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Original Article

NANOPRECIPITATION TECHNIQUE FOR PREPARATION OF STERICALLY STABILIZED RISPERIDONE NANOSUSPENSION: *IN VITRO* AND *IN VIVO* STUDY

HEBA F. SALEM[1], RASHA M. KHARSHOUM[1]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Beni-Suef University, Egypt
Email: rasha0mohd@hotmail.com

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ABSTRACT

Objective: Risperidone is an atypical antipsychotic drug used to treat schizophrenia; however it suffers from a poor aqueous solubility, which delays its onset of action. Therefore, the purpose of the present study is to utilize the nanotechnology to formulate nanoparticles that enhance the dissolution and hence the bioavailability of risperidone.

Methods: Nanosuspensions were prepared by nano precipitation method in the presence of selected stabilizers at different concentrations. The nanosuspensions were evaluated for their particle size, zeta potential, drug content and *In vitro* drug dissolution. The selected formula was freeze dried and characterized by scanning electron microscopy (SEM), fourier transforms infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffractometry studies (XRD) and pharmacokinetic study.

Results: The *in vitro* dissolution showed higher drug release compared to the pure drug. The optimum formula has an average particle size of 215.56±12.65 nm and zeta potential of-19.84±2.55mV. The bioavailability parameters in the rabbits were enhanced by 2 folds when compared with the marketed tablets (Risperidal®).

Conclusion: Nanoprecipitation method was successfully employed to produce stable risperidone nanosuspension by using the proper stabilizer (pluronic F 127). Risperidone nanoparticles may be a promising formula that improves the dissolution and hence oral bioavailability of poorly water soluble risperidone.

Keywords: Risperidone, Nanosuspension, Pluronic F127, Nanoprecipitation, Bioavailability

INTRODUCTION

Risperidone, a novel benzisoxazole-type atypical antipsychotic, is effective in the treatment of positive as well as negative symptoms of schizophrenia and has a low incidence of extrapyramidal side effects [1-3]. It is approved by the FDA for the treatment of schizophrenia bipolar disorder and irritability in children and adolescents ages [4]. Risperidone is practically insoluble in water, freely soluble in methylene chloride, and soluble in methanol [5, 6]. Nanosuspension is an is an efficient and intelligent approach used to deliver water-insoluble drugs where the drug is reduced to the submicron range as the saturation solubility and the surface area available for dissolution increased thereby increasing its dissolution rate and hence its bioavailability [7, 8]. Stabilizer plays an important role in the formulation of nanosuspensions. In the absence of an appropriate stabilizer, the high surface energy of nano-sized particles can induce agglomeration of articles. The main functions of a stabilizer are wetting the drug particles thoroughly, and preventing Ostwald's ripening [9]. Nanoprecipitation method presents numerous advantages, in that it is a straightforward technique, rapid and easy to perform. In this method, the drug is dissolved in an organic solvent such as acetone, acetonitrile, methanol or ethyl acetate. The organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type of stabilizer, concentrations of stabilizer, and homogenizer speed [10]. In the present work, nanosuspension is prepared by nanoprecipitation method in which drug is dissolved in a solvent, which is then added to non-solvent that cause precipitation of the fine drug particle and the system is stabilized by polymer and surfactant. The objective of this work was to formulate and optimize risperidone nanoparticles.

MATERIALS AND METHODS

Materials

Risperidone (Janssen-Cilag, Egypt), polyvinyl pyrrolidine (PVP K30), was kindly supplied by EPICO pharmaceutical company, Egypt. Pluronic F127 by Sigma-Aldrich, Inc., Germany. Tween 80 (Polyoxyethylene sorbitan monolaurate,) and Ethyl alcohol 95% V/V by EL-Nasr pharmaceutical chemical CO. (Egypt). Every additional chemical or reagent was of analytical grade or better.

Methods

Preparation of risperidone nanosuspensions

Nanosuspensions were developed according to the nanoprecipitation method [11-14]. In brief, risperidone (20 mg) and two different stabilizers (Pluronic F 127, PVP K30) were dissolved in an organic solvent (10 ml of 95% ethanol) to form a series of organic solutions at room temperature (25±1 ℃) containing different concentrations of stabilizers. Deionized water containing a surfactant (1% tween 80), which acts as the antisolvent system was cooled at low temperature (below 4 ℃). This was followed by adding the organic solution into aqueous solution at a very slow rate (0.5 ml/min) by the help of syringe, under high-speed mechanical agitation of 8000 rpm using Probe sonicator (model VC 505) for 10 min to get the desired nano suspension. The cooling was maintained throughout the process using an ice-water bath which controlled the precipitation rate. The batches were prepared according to the formulation design (table 1). The suspensions were kept under vacuum at 25 ° C for 2 h to remove organic solvents.

Characterization of the prepared nanosuspension

Particle size and poly dispersity Index

The prepared risperidone nanosuspensions were measured by Photon Correlation Spectroscopy (PCS) using a Zetasizer Nano ZS-90 instrument. In order to analyse, an aliquot of the nanosuspension was diluted before the measurement. Measurements were performed in triplicate at 90 ° scattering angle and at 25 °C.

