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RESEARCH ARTICLE

Nanocrystal-based drug delivery system of risperidone: lyophilization and characterization

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ABSTRACT

Objective: In the present work nanocrystal-based formulation of risperidone (RIS) was proposed to overcome solubility issue of RIS, while lyophilization technique was used effectively, for conversion of RIS nanosuspension to solid state.

Significance: RIS nanosuspension was developed and stabilized with a combination of polycaprolactone and Pluronic® F-68 as stabilizers. With focus on critical parameters like nature of cryoprotectants and effect of eutectic temperature on properties of nanosuspension, the suitability of lyophilization technique in improving the physical stability of prepared nanosuspension was also evaluated. Additionally, the developed nanocrystals were also assessed for their solid states properties.

Methods: Various process parameters affecting average particle size and polydispersity index (PDI), viz. drug to surfactant ratio, solvent to anti-solvent ratio, stirring speed, type of stabilizer were optimized. Assessment of lyophilization as a suitable solidification technique (for conversion to powder form) was done with selective cryoprotectants (trehalose dihydrate and sorbitol).

Results: The formulation was found to be stable at 4 °C for 3 months with size, PDI and zeta potential of 214 ± 3.4 nm, 0.120, and −10.2 ± 0.90 mV, respectively. Release profile of developed nanosuspension showed cumulative % release of ∼90% in initial 10 h whereas the value for the unprocessed drug was ∼11% in same time frame.

Conclusions: These findings suggest that developed formulation was able to enhance water solubility of the drug effectively and can be potentially used in the management of psychotic disorders.

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KEYWORDS
Psychotic disorders; nanosuspension; stabilizers; release studies; nanoprecipitation

Introduction

Psychotic disorders and schizophrenia are the conditions associated with impairment of social function, quality of life, cognitive and daily activities of the patient. Symptoms associated extend to diminishing interpersonal and vocational skills. This impairment is even reported after clinical recovery of patients which make these disorders to be additionally taken care of. Various categories of compounds classified as typical and atypical antipsychotic agents are used in the treatment of these psychotic disorders [1–3].

Risperidone (RIS), a second generation antipsychotic agent has been used for a long time in the management of psychological disorders including schizophrenia, bipolar mania, and irritability associated with autistic disorder [4,5]. Proposed mode of action for RIS (benzisoxazole derivative) is blocking of serotonin-2A (5-HT2A) and dopamine D2 receptors. Despite of its potential, RIS is reported to have higher log *p* values along with poor water solubility and is classified as biopharmaceutical classification system (BCS) class II drug [6]. This poor water solubility leads to limited oral bioavailability through gastrointestinal tract as well as by parenteral route and limits the use of aforementioned drug candidate [4]. Various solubility enhancement techniques can be used to enhance the apparent solubility of BCS class II drugs such as salt formation, solid state modification, use of co-solvents, hydro-trophy, complexation with cyclodextrins size reduction, etc. Researchers have reported solid dispersion [7], self-nano-emulsifying powder [8], and co-solvent [9] approach for enhancement of RIS water solubility. Use of sodium lauryl sulfate (SLS) in marketed formulation (Risperdal®) of drug presents the probability of solubility enhancement with the use of surfactant [10]. All these approaches have their advantages and limitations like toxicity issues associated with the use of co-solvents [11] (generally water miscible organic solvents) and surfactants [12]. Salem and Kharshoum have reported sterically stabilized nanosuspension of drug RIS, with 2fold enhancement in oral bioavailability [13], wherein surfactant amount used is beyond a particular level.

In the last decade, nanosuspension technology has emerged as potential solubility enhancement technique in the full-fledged form [14–17]. Nanocrystals are made up of the drug with the little amount of surfactant (below critical micelle concentration (CMC)) to stabilize formulation [18]. Most of the nanoparticles are made up of a large amount of excipients which is not the case with nanocrystals as most of the part is only the drug. Besides, the lower amount of stabilizers makes toxicity issues associated with nanosuspensions negligible and offers ease of scale-up and better physical stability compared to amorphous form [19,20]. Different methods are classified as top-down (high-pressure homogenization (HPH), media milling, and sonication) and bottom-up techniques (nanoprecipitation) for effective production of nanocrystals [21]. Development of nanocrystal-based formulation of RIS can be advantageous to tackle the problem of poor water solubility. Numerous solidification techniques are used to increase the physical stability of nanosuspensions as spray drying, lyophilization and

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many more based on the properties of drug and characteristics of the final formulation. Spray drying is more suitable for products that can withstand higher temperature, and thus may not be feasible for heat labile products. Among all these techniques, lyophilization is used predominantly for nanosuspension solidification, as it offers several advantages including, production of high value products without excessive damage, suitable for drying of heat labile product, enhanced stability on storage and easy reconstitutability prior to use [22].

The main focus of the present work was to explore lyophilization as the solidification technique for nanosuspension of RIS and to see the impact of this process variables on solid state properties of RIS nanosuspension. With focus on critical parameters, like nature of cryoprotectants and effect of eutectic temperature on properties of nanosuspension, the suitability of lyophilization technique in improving the physical stability of prepared nanosuspension was also evaluated. Additionally, the variables for nanosuspension production were optimized including the levels of steric stabilizer, ensuring that their levels are well within the IIG and CMC limits.

