Effect of Formulation Variables on Fabrication of Risperidone Loaded Nanoparticles for Sustained Drug Delivery

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ABSTRACT

Aim/ Background: Risperidone, an atypical antipsychotic agent, is used in the treatment of psychotic disorders due to its lower Extra Pyramidal side Effects (EPS) than conventional antipsychotics. Materials and Methods: The objective of the present investigation is to study the effect of formulation variables using $3^2$ factorial design; on preparation of nanoparticles to provide sustained drug release for a prolonged period of time; enhancing therapeutic efficacy by reducing dose dependent side effects. Study include formulation of nanoparticles by the nanoprecipitation method and optimization of the formulation parameters such as Amount of Polymer and Surfactant concentration as independent variables and Particle size and % entrapment efficiency as dependent variables using design of experiment; a statistical tool. Results: Optimized formulation of nanoparticles was characterized for various physicochemical parameters. The particle size was found to be 213.3 nm with 82.89% drug entrapment. Conclusion: In vitro drug release study and Pharmacodynamic study portend promising results of drug delivery for longer duration.

Key words: Schizophrenia, Risperidone, Nanoparticles, $3^2$ factorial design, Catalepsy.

INTRODUCTION

Schizophrenia; a chronic mental disorder can be characterized by symptoms like delusions, hallucinations, disorganized speech or behavior, difficulty in concentration etc. Management of such a disease condition requires long term treatment.

Long acting formulation is beneficial as an alternate treatment for patient who is non-adherent to Oral therapy. Risperidone is selective candidate drug due to lower risk of weight gain and less extrapyramidal side effects. Risperidone is BCS class II drug; reported to have 70% bioavailability and short half-life; 3-6 hr. Hence, long acting formulation is a need to be developed to provide controlled drug delivery which reduces dose dependent side effects. Currently, available marketed preparation of Risperidone is Solution, tablet, oral disintegrating tablet and powder for injection. An attempt has been made to develop spray dried Poly lactide co-glycolide (PLGA) nanoparticles, floating microparticles, self-nanoemulsifying drug delivery system, sustained release tablets, fast dissolving film, and chitosan nanoparticles. Due to very fine particle size, Nanoparticles are considered as potential carrier for efficient drug delivery and can be administered by various routes. Polymeric nanoparticles are more attentive due to its biodegradation, biocompatible and controlled release characteristics. Method of preparation was selected based on physicochemical properties of chosen drug and polymer. Some of the formulation variables like type and amount of polymer, type and amount of surfactant, volume of oil and aqueous phase etc. and Process variables like stirring rate, stirring time, rate of addition of oil phase etc. can affect entrapment, % yield and drug release pattern. The objective was to study the effect of formulation variables on preparation of...
Risperidone loaded nanoparticles using $3^2$ factorial design. Risperidone loaded PLGA nanoparticles are expected to reduce the frequency of administration, as well as dose and dose-dependent Extra Pyramidal side Effects (EPS).

**MATERIALS AND METHODS**

**Materials**
Risperidone and PLGA (Poly lactic acid: Glycolic acid: 75:25) were obtained as a gift sample from RPG Lifesciences, Mumbai and Purac biomaterials, Netherland; respectively. Poly Vinyl Alcohol (PVA) was obtained from Hi media, Mumbai. Other chemicals were obtained from S.D. Fine Chem, India.

**Methods**
Nanoparticles were prepared by Nanoprecipitation method (Sharma et al. 2014). Organic phase was prepared by dissolving weighed quantity of Risperidone and PLGA in Acetone. Aqueous phase was prepared by dissolving PVA in distilled water. Organic phase was added drop wise into aqueous phase at the rate of 1 ml/min with continuous stirring at 500 rpm on magnetic stirrer at room temperature. Stirring was continued for 5hr to remove organic solvent. Resulting nanoparticulate dispersion was centrifuged at 25000 rpm for 30 min at 4°C using cooling centrifuge (C-24 BL, Remi Instrument Pvt Ltd, Mumbai, India). Separated nanoparticles were washed twice with deionized water, following lyophilization (Heto Dry Winner, Denmark).

**Experimental Design**
A $3^2$ full factorial design was employed to study the independent and interactive effect of variables on nanoparticles at three levels i.e. low, medium and high. The design and statistical analysis were performed by Design – Expert® Software Version 10.0.1. Thirteen formulations (Table 1) of nanoparticles were prepared, which includes 9 design points and 4 centre points.

Data obtained using design expert software were subjected to fit quadratic model as shown in following equation.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_1^2X_{11} + b_2^2X_{22} + b_{12}X_1X_2$$  \ (1)

Where, $b$ is co-efficient in Polynomial equation which shows the magnitude of effect on independent variables. A high positive or negative value of $b$ may obtain a significant change in dependent variable. Checkpoint analysis was used to validate Polynomial equation. 23-26 multiple regression analysis and 3D response surface plots were used to study the main and interaction effects of the variables on responses.