Determination of zeta potential

The zeta potential of the nanosuspensions was measured by using an additional electrode in the same instrument used for particle size analysis (Malvern Zetasizer). Samples of formulations were diluted

with water and placed in the electrophoretic cell as the electrophoretic mobility was converted to zeta potential using the Smoluchowski equation [15]. Each sample was measured three times at 25 °C and average values were calculated.

Table 1: The composition of the prepared formulae of risperidone nanosuspension

Formula no.	Drug	Ethanol	Water	Stabilizer type	Stabilizer concentration	Surfactant (tween 80)
F1	20 mg	10 ml	100 ml	Pluronic F127	0.1%w/v	1% w/v
F2	20 mg	10 ml	100 ml	Pluronic F127	0.4%w/v	1% w/v
F3	20 mg	10 ml	100 ml	Pluronic F127	0.6%w/v	1% w/v
F4	20 mg	10 ml	100 ml	Pluronic F127	0.8%w/v	1% w/v
F5	20 mg	10 ml	100 ml	PVP* K30	0.1%w/v	1% w/v
F6	20 mg	10 ml	100 ml	PVP K30	0.4%w/v	1% w/v
F7	20 mg	10 ml	100 ml	PVP K30	0.6%w/v	1% w/v
F8	20 mg	10 ml	100 ml	PVP K30	0.8%w/v	1% w/v

*PVP K30 (Polyvinyl pyrrolidine K30)

Determination of the total drug content

The prepared formulae were assayed individually for the content of the drug as follows: An aliquot (0.5 ml) was dissolved in 10 ml phosphate buffer (pH 7.4) and filtered through 0.45 µm filter paper. The drug concentration was analyzed using UV spectrophotometer (UV-1600, Shimadzu, Japan) at λ_{max} 276.8 nm after proper dilution using phosphate buffer as a blank. Total drug content (TDC) and %TDC were calculated from the following equations:

TDC = [Vol. total/Vol. Aliquot] × Drug amount in aliquot × 100 (Equation 1)

% TDC = [TDC/TAD] × 100 (Equation 2)

Where, Vol. Total/Vol. Aliquot is the ratio of total Nanosuspension volume to the volume of an aliquot taken and the total amount of drug is taken for the formulation of nanosuspension, TAD (total added drug) [16].

***In vitro* dissolution studies of the prepared formulae**

The dissolution of each of the prepared formulae were studied under sink conditions then compared to the raw drug dissolution profile. Accurately weighed bulk drug and nanosuspensions (all equivalent to 2 mg of risperidone) were dissolved in (10 ml) phosphate buffer (pH7.4). The particles were added to a dialysis bag (Mw cut-off = 12,000Da). Following this, the formulae were released in a beaker containing phosphate buffered (500 ml) and stirred at a constant speed of (200 rpm) by a magnetic stirrer at 37±0.5 °C. Samples (5 ml) were withdrawn and replaced with a fresh medium at 5, 10, 15, 20, 30 and 45 min, filtered and measured spectrophotometrically at λ_{max} 276.8 nm. The experiments were done in triplicates for each of the selected formula. The drug release kinetics of the prepared formulae was performed for zero-order, first-order and Higuchi by using Microsoft Excel Add-InsDD Solver.

Lyophilization of selected nanosuspensions

In order to remove the water from the nanoparticle, freeze drying (also known as lyophilization) is most commonly used [17, 18]. The selected nanosuspension was frozen and lyophilized using a freeze dryer (FDU-7003, Kyeonggi-do, Korea). First, the nanosuspension was poured into ampoules and prefrozen in a deep freezer at–40 °C for 24 h (model FDU-7003, korea), then the ampoules were transferred to glass flasks, and the flask was attached to the vacuum adapter of lyophilizer (hetodry winner).

Optimization of risperidone nanoparticles

Process yield determination

The process yield was determined after lyophilization, using the following equation:

% process yield = [Recovered mass/Mass entered into the experiment] X100 (Equation 3)

Risperidone loading efficiency in the dry powders was determined by dispersing 1 mg of the lyophilized powder in 10 ml ethanol. The obtained suspension was sonicated in a bath-type sonicator for 30 min and centrifuged at 15,000 rpm for 30 min to remove insoluble ingredients. Then, the amount of risperidone in the supernatant was determined spectrophotometrically at λmax 276.8 nm. The produced mass was devided on the initially added mass of powder and calculated as a percent.

Scanning electron microscopy (SEM)

The surface morphology of the selected formula (F4) and the pure drug was examined by scanning electron microscopy (SEM) (Hitachi S800, Japan) at an appropriate magnification after palladium and gold coating of the sample. The pictures were taken at an excitation voltage of 20 kv.