Experimental

Materials

RIS was obtained as generous gift sample from Zydus-Cadila Pvt. Ltd. (Ahmedabad, India). Trehalose dihydrate (TD), sodium deoxycholate (SDC), polycaprolactone (PCL) (MW-45,000), Pluronic® F-68, hydroxy propyl beta cyclodextrin (HPβCD), L-arginine and Pluronic® F-127 were procured from Sigma-Aldrich (Darmstadt, Germany). Polyethylene glycol (PEG)-400, sorbitol, sucrose, Tween® 80, polyvinyl alcohol (PVA), and polyvinylpyrrolidone K-30 (PVP K-30) were acquired from HiMedia Laboratories (Mumbai, India). Mannitol and fructose were acquired from Fisher Scientific (Mumbai, India). Sodium chloride, sodium hydroxide, and disodium hydrogen phosphate were products of S D Fine Chemicals (Mumbai, India). Purified water was used in all experiments, obtained from a Milli®-Q Biocel, Millipore® (Burlington, MA). All other reagents and solvents were of analytical grade and used as such without further purification.

Methods

Drug-excipient compatibility studies

Physical compatibility of the drug with excipients was accessed using differential scanning calorimetry (DSC) [23,24]. All measurements were carried out on an Indium calibrated Auto DSC Q20 V24.9 Build 121 (TA Instruments, New Castle, DE) equipped with a refrigerated cooling system (RCS). Data acquisition and analysis were carried out using the Universal Analysis 2000 program (TA Instruments, New Castle, DE). The study was performed on RIS alone and physical mixture (1:1) of excipients with the drug. Samples (1–3 mg) were weighed into aluminum pans (TA Instruments, New Castle, DE) and heated under dry nitrogen (50 ml/min) in the scanning range between 25 and 190 °C at a rate of 10 °C/min using empty pans as a reference.

Preparation of nanosuspension

Nanocrystals of the drug were optimized by three methods comprising of one bottom up (nano-precipitation) and two combination techniques (nano-precipitation with probe sonication and nano-precipitation with HPH). The solvent–antisolvent method was used to achieve nano-precipitation. The solvents were screened from methanol, acetone, and dichloromethane (DCM). Polymers and surfactants as stabilizers were evaluated from Pluronic® F-68, Pluronic® F-127, PCL, PVA, and SDC alone and in combination. Processing conditions and material attributes, viz. type of stabilizer, drug to surfactant ratio, the solvent to antisolvent ratio, rotational speed were optimized to achieve desired size and polydispersity index (PDI) [25].

For combination approach using probe sonication, processing of nanoprecipitation was followed by probe sonication (Ultrasonic Processor VC505, Sonics & Materials Inc, Newtown, CT) for 5 min with processing conditions of 5 s impulse on, 3 s impulse off at 25% amplitude. In combination approach using HPH, same nanoprecipitation method was used to obtain a suspension which was subjected to HPH (PandaPLUS 1000, GEA NiroSoavi, Bedford, NH) for five cycles at a pressure of 500 bar followed by 15 cycles at 800 bar. All the batches were prepared in triplicates ($n = 3$) on the same day as well as on three successive days.

Solidification of nanosuspension

Various solidification techniques like spray drying [26] and freeze drying [21] are used for solidification of nanosuspensions as it is well reported that physical and chemical interactions take place faster in liquid form as compared to solid form [27]. An optimized batch of nanosuspension was lyophilized (Christ alpha 4d plus, Germany) to maintain the stability of nanosuspension. Compatibility of all cryoprotectants with the drug was evaluated (data not shown) as described in preceding sections. Mannitol, fructose, TD, sucrose, L-arginine, D-sorbitol, HPβCD, and PVP-K30 were screened for their use as cryoprotectant [21]. In initial trials, all the cryoprotectants were added in a concentration of 10% and nanosuspension was frozen at –80 °C for 48 h, followed by thawing at room temperature. Two such freeze–thaw cycles were performed. After thawing, particle size and PDI were determined, the cryoprotectants giving S_f/S_i ratio (where S_f and S_i are particle size after and before freeze–thaw cycles, respectively) of 1 ± 0.3 were selected for further optimization of their concentration [28].

To setup process parameters for lyophilization cycle, glass transition temperatures (T_g) of all these cryoprotectants were determined by DSC scans (an Indium calibrated Auto DSC Q20 V24.9 Build 121 (TA Instruments, New Castle, DE) equipped with an RCS). Data acquisition and analysis were carried out using the Universal Analysis 2000 program (TA Instruments, New Castle, DE). Thermal analysis was performed in a range of –70 to 20 °C with a cooling and heating rate of 10 °C/min for all cryoprotectant solutions and a mixture of cryoprotectant with nanosuspension.

For lyophilization, samples were frozen at –45 °C before primary and secondary drying steps. After freezing, primary drying was commenced at –30 °C at pressure of 250 mT (inbetween hold at –25 °C) for variable time periods. Finally, secondary drying was done at temperatures of 10 °C for 300 min utilizing pressures of 150 mT.

Characterization of formulation

Particle size and zeta potential analysis. Average particle size and PDI were measured by Malvern Zetasizer (Nano ZS90 series, Malvern, UK) working on the principle of dynamic light scattering. Zeta potential measurements were carried out using the same instrument. One milligram of lyophilized product was redispersed in 3 ml of Milli®-Q water and vortexing was done as per the need for uniform dispersion of sample. All the measurements were done in triplicate ($n = 3$).

Particle morphology. Particle morphology of lyophilized formulation was analyzed with scanning electron microscope (Nova SEM 450, Beaverton, OR) and atomic force microscope (Nova Scan, Beaverton, OR) under normal atmospheric conditions. Tapping mode atomic force microscopy (AFM) was applied in this study. The typical resonance frequency of these tip cantilever systems was about 134 kHz. Scan speed was set at 0.5 Hz and scan sizes were taken from 100 nm to 800 nm. Sample preparation was done by diluting sample to the ratio of 1:100 (1 mg in 100 ml) with double-distilled water and then dropping slightly onto the glass slide followed by subsequent drying at ambient conditions to circumvent the movement of particles. The data were represented in topographic and phase imaging. SEM analysis was carried out in instrument operated at an excitation voltage of 25 kV. The powder samples were mounted onto a steel stage using double sided adhesive tape before analysis.

