

Formulation development and pharmacokinetic studies of long acting *in situ* depot injection of risperidone

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Risperidone is an atypical antipsychotic drug widely prescribed all over the world due to its clinical advantages. The currently available long acting marketed depot formulation of risperidone is a microsphere based preparation using poly-[lactide-co-glycolide] (PLGA) as drug release barrier. It is however, a cold chain product due to thermal instability of PLGA at room temperature. After beginning the depot injection therapy it is administered every two weeks but associated with another drawback of about 3 weeks lag time due to which its tablets are also administered for three weeks so as to attain and maintain therapeutic drug concentration in the body. The present work attempts to develop a long acting depot delivery system of risperidone for once a month administration based on the combination of sucrose acetate isobutyrate and polycaprolactone dissolved in benzyl benzoate to provide an effective drug release barrier for one month without any lag time and which can be stored at room temperature precluding the requirement of cold supply chain. The developed depot formulation showed a sustained *in vitro* drug release profile with 88.95% cumulative drug release in 30 days with little burst release. The *in vivo* pharmacokinetic studies of the developed formulation conducted on rats showed attainment of mean peak plasma drug concentration of 459.7 ng/mL in 3 days with a mean residence time of 31.2 days, terminal half-life of 20.6 days, terminal elimination rate constant of 0.0336 per day, and a good *in vitro*- *in vivo* correlation.

Keywords: SAIB. Polycaprolactone. Risperidone depot injection. Benzyl benzoate.

INTRODUCTION

The psychological attitude of schizophrenic patients is generally negative and reluctant towards the drug therapy and, therefore, their long term treatment through oral drug therapy is always associated with poor patient compliance and non-adherence to drug administration and dosing schedule (Kane, Kishimoto, Correll, 2013). Valenstein *et al.* (2006) have reported that around 61% psychotic patients did not observe strict medication schedule in their four year study at some point of time during drug therapy with 18% patients being consistently poor in their dosage administration adherence and rest 43% showing

inconsistent adherence to prescribed drug therapy. This alarming situation would result into non-consistent drug levels in patients undergoing drug treatment causing ineffective therapy and thereby worsening the disease condition with upto five times increased risk of disease relapse in patients (Robinson *et al.*, 1999).

Administration of drug in the form of long acting depot injection eliminates the direct involvement of patients in drug administration and therefore effectively overcomes the above problem of patient non-compliance which is otherwise inevitable in the oral drug therapy (Kaplan, Casoy, Zummo, 2013). Studies have confirmed that the patients receiving long acting injectable antipsychotic therapy face lesser instances of disease relapses and hospitalizations (Offord *et al.*, 2013).

In the last five decades different types of depot technologies, viz., prodrugs, oil, microspheres,

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liposomes, and slow release powder based injections, etc. have been explored for designing depot injections. Each one of these techniques, however, has its own clinical and industrial merits, demerits, and limitations (Gulati, Gupta, 2011). Recently, an *in situ* gel forming technology has emerged as a simple, easy, patient-friendly, and low cost depot technology in which the depot system is formed *in situ* in the form of a gel matrix when the liquid composition of drug with a carrier and a solvent is injected into the body (Kempe, Mader, 2012; Dubey, Saini, 2018a). These systems are considered better formulations than the microsphere systems due to ease of administration with minimal invasion, better tissue compatibility, and simplicity of product manufacturing process (Agarwal, Rupenthal, 2013). Several approaches have been studied to achieve *in situ* gel forming systems (Packhaeuser *et al.*, 2004; Kempe, Mader, 2012), but majority of them have focused on the use of poly-lactic acid, poly-glycolic acid polymers, and their derivatives as release retardant polymers (Hatefi, Amsden, 2002). The PLGA derivatives, however, are expensive and heat sensitive polymers and therefore, the products developed using them are to be stored in cold conditions (Dong *et al.*, 2006) and their transportation obviously needs a cold supply chain mode which would significantly increase the product cost. The sucrose acetate isobutyrate (SAIB) is currently being explored as a new release retarding material in the designing of *in situ* gel forming injections as an alternative to the PLGA under a patented technology, SABER (Okumu *et al.*, 2002). SAIB is a hydrophobic, viscous fluid which when dissolved in small amounts of specific solvents transforms into a low viscosity injectable liquid (Strickley, 2004) which in contact with aqueous environment *in vitro* or *in vivo* converts into a viscous SAIB-drug matrix that acts as a drug reservoir for sustained drug release for extended period of time (Arthur, 2002). These *in situ* gel forming depot systems are simple, easy to manufacture, and cost effective formulations (Kempe, Mader, 2012). M/s DURECT Corporation, USA (2017) have successfully developed a sustained drug delivery injectable product of a local anesthetic agent (brand name Posimir[®]) by using SAIB and the product is under clinical trial phase III.

Risperidone, an atypical antipsychotic drug of benzisoxazole class, is quite effective against positive as well as negative symptoms of schizophrenia. Currently, a PLGA microspheres based intramuscular depot injection preparation of risperidone (Rispedal Consta[®] - Janssen) is available in the market in 25 mg, 37.5 mg, and 50 mg strengths with two weekly dosage regimen. However, the major limitation of this product is its ~3 weeks release lag time for achieving the therapeutic drug levels due to the slow and gradual hydrolysis of the PLGA copolymer used in the microspheres preparation (Eerdeken *et al.*, 2004). Owing to this, in the beginning of the drug therapy risperidone tablets are also required to be administered for 3 weeks along with the depot injection. Several approaches, viz., PLGA based microspheres (Su *et al.*, 2009; D'Souza *et al.*, 2014), microspheres co-encapsulated with magnesium hydroxide or arginine base (Hu *et al.*, 2011), PLGA based *in situ* depot systems (Wang *et al.*, 2012), PLGA and SAIB based *in situ* depot systems (Lin *et al.*, 2012), and, PLGA microspheres and SAIB based hybrid depot preparation (Lin *et al.*, 2015) etc. have been investigated to overcome this limitation.