**Characterization**

**Particle size and Zeta Potential**
Particle size analysis and Zeta Potential was performed in a triplicate using a zetasizer (Nano ZS, Malvern instrument, UK). Sample was prepared by diluting dispersion with distilled water to prevent aggregation and placed in a small disposable zeta cell.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Independent variables</th>
<th>Dependent variables</th>
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<tbody>
<tr>
<td></td>
<td>($X_1$) Surfactant Concentration (%)</td>
<td>($X_2$) Amount of Polymer (mg)</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>75</td>
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<tr>
<td>5</td>
<td>1</td>
<td>75</td>
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<tr>
<td>6</td>
<td>1.5</td>
<td>75</td>
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<tr>
<td>7</td>
<td>0.5</td>
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<td>8</td>
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<td>100</td>
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<td>12</td>
<td>1</td>
<td>75</td>
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<tr>
<td>13</td>
<td>1</td>
<td>75</td>
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</tbody>
</table>
Percentage Entrapment Efficiency

Percentage entrapment efficiency of Nanoparticles was determined by dissolving 10 mg of lyophilized nanoparticles in 10 mL of Acetonitrile followed by measuring the absorbance at 278 nm with a UV – Visible Spectrophotometer (UV – 1601, Shimadzu, Japan) after appropriate dilution with Acetonitrile.

\[
\% \text{EE} = \frac{\text{Actual weight of drug in sample}}{\text{Theoretical weight of drug in the sample}} \times 100
\]

\(2\)

%Drug Loading

The drug content in the Nanoparticles was determined by accurately weighing 10 mg of lyophilized Nanoparticles and adding them in 10 mL of Acetonitrile. Resulted dispersion was subjected to vortex and subsequently centrifugation to separate clear supernatant containing dissolved drug. Absorbance of the solution was then measured at 278 nm with a UV – Visible Spectrophotometer (UV – 1601, Shimadzu, Japan) after appropriate dilution with Acetonitrile.

Drug loading was calculated as follows:

\[
\text{D.L} (\%) = \frac{A}{B} \times 100
\]

Where A is the drug concentration in the Nanoparticles and B is the weight of NPs. Mannitol was used as cryoprotectant, which shows no interference in Risperidone peak obtained by UV-Spectrophotometric at 278nm.

Transmission Electron Microscopy (TEM)

Lyophilized Nanoparticles were stuck to a brass stub with double-sided adhesive tape. The stub was fixed into a sample holder and placed in the vacuum chamber of a Jeol JSM 1560 LV TEM (Jeol, Peabody, MA) and observed under low vacuum.

Differential Scanning Calorimetry (DSC)

DSC Thermogram of pure Risperidone, Polymer and drug loaded Nanoparticles was performed to study physical state of drug in Nanoparticles. Thermogram was obtained using 2910 MDSC instrument (V4.4E model, UK.). Few mg of sample was sealed in aluminum pan. Thermogram was recorded at heating rate of 10°C/m with temperature range from 20 to 200°C.

In vitro drug release study

Nanoparticles were dispersed by sonication in 1 mL of phosphate buffer saline (pH 7.4) and placed into previously soaked dialysis bag. The bag was placed into the bottle containing 25 mL of release medium maintained at temperature 37 ± 2°C under magnetic stirring at 50 rpm. At each selected time point, an aliquot of 1 mL was withdrawn and the same volume was replaced by fresh release medium. The sample was analyzed for drug content using a UV-visible spectrophotometer at 279 nm. Study was performed in triplicate. Drug release kinetic was also studied.

Stability study

As per ICH guidelines, optimized formulation was stored at room temperature (~25°C) and refrigerator (2°C to 8°C), at 75%RH; over a period of 3 months in stoppered glass vials. The sample was evaluated for particle size and percentage entrapment efficiency on selected point. Study was conducted in triplicate.

In vivo Catalepsy Study

Catalepsy is a state of behavioral immobility characterized by muscle rigidity and failure to correct an externally imposed posture for a prolonged period of time. It is clinically important as similar behavioral symptoms observed with treatment of Schizophrenia like extrapyramidal side effects. Healthy (18-25 g) Swiss Albino mice were divided into three groups of six animals (n = 6). Saline control, Risperidone solution (5 mg/kg, i.m.) and Risperidone loaded nanoparticles were administered to respective groups. Catalepsy was evaluated at 30 min intervals until 240 min. Catalepsy was assessed in the terms of the time for which the mouse remained an unusual position with both front limbs extended and resting on 4 cm high wooden bar (1.0 cm diameter). The endpoint was considered to remove the both front limbs from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 1100 sec was applied. All the observations were made in quiet room. If the animal maintained the imposed posture for at least 20 sec, it was considered to be cataleptic and given point. For every additional 20 sec of imposed posture, one extra point was given. The mice were evaluated twice at half an hour interval and only the greater duration of immobility was measured.