Characterization of physical state. X-ray diffraction (XRD) was applied to analyze the physical state of the sample. XRD patterns of the unprocessed drug, lyophilized drug alone without cryoprotectant and lyophilized nanosuspension were obtained using XRD (Xpert MPD, Philips, Amsterdam, Netherlands) employing Cu/Ni radiation. The diffractograms were scanned in the range from 0° to 50° 2θ at a rate of 2°/min. The sample was taken in the amount needed and crushed with mortar and pestle before applying to sample holders.

Stability studies. Stability studies for nanosuspension were commenced by storing formulation at 4 °C and withdrawing samples at predetermined time points of Day 1, 7, 15, 30, 60 and 90. Size and PDI value measurements for all the withdrawn samples were done with Malvern Zetasizer (Nano ZS90 series, Malvern, UK) with the same protocol mentioned in preceding sections. Studies were performed in triplicate ($n = 3$).

Analysis of drug content. The analytical method of the drug was developed using UV-VIS spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan) in phosphate buffer solution (PBS) pH 7.4. A stock solution of drug (100 μg/ml) was prepared by accurately weighing 10 mg of drug and dissolving it in 1 ml methanol and volume was made up to 100 ml with PBS pH 7.4 in volumetric flask. This stock solution was appropriately diluted to get a concentration in the range of 2–24 μg/ml which was scanned in the range of 400–200 nm to obtain λ_{max} values and a calibration curve was made. All readings were recorded in triplicate ($n = 3$). The calibration curve was validated with inter-day and intra-day measurements. Linearity, accuracy and precision were determined. Accurately weighed 5 mg of lyophilized sample was dissolved in 1 ml of Milli®-Q by vortexing and sample was analyzed for drug content by the aforementioned analytical method. Samples were filtered with 0.2 μm filter prior to analysis.

Analysis of residual solvent amount. The residual solvent acetone in lyophilized samples was analyzed by gas chromatography (GC) [29]. The system was an Agilent 7820A with a capillary column and a flame ionization detector (Agilent Technologies, Santa Clara, CA). Fifty milligrams of lyophilized sample was accurately weighed and dissolved in 10 ml N,N-dimethyl formamide (DMF, analytical grade). 1.0 ml of the solution was injected into the GC system at a flow rate of 2.5 ml/min with nitrogen as the carrier gas. Oven temperature was maintained at 100 °C, the injector and detector temperature were set to 220 and 250 °C, respectively.

In-vitro release studies. The dialysis bag diffusion technique was used to study the *in vitro* drug release of lyophilized powder and unprocessed drug. Lyophilized powder and unprocessed drug (dose equivalent; i.e. 2 mg) were placed in the separate dialysis bags (cellulose membrane, molecular weight cut off 12,000 Da, HiMedia Labs Pvt. Ltd, Mumbai, India), hermetically sealed and immersed into 250 ml of phosphate buffered saline (pH 7.4) maintaining sink condition. The entire system was maintained at 37 ± 0.5 °C with continuous magnetic stirring at 100 rpm/min. Five milliliters of sample was withdrawn from the receptor compartment at predetermined time intervals and replaced with fresh medium. Release study was carried out for 72 h. The amount of drug dissolved was determined by UV spectrophotometry at 279 nm [30] with the previously defined method.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) by GraphPad PRISM® (version 5.01, San Diego, CA). Data are expressed as mean ± standard deviation ($n = 3$). Statistical significance was considered for values $p < .05$.

Results and discussion

Drug-excipient compatibility studies

DSC curve of RIS showed an endothermic peak at 171.71 °C representing the melting point of the drug [31]. The endothermic event of the drug was found to be retained in all the physical mixtures with stabilizers (Tween® 80, Pluronic® F-68, Pluronic® F-127, PCL, SDC, PVA, PEG-400) except Tween® 80 and PEG 400 as shown in Figure 1. With Tween® 80 and PEG 400, melting endotherm was found to disappear which could be due to the solubilization of drug in Tween 80 and PEG 400. Hence stabilizers except Tween® 80 and PEG 400, were taken further for formulation optimization.

Preparation of nanosuspension

Nanoprecipitation method

Desired particle size and PDI of the final formulation were found to be dependent upon various parameters including solvent used, drug to surfactant ratio, solvent to antisolvent ratio, and stirring speed [25,32]. One parameter was optimized at a time by keeping other parameters as constant. Methanol, acetone, and DCM were primarily screened on the basis of miscibility with drug and drug solubility. Initial trials showed that DCM was found to be giving aggregated nanosuspension with larger particle size as compared to methanol and acetone. Probable reason of DCM giving larger size could be slower diffusion of DCM in water which will lead to lesser crystal nucleation and formation of larger crystallites [33]. Methanol produced nanosuspension whose particle size increased considerably after storage of 3 weeks, hence methanol was omitted and acetone was considered further for optimization of solvent to anti-solvent ratio. Table 1 summarizes average values of particle size and PDI.