The objective of the present study was to develop a 30 days sustained drug release intramuscular depot injection of risperidone without any significant drug release time lag and precluding the need of storage requirement of cold conditions and special supply chain management. The combination of polycaprolactone (PCL) and SAIB was used as release retarding agent for the development of *in situ* gel forming depot injection. The PCL is a safe, biodegradable, biocompatible, and bioabsorbable polymer and has a good thermal stability with a degradation temperature of 320°C (Patrício, Bártolo, 2013). Unlike PLGA polymers it does not generate any acidic environment during its degradation and generates non-toxic degradation products which are excreted through physiological pathways (Dasaratha Dhanaraju *et al.*, 2003). It is approved by Food and Drug Administration for use in implants and surgical absorbable sutures (Pohlmann *et al.*, 2013) and a PCL based long acting contraceptive delivery system of levonorgestrel (Capronor[®]) has already been commercialized (Ulery, Nair, Laurencin, 2011).

MATERIAL AND METHODS

Risperidone was supplied by M/S RPG Life Sciences Ltd., Mumbai, India, sucrose acetate isobutyrate by M/S Eastman Chemical Company, Kingsport, USA, and polycaprolactone (average molecular weight ~14000) by Piramal Healthcare, Mumbai, as gift samples. Benzyl benzoate (BB) was purchased from Qualigens Fine Chemicals, Mumbai. All other chemicals and solvents used were of analytical grade and purchased from market.

Particle size of risperidone powder:

The average particle size and polydispersity index of the risperidone drug powder was determined by light scattering technique using Malvern Mastersizer 2000 (Malvern Instruments, UK) using Millipore water containing 0.02% Tween 80 as a dispersant.

UV spectrophotometric analysis of risperidone:

The drug samples in different solvents except BB for solubility study were estimated spectrophotometrically at 280 nm on a double beam UV-visible spectrophotometer (UV-1700, Shimadzu, Japan). As BB shows its own absorption maxima at 229, 256, 263, 266, 272, and 280 nm and thus interferes in UV-visible analysis of risperidone at 280 nm (Hassan, Mossa, 1981), the drug samples in BB were estimated by HPLC method.

HPLC analysis:

The drug samples of developed formulation were analyzed by a validated reverse phase chromatographic method on the HPLC system (Waters Corporation, USA) at 280 nm employing a 4.6 × 250 mm; 5 µm BDS Hypersil Phenyl column (Thermo Fisher Scientific Inc., USA). The mobile phase was a mixture of phosphate buffer (pH 6.0) and acetonitrile (40:60 v/v) with a flow rate of 1 mL/min and 20 µL injection volume.

Solubility determination:

The solubility of risperidone was determined in millipore water, different organic solvents, and drug release test media [PBS (pH 7.4) + 0.5% SLS + 0.05% sodium azide]. An excess quantity of risperidone drug powder was added to each of the above solvents in stoppered glass test tubes and shaken on a shaker water bath at room temperature for 48 h. The saturated solutions were filtered through 0.45 µm membrane filter and analyzed.

Preparation of formulations:

Weighed quantity of PCL was dissolved in benzyl benzoate and weighed amounts of risperidone powder and SAIB were added in this solution using a vortex mixer (CM-101 Plus, Remi Lab World, India). Different formulation batches were prepared by varying the concentration of PCL.

EVALUATION

The developed depot formulation was evaluated employing following parameters:

Visual appearance:

The developed injection formulation was visually inspected for its color, consistency, homogeneity, and clarity.

Weight per mL:

One mL of the formulated injection was accurately pipetted into a pre-weighed micropipette tip fitted in a 1 mL micropipette (Eppendorf, Germany) and weighed on an analytical balance (Shimadzu, Japan). The difference of the weights of the filled and empty micropipette tip was taken as weight per mL of the formulation (n = 3).

Effect on the pH of fluid present at injection site:

The effect of formulated injection on the pH of the surrounding fluid present at injection site after its

injection was assessed by injecting 1 mL formulation into 10 mL distilled water and then monitoring pH of the resulting aqueous fluid by a calibrated pH meter (Cyberscan pH 510, Thermo Fisher Scientific, USA) for one hour as the depot formation would take place within this time.

Viscosity:

Viscosity of the formulated injection was determined ($n = 3$) by Brookfield Rheometer (R/S-CPS Plus™, Brookfield Engineering, USA). The formulation sample was placed between the bottom measuring plate and rotating measuring element of the rheometer which was operated at 300 rpm at room temperature and the viscosity was noted.

Syringeability:

The force required to inject the formulation through needle of a particular gauge injected at a definite rate was measured as its syringeability (Cilurzo *et al.*, 2011). One mL formulated injection was taken in a 2 mL plastic syringe attached with 16, 18, 20, and 21G needle, respectively and fitted on the Texture Analyzer (TA.XT.Plus, Stable Micro Systems, UK). The force required for displacing the plunger of the syringe by 5 mm at a speed of 1 mm/s was recorded by the Exponent Lite software as syringeability of the developed formulation.

In vitro drug release:

Measured volume of formulation was injected into a screw capped plastic tube of 15 mL capacity containing 10 mL drug release media [PBS (pH 7.4) + 0.5% SLS + 0.05% sodium azide] and shaken at 60 ± 5 rpm (Conti *et al.*, 1995) on an incubator shaker bath (SM Scientific Instruments, India) maintained at $37 \pm 1^\circ\text{C}$. Entire 10 mL drug release fluid was withdrawn after 1, 3, 7, 15, 22, and 30 days using a pipette coupled with a pipette controller taking care that the formulation is not disturbed. It was then slowly and carefully replenished with an equal volume of fresh release media using another pipette from the side of wall of the tube and the withdrawn fluid was analyzed by HPLC method.