RESULTS

Experimental Design

In present study, process variables have been kept constant and formulation variables has been optimized using statistical design. The effects of formulation variables on Nanoparticles were studied and optimized formula was proposed by design expert software upon selecting constraints.
Effect of independent variables on Particle size

Effect of selected variables on response Particle size can be depicted by following equation.

\[
Y_1 = 213.56 + 33.45A + 7.80B - 0.75AB - 2.27A^2 + 17.78B^2 - 0.45A^2B + 3.25AB^2
\]  \( (1) \)

Though Acetone is miscible with water, surfactant is required to reduce interfacial tension between organic phase and aqueous phase sufficiently, governing formation of fine droplet of drug in polymer solution when dropped into aqueous phase. PVA was less effective at lower concentration in reducing interfacial tension between aqueous phase and oil phase, resulted in coarser particles. At mid-level, Particle size was reduced significantly. At higher concentration of PVA, the viscosity of aqueous phase increases which leads to aggregation of droplets, consequences in the coarser particles.

Amount of polymer \( (X_3) \) had the prime influence on Particle size. With increase in amount of polymer, viscosity of oil phase increases; during emulsification this may lead to coarser droplet formation. After evaporation of organic solvent, coarser droplets resulted in formation of larger particle (Figure 1).

Effect of Independent variables on %Entrapment Efficiency

Influence of independent variables on %Entrapment efficiency was studied by equation 2.

\[
Y_2 = 82.41 + 2.91A + 2.50B - 0.32AB - 0.56A^2 - 0.15B^2 - 0.58A^2B + 0.46AB^2
\]  \( (2) \)

Applying higher concentration of PVA in the external aqueous phase resulted in higher % EE values. Selected drug and polymer are hydrophobic in nature; reduced interfacial tension improves the pathway for encapsulation of drug.

As the Polymer concentration increases, viscosity of oil phase also increases. Drug being more soluble in oil phase, increase in amount of polymer lead to drug enrich phase and thereby enhancing %Entrapment.

Selection of Optimized batch of nanoparticles is carried out by considering closeness of desirability factor near to 1. The experimental value for responses of optimized formulation was found in good agreement with the predicted values and thus assured the validity of Response Surface model.

Characterization

Particle size and Zeta Potential

Particle size of optimized batch of nanoparticles was found to be 221 nm with very less poly dispersity index which showed presence of uniform sized particles. PLGA being negatively charged polymer imparts anionic nature to nanoparticles where zeta potential values were found to be -25.4mV. Nanoparticulate dispersion found to be stable due to electrostatic repulsion between similar surface charges on particles which do not allow aggregation.

Drug loading

The drug (Risperidone) loading was found to be 82.89% w/w for optimized batch of nanoparticles.

Transmission Electron Microscopy (TEM)

TEM micrographs (Figure 2) showed that uniform NPs were successfully prepared by using the nanoprecipitation technique. Smooth surface and spherical shape of nanoparticles were observed which can be explained by stability of emulsion that polymer had adequately formed condense matrix around the drug molecules.

Differential Scanning Calorimetry (DSC)

Risperidone and polymer showed a sharp endothermic peak at 171°C and 59.69°C respectively (Figure 3) due to crystalline nature. No sharp endothermic peak observed with thermogram of nanoparticles which indicated amorphous form of drug and polymer. It can be supported by hypothesis of entrapment of drug within polymeric matrix of polymer.

In vitro drug release study

Drug release profile (Figure 4) followed biphasic pattern with an initial burst attributed to the drug associated near particles surface, followed by a linear release phase.
The drug release profile confirmed to the Higuchi model ($R^2 = 0.9822$), suggesting the drug release to be a diffusion controlled process based on the Fick’s first law of diffusion in which the diffusion coefficient depends upon both the concentration of drug and the time.

**In vivo Catalepsy study**

In the acute study, administration of the test drugs gave cataleptic scores similar to that of the vehicle-treated group at 30 min. However, from 60 min onwards, risperidone (5.0 mg/kg) and risperidone loaded PLGA nanoparticles resulted in significantly higher cataleptic scores than the vehicle-treated mice. On the other hand, risperidone loaded PLGA nanoparticles showed a marked alteration in cataleptic scores than conventional administration of risperidone group (Table 2).