The volume ratio of acetone to water was found to be a critical factor for process optimization. The solvent to anti-solvent ratio of 1:4 was required to achieve formulation with desired size and PDI. Decrease in this ratio resulted in increased particle size and PDI while further increase in ratio was associated with no further improvement in desired parameters (Table 1). The explanation for this phenomenon could be based on the equilibrium between nucleation and growth rates as increased anti-solvent to solvent ratio increases supersaturation and nucleation rate resulting in

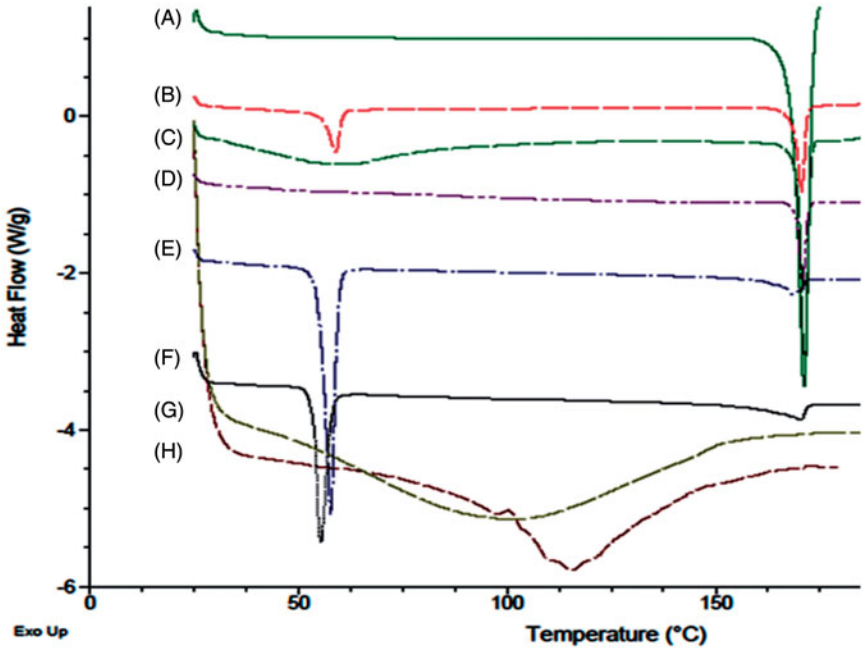

Figure 1. DSC curves of (A) RIS, (B) PCL and RIS, (C) SDC and RIS, (D) PVA and RIS, (E) Pluronic® F-127 and RIS, (F) Pluronic® F-68 and RIS, (G) PEG 400 and RIS, and (H) Tween 80 and RIS.

Table 1. Effect of various processing conditions on size and PDI of drug nanocrystals.

Nano-precipitation method (at 600 rpm)	Average particle size (in nm)	PDI
Solvent		
DCM	2034.0 ± 19.3	0.856
Methanol	718.0 ± 8.2	0.690
Acetone	245.6 ± 5.3	0.260
Drug concentration		
1 mg/ml	226.4 ± 4.6	0.230
2 mg/ml	206.2 ± 7.8	0.211
3 mg/ml	201.8 ± 5.9	0.232
4 mg/ml	213.0 ± 3.2	0.190
5 mg/ml	207.6 ± 6.0	0.200
Solvent to anti-solvent ratio		
1:8	226.0 ± 3.9	0.260
1:4	217.0 ± 3.6	0.120
1:2	219.0 ± 4.8	0.330
Stabilizer		
Pluronic® F-68	415.0 ± 9.7	0.970
Pluronic® F-127	443.1 ± 12.4	1.000
PVA	1786.0 ± 14.3	1.000
SDC	1902.0 ± 29.3	1.000
PCL	185.5 ± 7.1	0.290
Pluronic® F-68 + PCL	216.0 ± 4.7	0.120
Drug to stabilizer ratio (drug:PCL:Pluronic® F-68)		
4:5:20	242.0 ± 9.5	0.315
2:3:10	210.0 ± 3.9	0.180
1:2:5	150.3 ± 6.8	0.303
Nano-precipitation + probe sonication	197.6 ± 6.5	0.300
Nano-precipitation + HPH	431.4 ± 15.3	0.350

smaller size [34]. Drug concentration in the solvent was optimized to be 4 mg/ml as beyond that concentration decrease in zeta potential (data not shown) was observed. From 1 mg/ml to 4 mg/ml drug concentration, there was no significant difference ($p > .05$) among the values of size and PDI (Table 1). From various stabilizers, Pluronic® F-68 and PCL were found to be giving appropriate values for desired parameters. The combination of these stabilizers was thought to work better. Preliminary trials concluded in better results as compared to individual stabilizers and were optimized further to seek ratio of these two. Upon varying the amount of Pluronic® F-68, an increase in solubility of the drug in water was observed which was probably owing to enhancement of particle size due to Oswald ripening [35]. The amount of Pluronic® F-68 was kept constant and PCL amount was varied to optimize the final ratio of 3:10 (Table 1).

In summary, findings suggest that addition of 4 mg/ml of the drug in acetone with a solvent to anti-solvent ratio of 1:4 along with drug:PCL:Pluronic® F-68 ratio of 2:3:10 was needed to achieve appropriate values of particle size and PDI (Figure 2).

Combination methods (nanoprecipitation with probe sonication/ nanoprecipitation with HPH)

Both the combination techniques resulted in increased particle size as well as PDI (Table 1) which may be due to energies provided by probe sonication and HPH process which were sufficient enough to initiate direct collision of particles or to overcome steric barriers generated with the use of stabilizers. Some authors have reported increased particle size due to enhanced collision of particles resulting in increased particle size [36]. For this reason, combination methods were not taken further into consideration.