Stability:

The formulation was subjected to stability studies according to the ICH guidelines at $25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH (real time) and $40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH (accelerated) for 6 months in Type I glass vials of 5 mL capacity (ICH-Guidelines, 2003). The formulation was checked for physical appearance, resuspendibility, and drug content initially, and after 1, 3, and 6 months. To analyze the drug content, 100 mg preparation was dissolved in 1 mL methylene chloride, suitably diluted further with acetonitrile, filtered through filter paper of $0.45 \mu\text{m}$ pore size, and the filtrate was estimated for risperidone content by HPLC method.

In vivo pharmacokinetic study:

The *in vivo* pharmacokinetic study was conducted on six healthy male albino Wistar rats of 160–200 g body weight after due approval of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Approval number: CPCSEA/IAHVB/2017/01/02). The animals were housed in spacious cages for easy movement and given free access to food and water during the entire period of study. The developed depot injection formulation was subcutaneously injected (Turner *et al.*, 2011) to each rat using a 20G needle at a dose of 25 mg/kg body weight. The blood samples (0.3 mL) were collected from lateral tail vein at predefined time intervals, i.e., 1, 3, 7, 15, 22, 26, and 30 days respectively and transferred into centrifuge tubes containing a solution of disodium edetate as anticoagulant and centrifuged at 5000 rpm for 10 min at 4°C (Eppendorf 5415R Centrifuge, Eppendorf, Germany) to separate the plasma. The plasma samples were collected into fresh centrifuge tubes and stored at -20°C until analyzed.

Plasma sample preparation and analysis:

Each plasma sample stored at -20°C was first thawed at room temperature and mixed with three times the volume of acetonitrile on a vortex mixer (CM-101 Plus, Remi Lab World, India) for 5 min to precipitate

plasma proteins and then centrifuged at 5000 rpm for 10 min at 4 °C (Eppendorf 5415R Centrifuge, Eppendorf, Germany). The separated supernatant was collected in another tube and analyzed for drug content by a validated HPLC method on an RP-HPLC system (Waters Corporation, USA) using Waters 2996 photodiode array detector, Empower software, and a 4.6 × 250 mm; particle size 5 µm BDS Hypersil Phenyl column (Thermo Fisher Scientific Inc., USA). The mobile phase consisted of phosphate buffer (pH 6.0) and acetonitrile (40:60 v/v) with a flow rate of 1 mL/ min. The injection volume was 20 µL and the detection wavelength 280 nm. The method had lower limit of quantification of 100 ng/mL and was linear over the concentration range of 100-5000 ng/mL (Dubey, Saini, 2018b).

Statistical data analysis:

A plasma drug concentration–time curve was constructed by plotting the plasma drug concentration against time. The peak plasma drug concentration (C_{max}) and time to achieve peak plasma drug concentration (T_{max}) were directly obtained from the graph and the area under the plasma drug concentration–time curve (AUC_{0-30d}) was calculated by trapezoidal method (D'Souza *et al.*, 2014). The plasma drug concentration–time data were represented by non-compartmental pharmacokinetic model for extravascular administration and terminal elimination half-life ($t_{1/2}$), the terminal elimination rate-constant (λ_z), mean residence time (MRT), total apparent clearance of drug from plasma after extravascular administration (Cl/F), and apparent volume of distribution after extravascular administration (V_z/F) were calculated using Kinetica software (Version 5.0, Adept Scientific, UK) (Dubey, Saini, 2019).

In vitro- *in vivo* correlation:

The IVIVC between *in vitro* drug release and the plasma concentration data was established by fractional AUC method (Shen, Burgess, 2015). The fractional AUC was calculated by dividing AUCs at different time intervals with cumulative AUC till last time point. The percent fractional AUC was plotted against *in vitro*

cumulative percent drug release and the correlation coefficient was calculated to assess the extent of IVIVC (Prescott *et al.*, 2007).

RESULTS AND DISCUSSION

Particle size:

The d10, d50, and d90 values of risperidone drug powder used in the formulation were 6.422, 16.396, and 31.593 µm, respectively with a polydispersity index of 1.535.

Solubility study and drug release media selection:

The solubility of risperidone found in different solvents is reported in Table I.

As the solubility of risperidone in water and phosphate buffered saline (pH 7.4) was found to be very less, 0.5% sodium lauryl sulphate (SLS) was added in PBS to attain sink conditions in drug release study. Since release studies were to be conducted for one month, sodium azide (0.05%) was added in the release media as preservative. The stability of risperidone in this release media for one month duration was confirmed by its solution state stability study in which no degradation was detected.

TABLE I - Solubility of risperidone in different solvent media

| Medium | Solubility (mg/mL) |
|------------------------------------------------|--------------------|
| Water | 0.144 ± 0.0056 |
| Phosphate buffered saline, pH 7.4 (PBS) | 0.175 ± 0.0127 |
| Dimethyl sulphoxide | 4.827 ± 0.172 |
| Ethanol | 18.469 ± 1.513 |
| Triacetin | 176.419 ± 5.929 |
| Benzyl benzoate | 46.280 ± 1.745 |
| Drug release test medium (PBS + 0.5% SLS) | 1.744 ± 0.053 |
| Drug release test medium + Placebo formulation | 4.227 ± 0.114 |

± SD; n=3

Selection of solvent for SAIB:

SAIB solutions were prepared in ethanol, DMSO, triacetin, and BB. The preoptimization batches of *in situ* gel forming depot injections were formulated by dispersing risperidone in each of the above SAIB solutions. The amount of SAIB and solvent was kept in

the ratio of 80:20 as this was found to be their optimum proportion for sustaining the drug release for one month from a depot injection. A plain risperidone dispersion was also prepared by dispersing drug powder in drug release media [PBS (pH 7.4) + 0.5% SLS + 0.05% sodium azide] and it was used as control. The composition of designed depot formulations is shown in Table II.

