceptualization, Damineni Saritha and Gundawar Ravi; methodology, Damineni Saritha and Gundawar Ravi; software, P. Subhash Chandra Bose; formal analysis, Damineni Saritha and Gundawar Ravi; investigation, Damineni Saritha, Gundawar Ravi and P. Subhash Chandra Bose; resources, Damineni Saritha, Gundawar Ravi and Riyaz Ali M. Osmani; data curation, Damineni Saritha, Riyaz Ali M. Osmani, Iriventi Padmini and Sandeep Kanna; writing—original draft preparation, Gundawar Ravi and Iriventi Padmini; writing—review and editing, Damineni Saritha, P. Subhash Chandra Bose, Riyaz Ali M. Osmani and Sandeep Kanna. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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