Lyophilization of optimized nanosuspension

In order to optimize both type and concentration of cryoprotectants, often studies on a large number of formulations are needed, which can be very tedious and time consuming. Besides, lyophilization is an expensive process, freeze–thaw study is comparatively rapid and quick than freeze drying and thus can be used as a pretest for screening of type and concentration of cryoprotectants to be used. Freeze–thaw study is based on the principle that an excipient, which protects nanoparticulate formulation during the initial step of freezing, is likely to be an effective cryoprotectant, and the same was estimated by calculating S_f/S_i ratio. Nature of cryoprotectant and its concentration has major impact on S_f/S_i ratio [37]. In initial trials, all the cryoprotectants were added in a concentration of 10% and nanosuspension was frozen at –80 °C for 48 h,

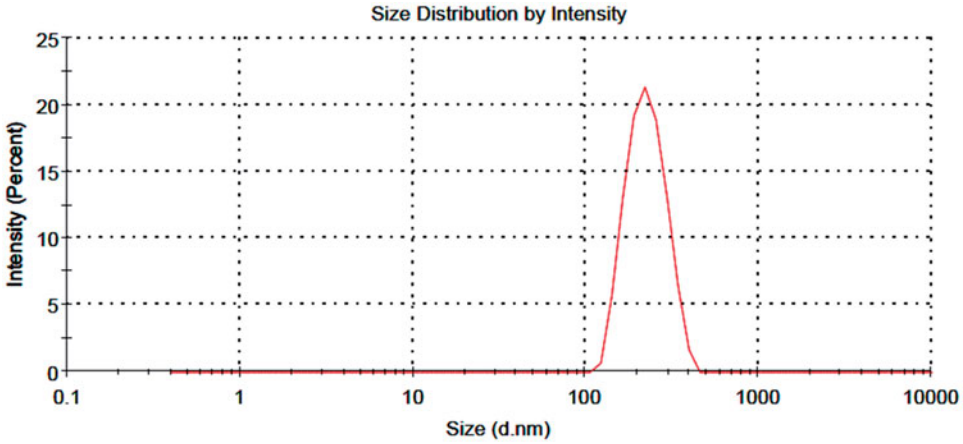

Figure 2. Frequency distribution curve showing size and particle size distribution for optimized nanosuspension.

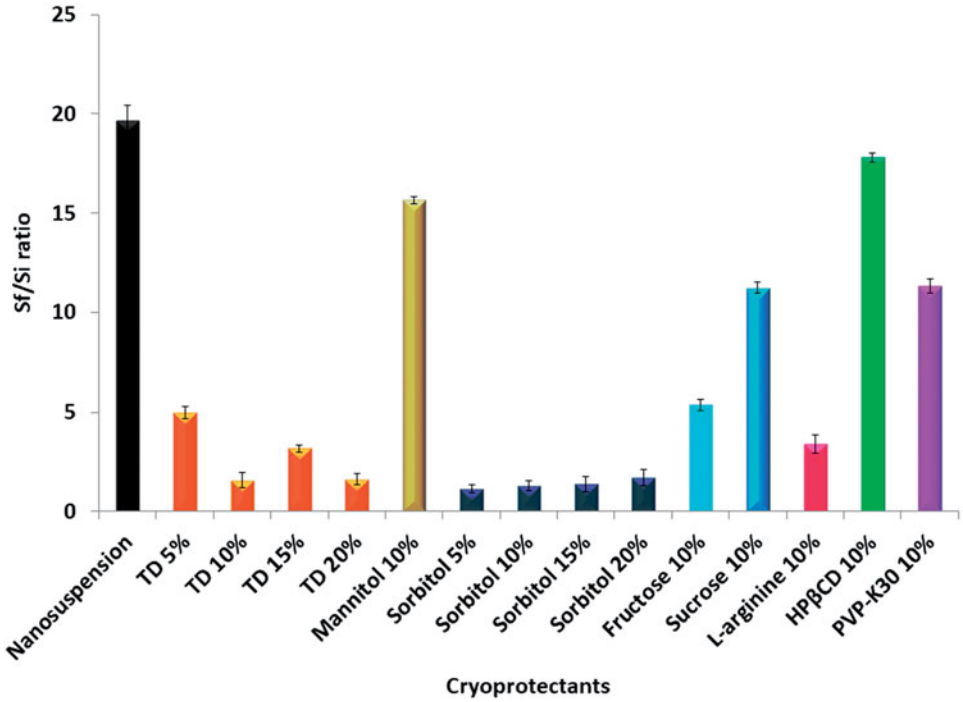

Figure 3. S_f/S_i ratio shown by various cryoprotectants in freeze thaw studies.

followed by thawing at room temperature. S_f/S_i values approaching 1 ± 0.3 suggest retention of initial particle size while values approaching two or more signify aggregation of nanocrystals [38]. Irreversible aggregation was detected for nanosuspension alone (devoid of cryoprotectant) justifying the need of cryoprotectants. Various S_f/S_i values of initial trials are shown in Figure 3. Initial trials with 10% concentration of all the cryoprotectants suggested sorbitol and TD to have a lower value of 1.28 ± 0.25 and 1.57 ± 0.4, respectively, as compared to other cryoprotectants. Both of these were taken further for optimization of effective concentration. Minimum effective cryoprotectant concentration for TD and sorbitol was found to be of 10% and 5%, respectively. Concentrations above were considered for final lyophilization process.

T_g determination for cryoprotectants alone and in combination with nanosuspension (Table 2) suggested a small shift in values for almost all the cryoprotectants screened. Lyophilization parameters were set accordingly. Determination of T_g of a product is an important step in establishing and optimizing the freeze drying process, as collapse temperature depends on it. Collapse temperature determines the maximum temperature that the product can withstand during primary drying without its melting or collapsing. Thermal analysis (DSC and freeze dry microscopy) is a common method used to determine this critical temperature of the product. The collapse temperature of amorphous products is typically a few degrees warmer than its glass transition temperature. Primary drying temperature in lyophilization cycle was set accordingly, taking into consideration experimental values obtained [39].

Table 2. Glass transition temperatures of cryoprotectant solution alone and in combination with nanosuspension.