TABLE II - Composition of formulation batches of risperidone depot injection for selection of SAIB solvent

| Formulation batch code | Drug (mg) | SAIB (%) | Solvent | | | |
|------------------------|-----------|----------|----------|-------------|---------------|--------|
| | | | DMSO (%) | Ethanol (%) | Triacetin (%) | BB (%) |
| RIS/IS/PRE1 | 10 | - | - | - | - | - |
| RIS/IS/PRE2 | 10 | 80 | 20 | - | - | - |
| RIS/IS/PRE3 | 10 | 80 | - | 20 | - | - |
| RIS/IS/PRE4 | 10 | 80 | - | - | 20 | - |
| RIS/IS/PRE5 | 10 | 80 | - | - | - | 20 |

SAIB- Sucrose acetate isobutyrate; DMSO- Dimethyl sulphoxide; BB- Benzyl benzoate

In vitro drug release:

The formulated preparations were subjected to *in vitro* drug release study for 30 days. The drug release data are graphically presented in Figure 1. The solvent selection was done on the basis of the results of *in vitro* drug release study of formulations.

The plain risperidone drug formulation (RIS/IS/PRE1) showed complete dissolution within three hours. Whereas, SAIB formulations containing DMSO, ethanol, triacetin, and BB (RIS/IS/PRE2-5) as the solvent showed 26.67%, 41.40%, 21.42%, and 18.47% drug release, respectively on the first day. These data, however, also indicated high burst release of risperidone from the formulations prepared using SAIB alone.

The time to release 50% and 90% drug, i.e., $T_{50\%}$ and $T_{90\%}$, respectively was calculated with the help of regression equations obtained from their release profile curves. The $T_{50\%}$ value for the formulations containing

DMSO (RIS/IS/PRE2), ethanol (RIS/IS/PRE3), triacetin (RIS/IS/PRE4), and BB (RIS/IS/PRE5) was 8.2 days, 2.9 days, 9.4 days, and 11.1 days, respectively while the $T_{90\%}$ values for these formulations were 21.3 days, 18.7 days, 22.8 days, and 24.9 days, respectively. The most prominent sustained drug release effect was observed in the formulation containing BB, whereas, the formulation containing ethanol was found to be least effective in this respect. This can be attributed to highly lipophilic nature of BB and on the other side highly hydrophilic nature of ethanol, which must have influenced the drug diffusion behavior from respective formulations after SAIB gel formation *in situ*. The hydrophilicity or high aqueous solubility of a solvent causes fast solvent diffusion into the fluid present at the site of injection leading to an accelerated drug release and vice versa (Lin *et al.*, 2012). Nevertheless, none of these formulations could adequately control and sustain the rate of drug release for a period of about one month.

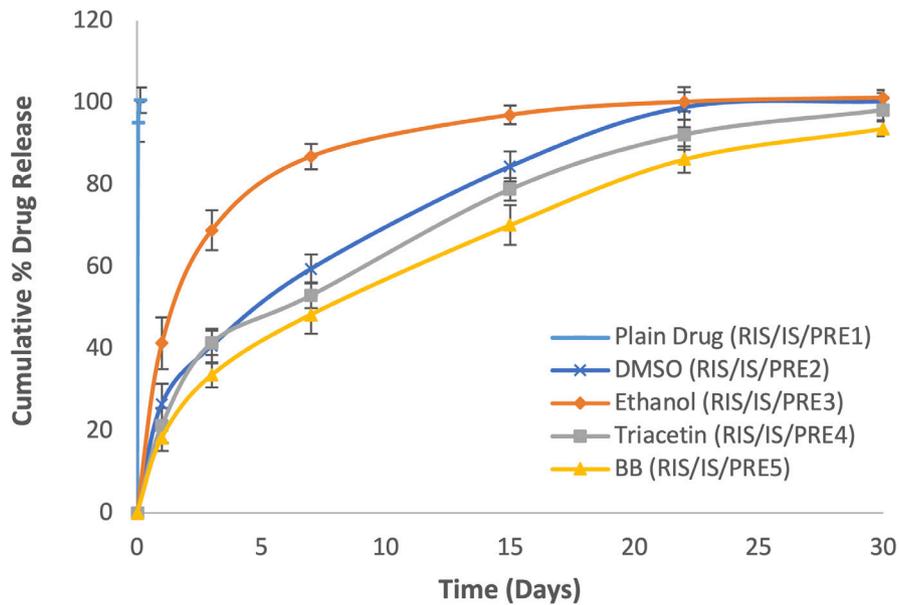


FIGURE 1 - *In vitro* cumulative percent drug release of formulation batches of risperidone depot injection for selection of SAIB solvent (mean \pm SD; n=3).

Effect of PCL as release retarding polymer:

As the SAIB alone was not able to retard the drug release from the developed formulation for sufficiently long period therefore, the effect of PCL, which is also a biodegradable release retarding polymer, in the SAIB based formulation was investigated. Different formulation batches of risperidone depot injection were, therefore, designed using combination of SAIB and PCL as drug release retarding material and benzyl benzoate as solvent.

Optimization of composition of depot injection formulation:

PCL solutions in varying concentrations were prepared by dissolving PCL in BB and weighed quantity of risperidone powder was dispersed uniformly in each of the above PCL solutions followed by addition of weighed quantity of SAIB. The resulting mixture was mixed on a vortex mixer (CM-101 Plus, Remi Lab World, India) to get a uniformly dispersed depot preparation (Figure 2).