**Stability study**

Result showed significant increase in particle size was observed during storage may be due to aggregation of nanoparticles at room temperature. %EE of nanoparticles was also found to be significantly reduced at room temperature due to degradation of nanoparticles. Hence, it can be concluded that nanoparticles should be stored at freeze temperature (2-8°C) for better stability.

**DISCUSSION**

The objective of Present Investigation was to study the effect of formulation variables on formulation of Risperidone loaded nanoparticles and to study the efficacy of preparation in management of Schizophrenia upon intramuscular administration. Effect of two independent variables i.e. Surfactant concentration and Amount of Polymer were studied for Particle size and % Entrapment Efficiency of nanoparticles using $3^2$ full factorial design. Optimized batch of nanoparticles was evaluated for various characterization parameters. Selected batch showed Particle size 213.3 nm with 82.8% Entrapment efficiency which is suitable for intramuscular administration.

In vitro drug release study showed extended drug release pattern for around 168hr and confirmed by performing Catalepsy study. Nanoparticle formulation was found to be stable with no significant change in Particle size and %Entrapment efficiency when stored at freeze temperature.

**CONCLUSION**

The results of the investigation conclusively demonstrated that nanoparticles prepared by nanoprecipitation method showed sustained release of drug over a period of 168hr and reduce in dose dependent side
Table 2: Effects of Risperidone loaded PLGA nanoparticles on cataleptic scores.

<table>
<thead>
<tr>
<th>Time after haloperidol (min)</th>
<th>Control (10 ml/kg) (a)</th>
<th>Risperidone solution (5 mg/kg) (b)</th>
<th>Risperidone loaded PLGA nanoparticles (5 mg/kg) (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.0 ± 0.74</td>
<td>9.78 ± 1.95</td>
<td>8.89 ± 1.40</td>
</tr>
<tr>
<td>60</td>
<td>1.4 ± 0.24</td>
<td>16.00 ± 1.00***</td>
<td>14.75 ± 1.70**&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>1.2 ± 0.58</td>
<td>22.75 ± 1.81**&lt;sup&gt;†&lt;/sup&gt;</td>
<td>18.0 ± 1.65&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>1.3 ± 0.84</td>
<td>28.83 ± 1.67**&lt;sup&gt;†&lt;/sup&gt;</td>
<td>23.65 ± 1.90&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>240</td>
<td>1.0 ± 0.78</td>
<td>21.85 ± 1.17**&lt;sup&gt;†&lt;/sup&gt;</td>
<td>14.75 ± 1.18&lt;sup&gt;††&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SEM

Effects observed by catalepsy model; and their physical properties promise their use in the development of intramuscular sustained release injection. However, the findings of this investigation can only be settled after animal experimentation on additional animal models followed by extensive clinical study.

ACKNOWLEDGEMENT

Authors are thankful to RPG Life Science, Mumbai and Purac Biomaterials, Netherland for providing drug and excipient materials to carry out the research work.

CONFICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

NPS: Nanoparticles; EPS: Extra Pyramidal side Effects; BCS: Biopharmaceutical Classification; PLGA: Poly lactide co-glycolide; PVA: Poly Vinyl Alcohol; 3D: Three dimensional; UV: Ultra violet; D.L.: Drug loading; TEM: Transmission Electron Microscopy; DSC: Differential Scanning Calorimetry; RH: Relative humidity; i.m.: intramuscular; mV: milli volt.

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PICTORIAL ABSTRACT

From the present study, an attempt was made to curtail the problems associated with the use of currently available Risperidone preparation in management of Psychosis (Schizophrenia) by preparing Risperidone loaded nanoparticles. Nanoparticles were prepared by Nanoprecipitation method and optimized for formulation parameters using 3² full factorial design. Prepared nanoparticles were characterized for particle size analysis, zeta potential, drug loading, Transmission electron microscopy (TEM) and Differential scanning calorimetry (DSC) study in order to check the nanoparticles dispersion suitability to be given by intramuscular injection. Optimized batch of nanoparticles showed retarded release of drug from the polymer due to reduced mobility of drug. Pharmacodynamic study confirmed extended release pattern and has reduced EPS liabilities compare to Risperidone solution. Stability study showed there was no significant increase in Particle size after reconstitution of lyophilized batch.

SUMMARY

Dr. Dipti Gohil is Associate Professor in Department of Pharmacy, Sumandeep Vidyapeeth (Deemed to be University), Vadodara, India. She has guided more than 15 students at PG level. She has published a book and 2 book chapters in reputed Publishing House. Her Area of Research Interest is Formulation development, optimization and evaluation of Nanoparticulate, topical drug delivery system.

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Cite this article: Gohil D, Shah N, Maheshwari RA. Effect of Formulation Variables on Fabrication of Risperidone Loaded Nanoparticles for Sustained Drug Delivery. Indian J of Pharmaceutical Education and Research. 2022;56(1):58-64.