Cryoprotectants	T_g of cryoprotectant solution (°C)	T_g of cryoprotectant solution with nanosuspension (°C)
TD	−26.21	−26.27
Sorbitol	−18.45	−19.53
L-Arginine	−17.74	−20.71
PVP K-30	−18.36	−21.06
Mannitol	−31.20	−33.70
HPβCD	−26.56	−20.94

Formulation characterization

Particle size and zeta potential analysis. Average particle size, PDI, and zeta potential of finally optimized nanosuspension were found to be 214 ± 3.4 nm, 0.120, and −10.2 ± 0.90 mV, respectively. Obtained values of size and PDI were found to be in acceptable range [40]. Zeta potential plays an important role in the stability of nanosuspension and is a measure of electrostatic stabilization. Despite of having aforementioned values of zeta potential, formulation was found to be quite stable as shown in storage dependent stability data. This was owing to the use of nonionic surfactants in formulation which often results in lower zeta potential values [41].

Analysis of particle morphology. SEM was performed to analyze the surface morphology of unprocessed drug and lyophilized nanosuspension formulation. The unprocessed drug was found to show

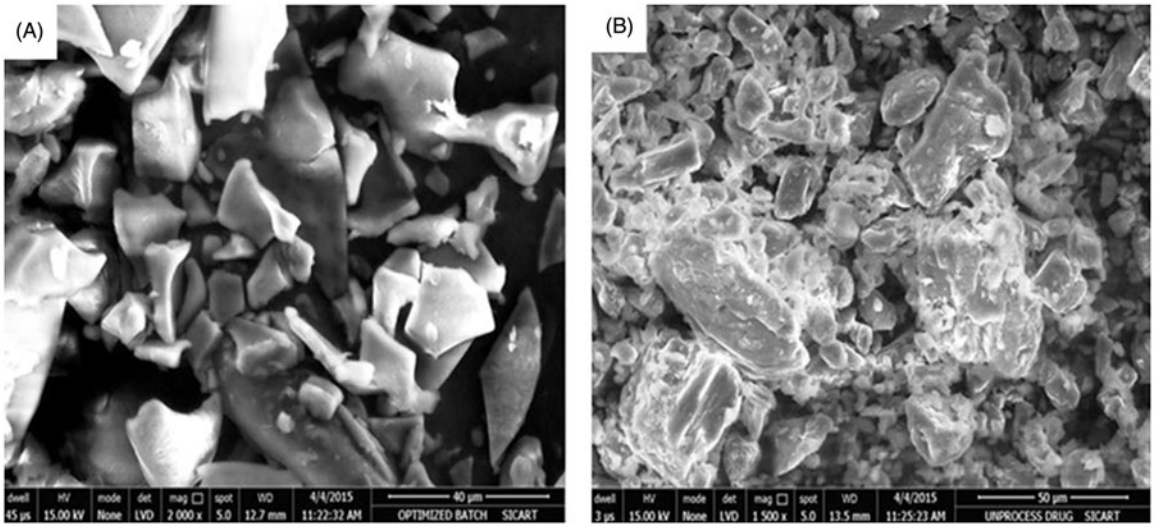

Figure 4. SEM images of (A) unprocessed drug and (B) lyophilized nanosuspension.