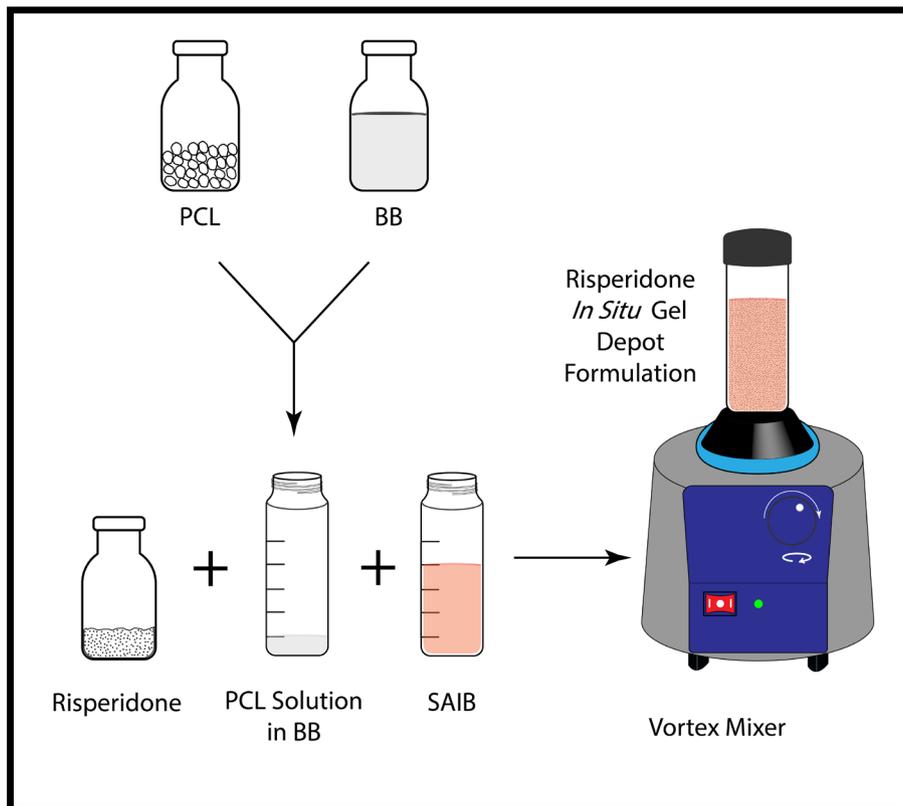


FIGURE 2 - Schematic representation of risperidone depot injection formulation preparation. PCL- Polycaprolactone; BB- Benzyl benzoate; SAIB- Sucrose acetate isobutyrate

The lower limit of PCL concentration in the formulations was kept as 4% because the burst release from the formulations containing less than 4% PCL was high. On the other hand the upper limit of PCL concentration was kept as 7%, as this was the maximum amount of PCL that could be incorporated into the formulations in the dissolved form. All the trial formulation batches contained 10 mg risperidone, 80%SAIB, and requisite amount of PCL. A formulation batch containing only PCL solution without SAIB was also prepared (RIS/IS/OP5). The composition of all the formulation optimization batches designed is shown in Table III.

The prepared formulations were subjected to *in vitro* drug release study for 30 days and the cumulative percent drug release at different time points is reported in Table IV and graphically represented in Figure 3.

TABLE III - Composition of formulation optimization batches of risperidone depot injection

| Formulation batch code | Drug (mg) | SAIB (%) | PCL (%) | BB (%) |
|------------------------|-----------|----------|---------|--------|
| RIS/IS/OP1 | 10 | 80 | 4 | 16 |
| RIS/IS/OP2 | 10 | 80 | 5 | 15 |
| RIS/IS/OP3 | 10 | 80 | 6 | 14 |
| RIS/IS/OP4 | 10 | 80 | 7 | 13 |
| RIS/IS/OP5 | 10 | 0 | 7 | 93 |

SAIB- Sucrose acetate isobutyrate; PCL- Polycaprolactone; BB- Benzyl benzoate

TABLE IV - In vitro drug release from formulation optimization batches of risperidone depot injection

| Formulation batch code | In vitro cumulative % drug released (n = 3) | | | | | |
|------------------------|---------------------------------------------|------------|------------|------------|-------------|-------------|
| | Day 1 | Day 3 | Day 7 | Day 15 | Day 22 | Day 30 |
| RIS/IS/OP1 | 20.56±4.24 | 26.75±3.48 | 42.38±3.59 | 62.75±4.65 | 79.56±2.77 | 92.74±2.73 |
| RIS/IS/OP2 | 12.77±2.41 | 22.31±2.94 | 35.79±3.19 | 55.67±4.08 | 71.26±3.93 | 88.95±4.17 |
| RIS/IS/OP3 | 10.31±1.83 | 13.88±2.54 | 21.50±3.86 | 38.19±3.79 | 52.75±4.07 | 68.75±4.25 |
| RIS/IS/OP4 | 9.63±3.11 | 12.94±2.29 | 20.50±2.53 | 30.75±3.34 | 42.31±4.65 | 51.31±4.03 |
| RIS/IS/OP5 | 23.27±4.83 | 41.25±4.68 | 64.69±4.91 | 90.06±5.37 | 100.44±3.82 | 100.85±1.66 |