Transmission electron microscopy (TEM)

The high-resolution transmission electron microscope (TEM, JEM-1400, and Japan) was used to evaluate the surface of the optimized formulation (F4) nanosuspension. A drop from the suspension was loaded onto a carbon-coated copper grid. The excess suspension was removed immediately using filter paper. Negative staining using (2%w/v) aqueous solution of phosphotungstic acid was directly added to the grid and left for 45 seconds. Then, samples were dried for 1 hour. After drying, the grid was directly investigated and photographed using (Jeole camera, JEM-1400, Japan)

Fourier transforms infrared spectroscopy (FTIR)

The Fourier transforms infrared (FTIR) spectra of pure risperidone and nanoparticles (F4) were recorded using FTIR spectro-photometer. Powders were mixed with potassium bromide (spectroscopic grade) and compressed into disks using hydraulic press before scanning from 5000 to 500 cm^{-1}.

Differential scanning calorimetry (DSC)

The pure risperidone and risperidone nanoparticles (4-6 mg) were sealed in the flat-bottomed aluminum pan of the differential scanning calorimeter (Shimadzu DSC-50, Japan). Data collection was carried out at a temperature range of 0–300 °C, and the heating rate was 5 °C/min under nitrogen gas at a flow rate of 25 ml/min. The transition and melting point measurements were assessed using the device software.

X-ray powder diffractometry studies

The X-ray diffraction pattern of the prepared formula was compared to that of the plain drug. Pure risperidone and risperidone nanoparticles (F4) were exposed to Cu kα radiation at a voltage of 45 kV and a current of 40 mA at a rate of 8 deg/min. A fixed slit system was employed with following slit parameters: divergence 1^{o}, scatter 1^{o} and receiving 0.3 mm. A sample equivalent to 60 mg was placed in sample holder groove and tightly packed [19].

***In vivo* drug absorption study**

Selection of animals

Twelve albino male rabbits, weighing between 2.5 and 3 kg, were used in the *in vivo* study and were divided into two groups, each containing 6 rabbits. Based on the previous results (F4) was selected for the *in vivo* study. Animals were acclimatized to laboratory

conditions for at least 7 d prior to the study. They were housed individually in stainless steel cages, fed a commercial laboratory rabbit diet, and allowed water and libitum. The rabbits were fasted for overnight (>12 h) prior to dose administration. Animals were held in rabbit restrainers during blood sampling, and they were conscious throughout the duration of the experiments. The tested formulae were orally administered to rabbits as follow: The tablets were crushed and suspended in water then giving a certain volume of the suspension corresponding to the required dose orally to each rabbit using a bulb syringe. Group, I took the Risperdal® tablets 2 mg; Janssen-Cilag (reference) and group II took the nanoparticles (F4) at a dose of 0.2 mg/Kg of the body weight [20]. Each preparation was dispersed into 1 ml of distilled water overtaxing for 20 seconds immediately prior to dosing.

Blood samples

Blood samples (0.5 ml) were collected after the administration of the drug from the marginal ear vein of the rabbits at time intervals of 0.5, 1, 1.5,2, 3, 4, 6, 8, 10, 12 and 24 h after drug administration. The blood samples were then centrifuged at 3000 rpm for 10 min, and the clear plasma was then collected in polyethylene capped tubes and deep frozen at-20 °C till required for extraction and analysis.

Chromatographic conditions

Analysis of samples was performed using a Shimadzu HPLC system equipped with the spectrofluorometric detector. Samples were directly injected into HPLC C18 column. (3.9 mm X 300 mm, particle size 5 Â µm), the mobile phase was a mixture of methanol and 0.02 N acetic acid (40:60 V/V). The pH of the mobile phase was adjusted to 6 with 30% acetic acid. After filtration through a membrane filter, the mobile phase was degassed and pumped at flow rate 1 ml/min and the UV detector was adjusted at 277 nm. A calibration curve was plotted for risperidone in the range of 30–300 ng/ml. Duloxetine was used as internal standard, and the element peaks were investigated using peak height ratio; all assays were performed at ambient condition.

Pharmacokinetic data analysis

The C_{max} and T_{max} were calculated using the plasma concentration-time curve in the WinNonlinTM Nonlinear Estimation Program. One way analysis of variance was employed to assess the significance of the difference between the T_{max}, C_{max}, $AUC_{(0-24)}$, K_{el} and MRT data from the tested nanoparticles formulation (F4) and the reference at a level ($p<0.05$) using the SPSS program [21].