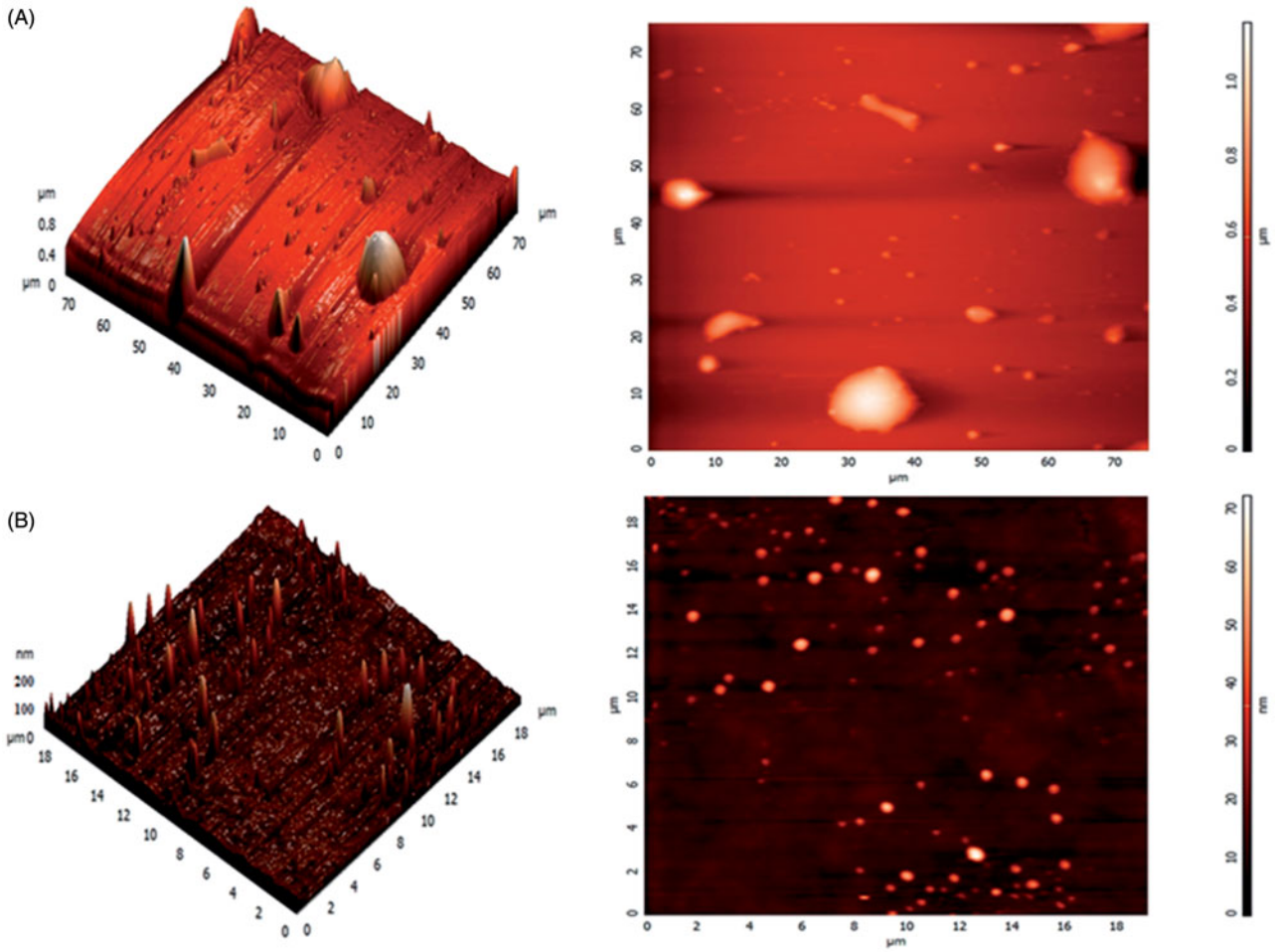

Figure 5. AFM images of (A) unprocessed drug and (B) nanosuspension.

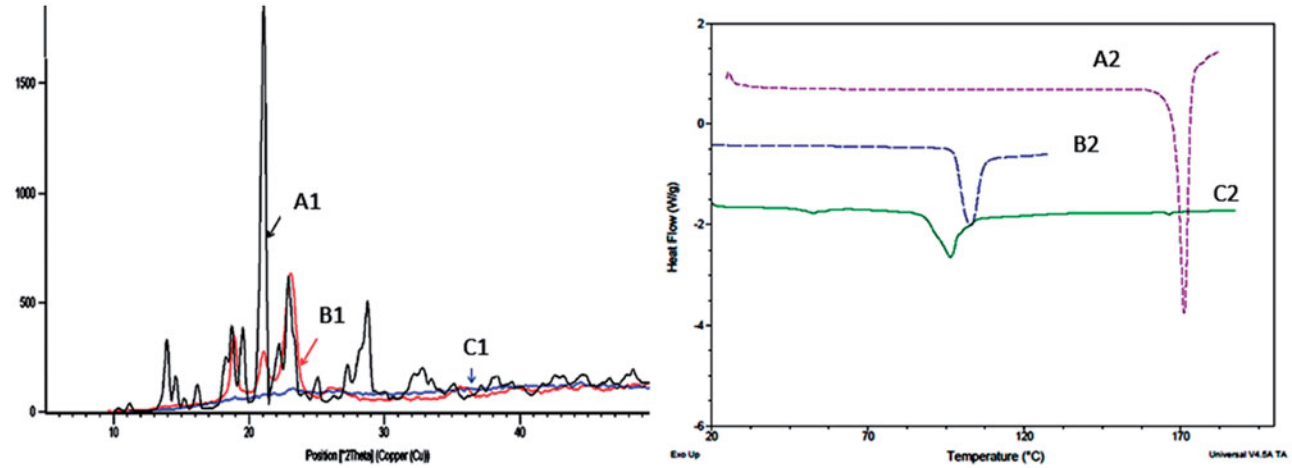

Figure 6. XRD spectra of (A1) RIS, (B1) physical mixture, and (C1) lyophilized sample, DSC curves of (A2) RIS, (B2) cryoprotectant and (C2) final lyophilized formulation.

irregularities on the surface while the lyophilized formulation showed less irregular appearance (Figure 4). Surface roughness was verified with AFM analysis. Results suggested that unprocessed drug had average particle size of 789 nm and average roughness of 67.8 nm. An optimized batch of drug nanosuspension was found to be showing average particle size of 200.87 nm and average roughness of 2.12 nm. It was concluded that the roughness of the particle surface was reduced considerably after processing and the nanoparticle size as observed by AFM correlated well with size measured by dynamic light scattering (Figure 5). Gao et al. in their work on drug nanocrystal have reported reduction in surface roughness for smaller size nanocrystals of drug amitriptyline hydrochloride as compared to larger size agglomerates [42].

Physical state characterization. XRD analysis revealed characteristic peaks of drug at 2θ values of 18.76, 19.55, 21.11, 23.4 and 25.08 suggesting a drug to be present in the crystalline form [43]. After lyophilization, intensity of these peaks was found to be decreased drastically showing partial amorphization of drug present in the lyophilized sample as shown in Figure 6. Many researchers have reported amorphization/partial amorphization of the active ingredient after lyophilization [44,45]. The possible reason behind this could be stressful freeze drying steps like crystallization of water followed by sublimation. During this cooling process for conversion to solid form, some solutes which cannot crystallize, are converted to amorphous form when the cooling temperature goes beyond T_g of concentrated solids [46,47].

The XRD results were further corroborated using DSC (Figure 6), which showed that partial amorphization was seen in case of samples with cryoprotectant where only a small peak of drug was seen in its original position. However, a eutectic peak was observed at temperature below that of both drug and cryoprotectant, indicating that drug was molecularly dispersed. It is reported that when the nanoparticle dispersion containing cryoprotectant is frozen below glass transition temperature, the cryoprotectants forms a glassy/vitreous coating around the nanoparticles and protects them from mechanical stress of ice crystals, thus preventing aggregation. Insufficient concentration of cryoprotectant often leads to incomplete coating of glassy matrix around nanoparticles thereby leading to aggregation, and subsequently crystallization under further stressed conditions [37]. Stability of the lyophilized products for the duration studied revealed that no such partially amorphous to crystalline conversion was occurring upon storage indicating that the system was stable.