± SD; n=3

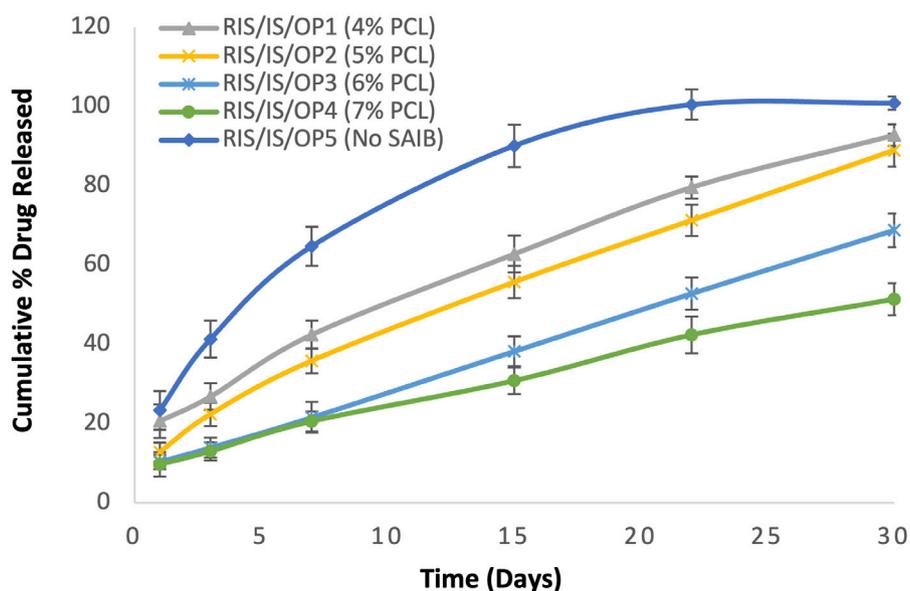


FIGURE 3 - In vitro drug release from formulation optimization batches of risperidone depot injection (mean ± SD; n=3).

The drug release data shows that as the concentration of PCL in the formulations was increased the drug release rate was progressively decreased. The first day burst release for the formulation batches RIS/OP/1-4 which contained 4%, 5%, 6%, and 7% PCL was 20.56%, 12.77%, 10.31%, and 9.63%, respectively. The burst release from the formulation batch containing only 7% PCL and no SAIB (RIS/IS/OP5) was 23.27%, which shows that the PCL alone also is not efficient enough in retarding the drug release from the formulation.

The calculated $T_{50\%}$ values for the formulations containing 4%, 5%, 6%, and 7% PCL (formulation

batches: RIS/IS/OP1-4) were 11.4 days, 13.9 days, 20.8 days, and 28.3 days, respectively while the $T_{90\%}$ values were 27.2 days, 29.5 days, 40.5 days, and 55.9 days, respectively. The formulation without SAIB (formulation batch: RIS/IS/OP5), however, produced the $T_{50\%}$ and $T_{90\%}$ values as 5.35 days and 20.57 days, respectively which confirmed that neither PCL nor SAIB alone could adequately sustain the release of risperidone from the formulation, their combination, however, could achieve the one month sustained release objective of depot formulation more effectively.

The formulation batch RIS/IS/OP2 containing combination of 5% PCL and 80% SAIB showed the most prominent sustained release profile. It achieved 50% and 90% of the total observed drug release in about 14 days and 30 days, respectively, and yielded a cumulative release of ~89% of the loaded drug. This formulation composition (RIS/IS/OP2) was therefore selected as the optimized formulation of the risperidone depot injection. It exhibited a 12.77% initial burst release on day one facilitating the drug release from first day itself with a drug release phase lasting up to 30 days. On the contrary, the drug release exhibited by the reported *in vitro* drug release profile of the available marketed formulation (Risperdal Consta®) showed only a 1.6% release on day one followed by a release time lag of about three weeks and then a drug release phase up to 40 days after administration of depot injection (Rawat *et al.*, 2011).

Drug release kinetics:

The 30 days cumulative percent drug release- time profile data of the optimized formulation (RIS/IS/OP2) were fitted into different kinetic models namely zero-order, first order, Higuchi, Hixson- Crowell, and Ritger-Peppas models and the correlation coefficient value for each model was determined. The data best fitted in the Higuchi model ($r^2 = 0.9911$) which demonstrated that the drug release from the developed depot injection system followed fickian diffusion (Su *et al.*, 2009). The r^2 values for other models were found to be 0.9696, 0.9641, 0.9869, and 0.9367 for zero-order, first-order, Hixson-Crowell, and Ritger-Peppas models, respectively. It is reported that when the temperature of study is above glass-transition temperature (T_g) of the polymer, the fickian diffusion is observed (Masaro, Zhu, 1999) and as PCL has a T_g of -60°C (Woodruff, Hutmacher, 2010) the diffusion controlled drug release is further confirmed. The drug release-time profile for 1-30 days period showed best fit for zero-order kinetic model with r^2 value of 0.99 suggesting that after the first day, the depot system maintained a uniform and sustained drug release upto a period of 30 days as can be seen in the drug release curve for the optimized formulation (RIS/IS/OP2) in Figure 3. This may be attributed to the attainment of uniform flux rate

after the initial burst release phase of day one. The slope of the drug release curve for the optimized formulation (RIS/IS/OP2) signified a drug release flux of 2.56% per day which is equivalent to 0.256 mg drug per day.

EVALUATION OF THE DEVELOPED FORMULATION

Visual appearance:

The developed injectable depot formulation was a white coloured injectable viscous liquid dispersion.

Viscosity:

Viscosity of the formulation was found to be 75.45 ± 0.107 mPas ($n = 3$).

Weight per mL:

The weight per mL of the developed risperidone depot injection was found to be 1.115 ± 0.004 g/mL ($n = 3$).

Effect on the pH of fluid present at injection site:

The developed risperidone depot injection was injected into 10 mL distilled water to form an *in situ* gel depot, and the pH of the surrounding fluid was measured for one hour as the formation of depot after injecting the formulation is expected to get completed within this time duration. The pH of water remained unchanged at 7.0 ± 0.48 during 1 h of study. This implies that the presence of the depot injection at the site of injection would not alter the pH of the biological fluid present at the injection site.