K_{el}: the elimination rate constant was calculated from the negative slope of the log-linear terminal portion of the plasma concentration-time curve. The level of absorption from the nanoparticles formulation (F4) relative to the reference was calculated as the relative bioavailability by using the formula[22]; Relative bioavailability (%) = ($AUC_{(0–24)}$ test/$AUC_{(0-24)}$ reference) x 100 (equation 4)

RESULTS AND DISCUSSION

Particle size and poly dispersity index (PDI)

The choice of suitable stabilizers and the concentration of it are the key factors to control the size and stability of the nanosuspension during nanoprecipitation process [23, 24] in this study; two stabilizers were examined at different concentration (table 1). The results showed reduced with the increasing of pluronic F127 concentration as the particle size of formula F1 which contains 0.1% pluronic F127 was 611.45±46.45 nm compared with 215.56±12.65 nm for F4 which contains 0.8% pluronic F127 (table 2). This could be attributed to the increase in the molar substitution ratio (MSR) of the polymer per drug. The increase of the hydrophilic corona surrounding the polymer to protect the nanoparticles enhances the stability and prevents particles from coalescence and preventing aggregation [25, 26]. On the other hand, the particle size increased with the high concentration of PVP K30 which might be due to the higher viscosity of the resulting solution that might hinder particle attrition at the same milling energy. moreover Ostwald ripening might cause agglomeration, and consequently, higher particle size values resulted [27] and this has been explained theoretically by Kablanov *et al.* [28]. On the other side, the poly dispersity index (PI) values were ranged from 0.09-0.512 which indicates acceptable uniformity level for most of the preparations [29].

Zeta potential analysis

Zeta potential analysis was performed to investigate the surface properties and stability of nanosuspension. Zeta potential of the prepared was observed between-9.76 to-19.84 (table 2). Zeta potential of risperidone nanosuspensions was relatively low due to the shielding effect of the hydrophilic chains of the polymers used. These chains formed what is called hydrophilic corona that is surrounding the particles and prevent the true measure for the zeta potential [30]. On the other hand, the importance of the colloidal stability of the nanosuspension is reduced because these formulae will be kept in dry state which is reducing the importance of zeta potential as a controlling factor. (F4) was found to be-19.84±2.55mV (table 2) that concluded that the system had sufficient stability even with escalated amounts of pluronic used [31].

The percent of the total drug content

The drug content for the prepared formulae was shown in (table 2). The drug content for all formulae was calculated as a percent of the initially added drug. The amount of the drug within the formulations was more than 85 % in all samples.

Table 2: Physicochemical characterization of risperidone nanosuspensions

Formulation	Particle size (nm) ±*SD	PDI±*SD	ZP (mV)±*SD	Total drug content (%)
F1	604.17±46.45	0.512±0.09	-10.39±1.87	86.43
F2	523.86±28.41	0.154±0.18	-11.97±1.71	92.58
F3	313.59±19.58	0.271±0.17	-15.35±2.55	91.67
F4	215.56±12.65	0.198±0.15	-19.84±2.55	94.67
F5	638.23±25.64	0.336±0.16	-17.38±2.87	90.89
F6	441.43±29.26	0.09±0.11	-15.64±4.11	92.95
F7	491.64±33.45	0.158±0.17	-10.12±2.35	87.84
F8	611.45±28.08	0.501±0.12	-9.76±3.76	91.638

PDI-polydispersity index, ZP-Zeta potential, each sample was analysed in triplicate (n = 3) *mean±SD

***In vitro* dissolution**

The most important feature of nanoparticles is the increase of the dissolution rate not only because of increase in surface area but also because the use of hydrophilic surfactant. The *In vitro* dissolution of risperidone was carried out for all of the prepared nanosuspensions formulations and then compared to that of the pure drug powder (fig. 1). The cumulative percentage of the drug dissolved was 98.46 % at 45 min for selected nanosuspension (F4) while the cumulative percentage of the pure drug was 35.52 at 45 min. The difference was significance at $p<0.05$ when t-test for unpaired data was applied, and the release kinetics was found to obey first-order kinetics with $R^2>0.98$ (table 3).

Process yield determination

The yield of the mass recovered for processed nanoparticle was determined after lyophilization process and was considerably high (94±2.75%) which indicated efficient processing with minimum batch variability, thus representing a negligible loss of drug during preparation.

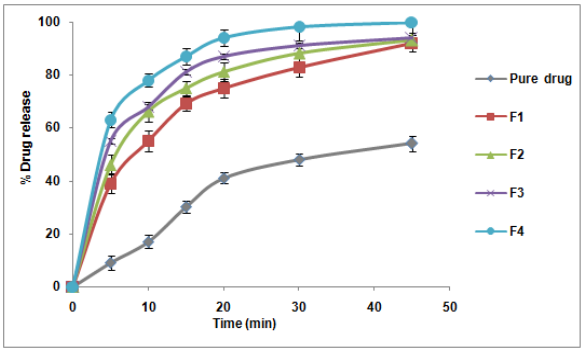

Fig. 1 a: ***In vitro*** **release profile of risperidone F1, F2, F3 and F4 in phosphate buffer pH 7.4 in comparison to pure risperidone**