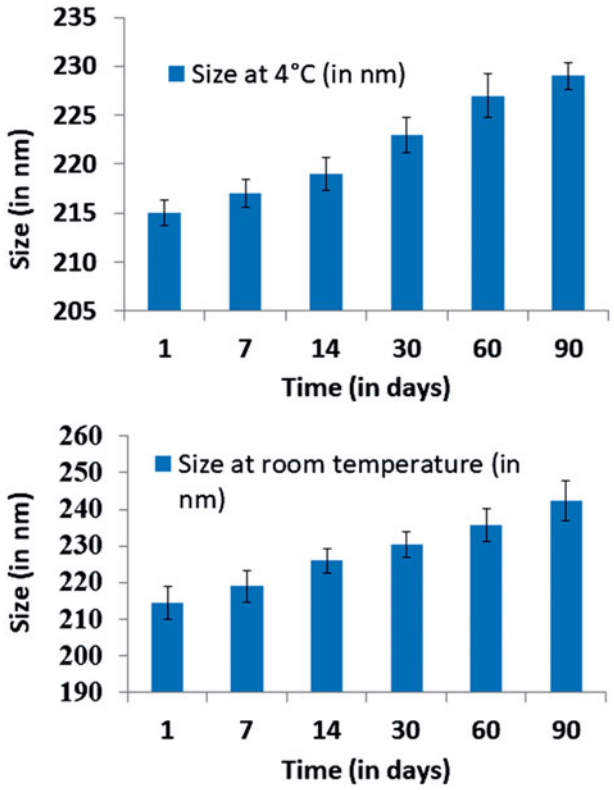

Figure 7. Stability studies of nanosuspension at 4 °C and 25 °C.

Stability studies. Drug nanosuspension was found to be stable at 4 °C with slight increase in size. Study was conducted for a time period of 3 months and sampling at various time point suggested little increase in size and PDI over the time period of study as shown in Figure 7. Ostwald ripening a common phenomena, which leads to formation of larger crystallites at the expense of smaller ones, is a well-known cause of instability in nanosuspension. Increase in size and PDI (growth of nanocrystals in micron range and non-homogeneity of sample) over storage time generally indicates physically unstable nanosuspension [48]. However, the nanosystem was found to be physically stable at storage conditions providing slight increase in size and PDI. Results obtained from stability studies at 25 °C suggested little more increase in particle size in comparison to samples stored at 4 °C due to accelerated storage conditions. Probable reason behind this could be higher kinetic

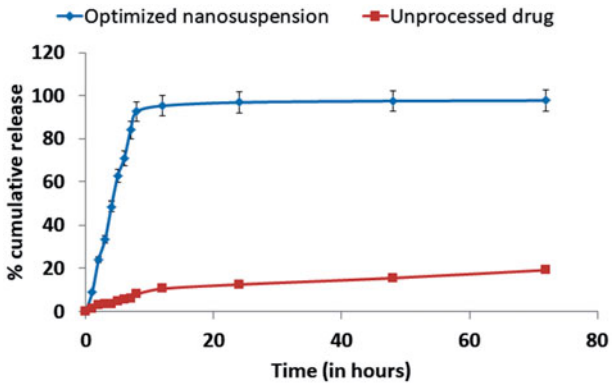

Figure 8. Cumulative % drug release with respect to time.

energy imparted by temperature leading to collision and enhanced chances of aggregation.

Analysis of drug content. The drug content in the lyophilized sample was analyzed with the developed analytical method using UV-VIS spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan) in PBS pH 7.4 and distilled water. Results obtained revealed that drug content in the lyophilized formulation was found to be 70.23%, which was considerably good amount of loading.

Analysis of residual solvent amounta. Acetone, used in the formulation of drug nanosuspension belongs to class III according to International Conference on Harmonization (ICH) and has values of daily limits as 5 mg [49]. Gas chromatography was used to analyze the amount of it as residual solvent in the lyophilized sample. Results suggested that it was either found to be absent or below-permitted levels. Presence of residual solvents can occasionally provide favorable situation for amorphous solid to convert back into crystalline material, hence estimation of their levels is desirable.

In-vitro *release study.* Drug release from nanosuspension was showing around 90% of drug release in initial 10 h while unprocessed drug gave 19% release after study of 72 h. Prepared nanosuspension showed ~4-fold increase in drug release which shows that our drug nanosuspension can provide better release characteristics as compared to the unprocessed drug. Nanocrystallization is known to reduce diffusion distance owing to tremendous decrease in particle size and increase in surface area, thereby enhancing dissolution rate and solubility. This enhanced release could be due to the increase in apparent solubility of drug present in nanocrystalline form (Figure 8). Release profile of unprocessed drug showed only slight increase in amount released over a time period of 10 h whereas formulation revealed a gradual increase in drug release for the same time frame.

Conclusions

Stable nanosuspension formulation of drug RIS was successfully developed. Drug nanosuspension (with average particle size, PDI, and zeta potential values of 214 ± 3.4 nm, 0.120, and −10.2 ± 0.90 mV, respectively) was prepared by the simple method of nano-precipitation with the aid of PCL and Pluronic® F-68 as surfactants. The formulation was found to be stable at temperatures of 4 °C with only little increase in size. Effect of lyophilization as solidification technique was assessed with selective cryoprotectants (TD and sorbitol). Data from *in vitro* release studies revealed about ~90% of drug release in initial 10 h which was very high as compared to the unprocessed drug (~19%). The overall developed formulation has shown potential for enhancing the apparent solubility of drug RIS using nanonization technique.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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