Syringeability:

The injection force required to expel the formulated depot injection from the syringe was found to increase with an increase in the needle gauge size and was in order of $22\text{G} > 21\text{G} > 20\text{G} > 18\text{G} > 16\text{G}$ and was measured as 175 N, 120 N, 55 N, 16-26 N, and 6 N, respectively for 22G, 21G, 20G, 18G, and 16G needles. The needle gauge size recommended for intramuscular administration is 20-23G (Heller, Veach, 2008) and the formulated depot injection was readily syringeable through 20G and 21G needles.

Stability:

The stability study results against the set in-house limits are reported in Table V.

The formulation was found to be stable with respect to physical appearance, resuspendibility, and drug content for up to 6 months at the real time as well as accelerated storage conditions.

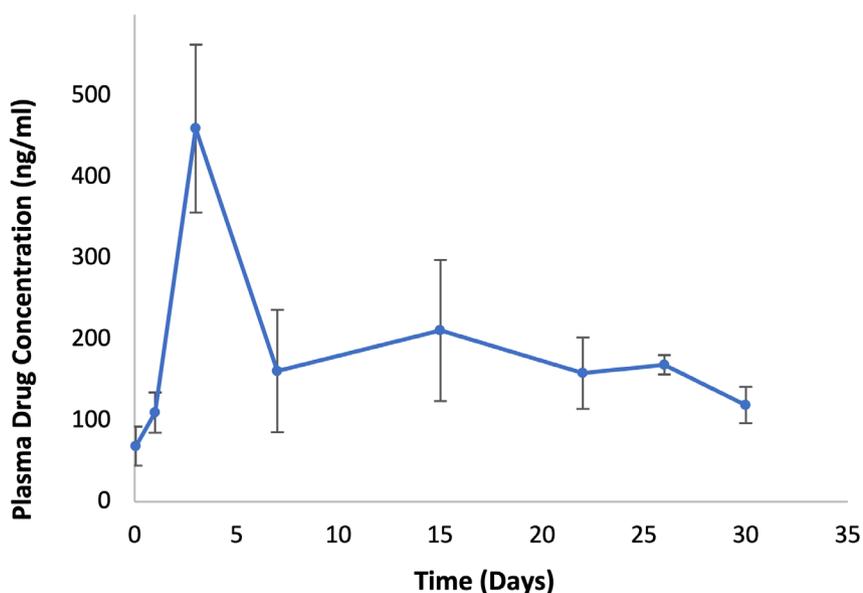
TABLE V - Stability study data of the developed risperidone depot injection

| Test parameter | In-house Limit | Initial | Storage Condition | | | | | | |
|---------------------|--------------------------|-----------|-----------------------|-----------|-----------|---------------------|-----------|-----------|-----------|
| | | | 25 ± 2° C/ 60 ± 5% RH | | | 40 ± 2°C/75 ± 5% RH | | | |
| | | | Time (Month) | | | Time (Month) | | | |
| | | | 1 | 3 | 6 | 1 | 3 | 6 | |
| Physical appearance | White viscous dispersion | No change | No change | No change | No change | No change | No change | No change | No change |
| Resuspendibility | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| Assay (%) | 90-110 | 100.76 | 99.42 | 98.19 | 97.08 | 99.14 | 97.36 | 94.70 | |

***In vivo* pharmacokinetic study:**

The plasma levels attained by the developed risperidone depot injection in rats are shown in Figure 4. The mean peak plasma drug concentration of 459.7 ng/mL attained after 3 days of drug administration came down to 160.8 ng/mL on 7th day. The plasma drug concentration,

by and large, was maintained between 158.3 ng/mL and 211.1 ng/mL concentration range after 7th days to 26 days of administration and subsequently decreased to 119.0 ng/mL on 30th day. This confirmed that the developed risperidone depot injection sustained the drug release for the period of 30 days.


FIGURE 4 - Mean plasma concentration - time profile of risperidone after subcutaneous administration of the developed risperidone depot injection into rats (mean ± SD; n = 6).

The steady state plasma drug concentration of risperidone is reported to be in range of ~10-30 ng/mL while its recommended therapeutic concentration range lies between 20-60 ng/mL (Baumann *et al.*, 2004). The plasma concentration obtained from the developed product in present study is above this therapeutic concentration range for all the studied time points during the period of one month study. The higher plasma drug concentration attained cannot be of any toxicity concern as the dose administered in present study, i.e., 25 mg/kg

is much lesser than the reported LD₅₀ value of 172 mg/kg for subcutaneous risperidone injection in male rats (Product Monograph, 2004).

The pharmacokinetic parameters obtained from plasma drug concentration data of the developed risperidone depot injection are presented in Table VI. The C_{max} and AUC_{0-30d} are reported as mean ± standard deviation while the other pharmacokinetic parameters were obtained from Kinetica software using mean values.