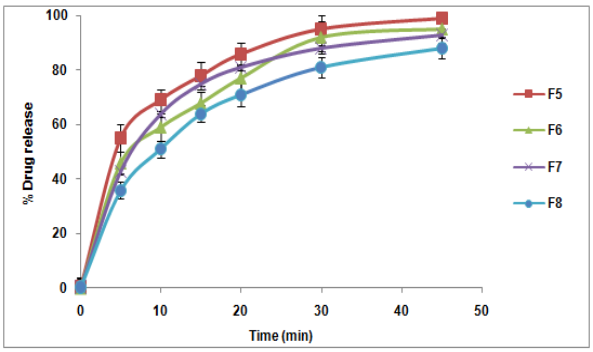

Fig. 1b: ***In vitro*** **release profile of risperidone F5, F6, F7 and F8 in phosphate buffer pH 7.4**

Table 3: Correlation coefficient R^2 values of various kinetic models used for analysis of the release data of risperidone nanosuspension

Formulation	Zero order	First order	Higuchi diffusion
F1	0.901	0.981	0.971
F2	0.885	0.994	0.943
F3	0.943	0.987	0.953
F4	0.931	0.985	0.932
F5	0.899	0.997	0.963
F6	0.922	0.985	0.981
F7	0.951	0.982	0.911
F8	0.969	0.992	0.926

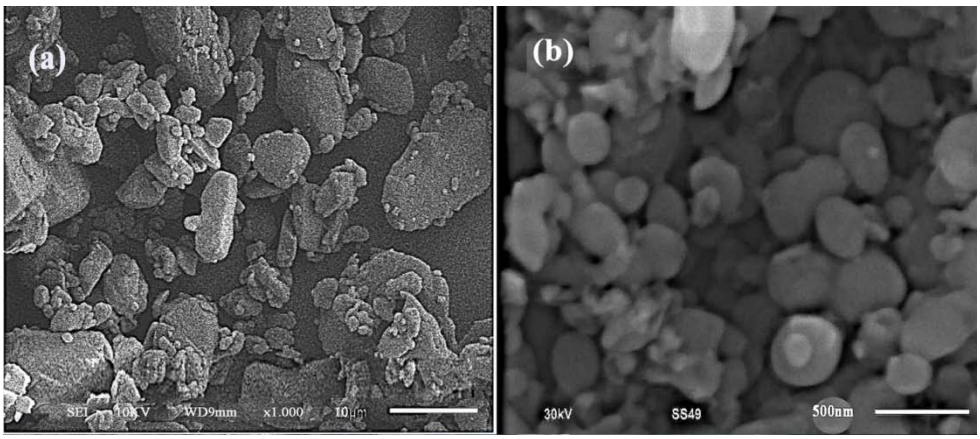

Fig. 2: Scanning electron microscopy (SEM), of (a) pure risperidone and (b) risperidone nanoparticles (F4)

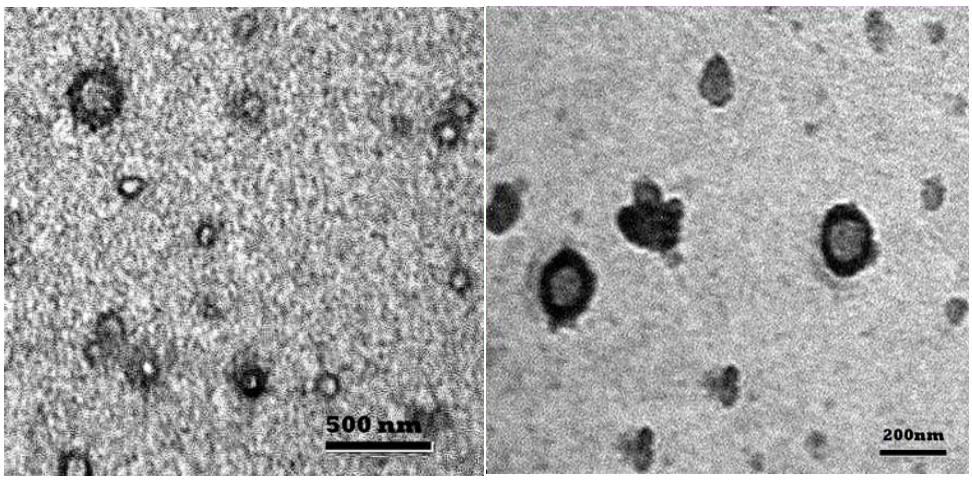

Fig. 3: Transmission electron microscopy of the optimized formula (F4) nanosuspension with different magnification

Morphology evaluation

In order to study the further transformation of selected nanosuspension (F4) into solid intermediates and their redispersion in water, the suspensions were lyophilized after preparation. The morphological characteristics were investigated using scanning electron micrographs (SEM). The SEM image of the drug and nanosuspension showed a significant difference in the morphology of these particles. Nanosuspension sample was appeared to be spherical with the mean particle size of 215 nm. They are having narrow distribution index (fig. 2b), while SEM of the drug showed coarse, irregular, more elongated, and within a micro range (fig. 2 a).

A close up look for the surface of F4 was carried out using the TEM (fig. 3) to determine the thickness of Pluronic 127 layer that adsorbed onto the surface of the nanoparticles. The results showed the formation of uniform non-aggregated particles that adsorb the hydrophilic corona around them. Two distinct layers are shown, where the hydrophobic part of the polymer is directed inward the particles and the hydrophilic part of the polymers are directed outward.

Fourier transform infrared spectroscopy (FTIR)

Risperidone spectrum showed a prominent band at 3058.1 cm^{-1} corresponding to the aromatic C–H stretching, 2757.1 cm^{-1} band due to aliphatic C–H stretching and a prominent band at 1651.6 cm-1 corresponds to C=O stretching of the aryl acids (fig. 4). The IR spectra of nanoparticles (F4) showed the characteristic peaks of risperidone, and this suggested that there was no change in the risperidone chemical structure during processing.

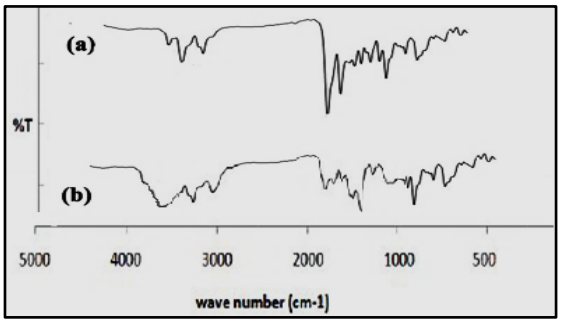

Fig. 4: Fourier transforms infrared spectroscopy (FTIR):(a) pure risperidone, (b) nanoparticles (Lyophilized nanosuspension)

Differential scanning calorimetry (DSC)

The DSC thermogram of pure risperidone (fig. 5) shows a characteristic sharp endothermic melting peak at about 170.12 °C with peak onset at 162.91 °C and peak end at 167.17 °C and the heat of transition was (15.06 J/g). The thermogram of risperidone nanoparticles (F4) shows endothermic melting peak at 155.74 °C which is close to the expected value for the drug addition, melting enthalpies of endotherm were at a lower-energy state of 4.934 J/g as compared to a crystalline form of the drug. The shift in the risperidone peak to a lower temperature and the decrease in the area of the peak in the nanoparticles compared to pure risperidone might be due to smaller risperidone crystals [32]. Additionally, this decrease in enthalpy value indicates low lattice energy, and it was very well reported that the particles with lower lattice energy are easier to dissolve [33].

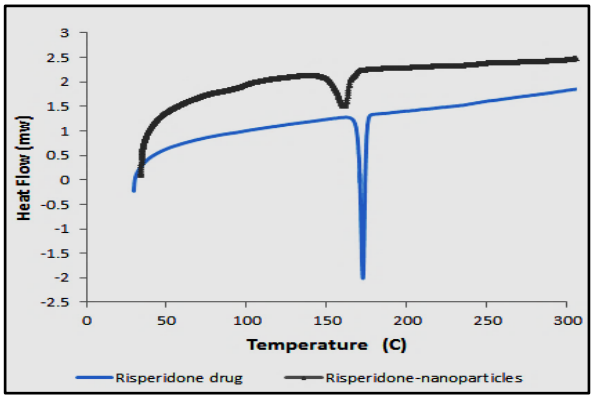

Fig. 5: Differential scanning calorimetry of pure risperidone and nanoparticles (lyophilized nanosuspension)

X-ray diffraction (XRD)

Crystallinity evaluation was carried out using XRD, which was carried out to examine the risperidone powder and the nanoparticles. The diffraction pattern of risperidone powder contained sharp peaks which is indicative of its crystallinity with two prominent peaks of high intensity at 2θ =14.21 ° and 2θ =21.25 (fig. 6 a). The obtained pattern (fig. 6 b) reveal that the drug crystallinity of nanosuspension formulation was not affected significantly [34], however reduction of the crystallinity was observed in the case of the nanosuspension due to the reduction of the peak at 14.21 °, suggesting that incorporation of the hydrophilic polymers may affect the crystalline state of the drug and gave an explanation for the increase in the dissolution rate of it. The data show that both freeze drying and sonication process had no significant effect on the risperidone nanoparticles.

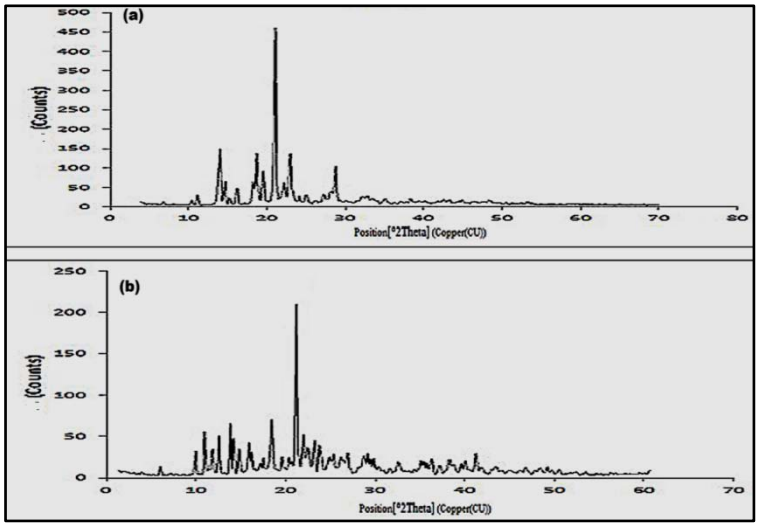

Fig. 6: X-ray diffraction (a) pure risperidone, (b) nanoparticles (lyophilized nanosuspension)