TABLE VI - Pharmacokinetic parameters of developed depot injection of risperidone

| Pharmacokinetic Parameter | C _{max} (ng/mL) | T _{max} (d) | AUC _{0-30d} (ng.d/mL) | λ _z (d ⁻¹) | t _{1/2} (d) | MRT (d) | V _z /F (L/kg) | Cl/F (L/d.kg) |
|---------------------------|--------------------------|----------------------|--------------------------------|-----------------------------------|----------------------|---------|--------------------------|---------------|
| Value | 459.7 ± 104 | 3 | 5906.4 ± 1801 | 0.0336 | 20.6 | 31.2 | 79.7 | 2.7 |

C_{max}- Peak plasma drug concentration; T_{max}- Time to achieve peak plasma drug concentration; AUC_{0-30d}- Area under the plasma drug concentration-time curve; λ_z- Terminal elimination rate-constant; t_{1/2}- Terminal elimination half-life; MRT- Mean residence time; V_z/F- Apparent volume of distribution after extravascular administration; and Cl/F- Total apparent clearance of drug from plasma after extravascular administration

The mean residence time of 31.2 days indicated that the drug remained in the body for this much time duration. The terminal elimination half-life of risperidone after administration of the developed formulation was 20.6 days and indicates the elimination and distribution of risperidone from the depot due to degradation of SAIB-polymer matrix. The volume of distribution, i.e., 2.7 L/d.kg indicates extensive distribution of risperidone in the body (Grant, Fitton, 1994). The sustained drug release observed from the formulation can be mainly attributed to the release retarding barrier properties of viscous SAIB-PCL polymer matrix gel formed *in situ* at the site of injection. The drug particles suspended in this matrix acted as the drug reservoir and slowly and continuously dissolved to maintain a constant flux of drug release into the surrounding fluid (Lu, Yu, Tang, 2007) and resulted into a predominantly zero order drug release profile (Chien, 1982). After four days of the drug

administration, the SAIB present in the polymer matrix would also gradually start degrading (Lin *et al.*, 2015) and then would support in the drug release from the formulation. The observed initial burst release produced by the developed formulation can be ascribed to the release of some amount of drug present in the formulation in dissolved state.

In vitro- in vivo correlation:

The graph between fractional AUC of plasma concentration profile and *in vitro* cumulative percent drug release of the formulated depot injection at different time points shown in Figure 5 is linear over 1-30 days and 0-30 days with correlation coefficients of 0.9987 and 0.9590, respectively. This demonstrates a good correlation between the *in vitro* and *in vivo* drug release profiles.

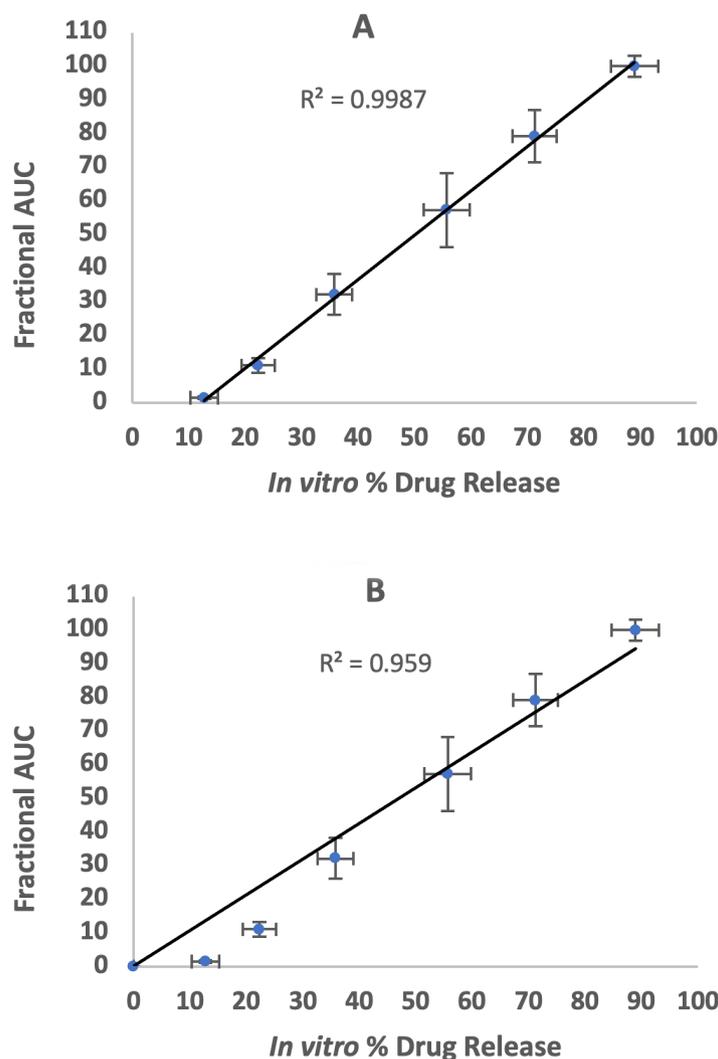


FIGURE 5 - *In vitro*- *in vivo* correlation of the developed risperidone depot injection over [A]: 1-30 days and [B]: 0-30 days (mean \pm SD; n=3: *in vitro* data; n=6: *in vivo* data).

The pharmacokinetic profile of the developed risperidone depot injection exhibited sustained drug release lasting for one month duration without any lag phase. On the other hand the intramuscular administration of the marketed depot product (Risperdal Consta[®]) is reported to show a lag phase of three weeks before the drug release phase (PrescribingInformation, 2007). Also, the developed product was found to be stable at room temperature without requiring cold storage conditions while the recommended storage condition for the marketed product (Risperdal Consta[®]) is 2-8°C and therefore it has to be transported through cold supply chain (PrescribingInformation, 2007).

CONCLUSION

An *in situ* gel forming depot injection of risperidone was developed using SAIB and PCL as biodegradable release retardants. The optimized injection formulation mainly consisted of 80% SAIB and 5% PCL in benzyl benzoate as solvent. The formulation had acceptable viscosity and syringeability and exhibited a predominantly zero order *in vitro* drug release profile with 89.95% cumulative drug release in 30 days. The *in vivo* pharmacokinetic studies demonstrated a sustained drug release for one month having a mean residence time of 31.2 days, half-life of 20.6 days, and a good *in vitro*- *in vivo* correlation. This developed

depot injection obviates the use of heat sensitive polymer like PLGA thereby eliminating cold temperature storage and cold supply chain transportation requirements and effectively resolves the drawback of lag time of depot formulation presently available in the market.

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