***In vivo* pharmacokinetics study**

A pharmacokinetic study in rabbits proved that the bioavailability was enhanced when nanoparticles formulation phenomenon of risperidone was compared to the market formula (Risperdal®). The mean plasma concentration–time data of risperidone following the administration of the marketed Risperdal® (2 mg tablets) and formula (F4) is shown in (fig. 7).

There was a statistically significant difference in the T_{max}, C_{max}, $AUC_{(0-24)}$ and MRT data between the market formula and the nanoparticles (F4). The C_{max} value of risperidone nanoparticles was significant ($p<0.05$) higher than market formulation (table 4). The $AUC_{(0-24)}$ value of risperidone nanoparticles after oral administration was almost 2 folds higher than those obtained of the marked formulation.

Table 4: Pharmacokinetic parameters after oral administration of risperidone formulations to rabbits (n =6)

Pharmacokinetic parameters	Formulations	
	Marketed formulation (Risperdal®)	Nanoparticles (F4)
C_{max} (ng/ml)	52.35±7.89	125.65±11.17 20.17739 20.17739
T_{max} (h)	2.83±0.718	1.833±0.389
$AUC_{(0-24)}$ (ng. h/ml)	243.5±59.72	472.87±86.98
% Relative Bioavailability	-	194.61 %
K_{el} (h^{-1})	0.143±0.09	0.113±0.08
MRT (h)	8.93±3.63	12.55±2.88

*Data expressed as mean±standard deviation, K_{el}–Elimination rate constant, MRT-mean resident time

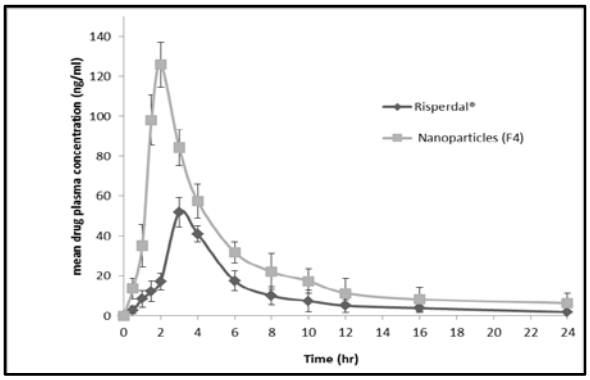

Fig. 7: Plasma concentration of risperidone following the administration of Risperidal®tablets and risperidone nanoparticles (F4), (n=6±SD)

CONCLUSION

Nanoprecipitation method was successfully employed to produce stable risperidone nanosuspension by using the proper stabilizer (pluronic F 127). It has been concluded that the nanosuspensions were easily formulated and recovered to form nanoparticles. The dissolution was significantly enhanced in the prepared nanosuspension. Consequently, risperidone nanoparticles represent promising new drug formulation for oral drug delivery with enhancement the oral absorption.

CONFLICT OF INTERESTS

The authors declared no conflict of interest

REFERENCES

1. Megens F, Awouters L, Schotte A. Survey on the pharmacodynamics of the dopamine-D2 antagonistic properties. J Pharmacol Exp Ther 1988;244:685–93.
2. Anthony D, Nick C, Patel M, Lynn C. Antipsychotic drugs. Clin Ther 2004;26:649-66.
3. Leysen JE, Stoof JC, Janssen PA. Biochemical profile of risperidone, a new antipsychotic. J Pharmacol Exp Ther 1988;247:661–70.
4. Bronstein AC, Spyker DA, Cantilena LR. Annual report of the American association of poison control centers' national poison data system (NPDS), 27th annual report. Clin Toxicol 2010;48:979.
5. Megens F, Awouters HL, Schotte A. Survey on the pharmacodynamics of the new antipsychotic risperidone. Psychopharmacology 1994;114:9–23.
6. Martindale, The complete drug reference. SC Sweetman. Editor. Pharmaceutical Press: London, Chicago; 2009. p. 1024-7.
7. White SR. Acute dystonic reaction and the neuroleptic malignant syndrome. J Pharm Practice 2005;18:175-83.
8. Janssen CJ, Niemegeers F, Awouters KH, Schellekens A, Meert TF. Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S2 and GG and Conzentino P. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int J Pharm 1995;125:309-13.
9. Moschwitzer J, Achleitner G, Pomper H, Muller RH. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspensions. Eur J Pharm Biopharm 2004;58:615-9.
10. Wongmekiat A, Tozuka Y, Oguchi T, Yamamoto K. Formation of fine drug particles by co-grinding with cyclodextrin. I. the use of β-cyclodextrin anhydrate and hydrate. Pharm Res 2002;19:1867-72.
11. Itoh K, Pongpeerapat A, Tozuka Y, Oguchi T, Yamamoto K. Nanoparticle formation of poorly water soluble drugs from ternary ground mixtures with PVP and SDS. Chem Pharm Bull 2003;51:171-4.
12. Vikram M, Jayvadan K, Dhaval J. Formulation, Optimization and characterization of simvastatin nanosuspension prepared by nanoprecipitation technique. Der Pharm Lett 2011;3:129-40.
13. Kipp JE, Wong J, Doty M, Werling J, Rebbeck C, Brynjelsen S. Method for preparing submicron particle suspensions. US Patent, 0031719 A1; 2003.
14. Matteucci ME, Brettmann BK, Rogers TL, Elder EJ, Williams RO, Johnston KP. The design of potent amorphous drug nanoparticles for rapid generation of highly supersaturated media. Mol Pharm 2007;4:782–93.
15. Keck CM, Muller RH. Drug nanocrystals of poorly soluble drugs produced by high-pressure homogenization. Eur J Pharm Biopharm 2006;62:3-16.
16. Chorny M, Fishbein I, Danenberg HD, Golomb G. Lipophilic drug loaded nanospheres prepared by nanoprecipitation: effect of formulation variables on size, drug recovery, and release kinetics. J Controlled Release 2002;83:389-400.
17. Kumar MP, Rao YM, Apte S. Formulation of nanosuspensions of albendazole for oral administration. Curr Nanosci 2008;4:53-8.
18. Zhang D, Tan T, Gao L, Zhao W, Wang P. Preparation of azithromycin nanosuspensions by high-pressure homogenization and its physicochemical characteristics studies. Drug Dev Ind Pharm 2007:33:569-75.
19. Rahman Z, Zidan AS, Khan MA. Risperidone solid dispersion for orally disintegrating tablet: its formulation design and non-destructive methods of evaluation. Int J Pharm 2010;400;49-58.
20. Harkness JE WJ. The biology and medicine of rabbits and rodents. 3rd Edition. Lea and Febiger, Philadelphia; 1989.

21. Rajan S. A simple sample preparation with HPLC-UV method for estimation of amlodipine from plasma: application to bioequivalence study. Open Chem Biomed Methods J 2008;1:22-7.
22. Peter G, Francis LS, Shrikant V. Dighe. Pharmaceutical Bioequivalence. Drugs and the Pharmaceutical Sciences. New York, N. M. D; 1991. p. 48.
23. Gao L, Zhang D, Chen M, Zheng T, Wang S. Preparation and characterization of an oridonin nanosuspension for solubility and dissolution velocity enhancement. Drug Dev Ind Pharm 2007;33:1332-9.
24. Kocbek P, Baumgartner S, Kristl J. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. Int J Pharm 2006;312:179-86.
25. Van Eerdenbrugh B, Vermant J, Martens JA, Froyen L, Van Humbeeck J, Augustijns P. A screening study of surface stabilization during the production of drug nanocrystals. J Pharm Sci 2009;98:2091-103.
26. Merisko-Liversidge E, Sarpotdar P, Bruno J, Hajj S, Wei L. Peltier N, *et al.* Formulation and antitumor activity evaluation of nanocrystalline suspensions of poorly soluble anticancer drugs. Pharm Res 1996;13:272-8.
27. Merisko E, Liversidge G, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci 2003;18:113-20.
28. Kabalnov AS, Pertzov AV, Shchukin ED. Ostwald ripening in emulsions: I. Direct observations of Ostwald ripening in emulsions. Colloids Surfactant 1987;118:590–7.
29. Patravale VB. Nanosuspensions: a promising drug delivery strategy. J Pharm Pharmacol 2004;56:827-40.
30. Saindane. Nanosuspension based in situ gelling nasal spray of carvedilol: development, *in vitro* and *in vivo* characterization. AAPS PharmSciTech 2013;14:189-99.
31. Vikram M. Formulation, Optimization, and characterization of simvastatin nanosuspension prepared by nanoprecipitation technique. Pharm Lett 2011;3:129-40.
32. Eerdenbrugh VB. Microcrystalline cellulose, a useful alternative for sucrose as a matrix former during freeze-drying of drug nanosuspensions a case study with itraconazole. Eur J Pharm Biopharm 2008;70:590-6.
33. Wei L, Yonggang Y, Yongshou T, Xinlan X, Yang C, Liwei M, *et al.* Preparation and *in vitro*/*in vivo* evaluation of revaprazan hydrochloride nanosuspension. Int J Pharm 2011;408:157–62.
34. Shivanand Pandey. Itraconazole nanosuspension meant for oral use: development of formulation, characterization and *in vitro* comparison with some marketed formulations. Pharm Lett 2010;2:162-71.