DOI: https://doi.org/10.31351/vol31iss1pp43-56

Improvement of the Solubility and Dissolution Characteristics of Risperidone via Nanosuspension Formulations.

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Abstract

Risperidone is an atypical antipsychotic drug that is used for treating schizophrenia, bipolar mania, and autism. Risperidone rebalances dopamine and serotonin to improve thinking, mood, and behavior by working on dopamine and serotonin $\alpha_2$receptor antagonism. Risperidone has poor solubility and high permeability through the intestine, so it belongs to Biopharmaceutical Classification System (BCS) class II exhibits poor oral biopharmaceutical properties. The aim of the present work was to improve solubility and dissolution of risperidone by preparing nanosuspension using different stabilizers and different solvents in a method known as solvent-antisolvent precipitation method. Twenty-eight formulas were prepared and evaluated particle size, PDI, (EE), zeta potential, and *in-vitro* dissolution studies. The results showed that particle size of nanosuspension was nanosized for all formulations. The best formula (F13) has particle size (40.9) nm containing a (Soluplus) as a stabilizer in ratio 1:1 with drug by using acetone as a solvent in ratio 1:5 with water which was act as anti-solvent with stirring speed 1000 rpm and E.E % was 98%. For self-dispersible dry nanosuspension, the selected formula (F13) shown fast dispersibility of less than 1 min. and complete *in-vitro* dissolution to about 30 min. in ٠,١N HCl. XRD and DSC indicate the transformation of a crystalline form of risperidone into an amorphous form. The stability studies of the best formula (F13) suggest that estimated shelf life was about 4 years.
In conclusion; the formulation of poorly water-soluble risperidone as nanosuspension significantly improved the dissolution rate of drug and enhanced its solubility.
Keywords: Risperidone, Nanosuspension, Solubility, Particle size.

تحسين خصائص الذوبان وتحلل خارج الجسم للريسبيريدون عن طريق تركيبات معلقة نانوية

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الخلاصة

دواء الريسبيريدون هو دواء غير نمطي لمرض الذهان يستخدم لعلاج انفصام الشخصية والاضطراب ثنائي القطب والتوحد. الريسبيريدون يعيد توازن الدوبامين والسيروتونين ليدعم وظائف التفكير والمزاج والتصرف من خلال عمله على مستقبلات الدوبامين والسيروتونين ومستقبلات المعاكسة الفا. الريسبيريدون قليل الذوبان في الماء وله نفاذية عالية من خلال الأمعاء ولذلك يصنف بالتصنيف الثاني لنظام تصنيف الصيدلانيات البيولوجية كدواء له خصائص ضئيلة بالاستخدام الفموي. الهدف من البحث هو دعم ذوبانية وتحلل الريسبيريدون من خلال تحضير كصيغة معلق نانوي بطريقة ترسيب بالمذيب ومضاد المذيب لتحضير المعلق النانوي. بأستخدام مثبتات Soluplus, poloxamer 188, (poloxamer 407, pvp k30) ومذيبات مختلفة(ميثانول, اسيتون أو الايثانول) بطريقة ترسيب المذيب. ثمانية وعشرون تركيبة قيم لها الحجم الحبيبي ونسبة انحباس الدواء وجهد الزيتاودراسة تحلل المادة خارج الجسم.. النتائج أظهرت أن الحجم الحبيبي لمعلق الريسبيريدون كان ضمن الحجم النانوي. وأفضل تركيبة كانت (F13)ولها حجم حبيبي nm (40.9) والتي تحوي Soluplus كمثبت بنسبه 1:1 مع الدواء الخام بإستخدام الاسيتون كمذيب بنسبة 1:5 مع الماء مضاد للمذيب بسرعة دوران ١٠٠٠ دورة بالدقيقة وبنسبة إنحباس دوائي تقدر ب ٩٨٪.للمعلق النانوي الجاف ذاتي الذوبان تظهر سرعة ذوبانية أقل من دقيقة وتحلل كامل خارج الجسم بحوالي ٣٠ دقيقة في وسط الحامضي 0.1N HCl. XRDو DSC أشارا تحول الشكل البلوري للريسبيريدون الى غير متبلور. دراسة استقراريه الدواء تشير إلى أن العمر الافتراضي للتركيبة (F13) ما يقارب الأربع سنوات. نستنتج ان تحضير الريسبيريدون قليل الذوبان في الماء كمعلق نانوي أدى الى تحسن كبير في حل الدواء وعزز قابليته في الذوبان.
الكلمات المفتاحية: ريسبيريدون، معلق نانوي، حجم الجسيم، ذوبانية.

Introduction

Biopharmaceutical Classification is such a system that provides a profile for each drug substance based on its permeability and solubility at several media. Accordingly, drugs are classified into four groups and become a benchmark in the regulation of bioequivalence of oral drug products. These classes are the following [(1)]: class I (highly soluble, highly permeable), class II (poorly soluble, highly permeable), class III (highly soluble, poorly permeable), and class IV (poorly soluble, poorly permeable). Decrease particle size leads to increase solubility and enhance dissolution. Many techniques were used to decrease particle size. Nanotechnology-based approaches used to enhancement of drug solubility rely on reducing drug size to nanoparticles [(2)]. Nanosuspension is one of nanotechnology approaches, which is a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the

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Received: 19/2/2021
Accepted: 25/7 /2021

suspended particle is less than 1µm in size [3]. An essential role of stabilizers is to compensate for the additional non-bound energy of newly revealed surfaces. Rigorously wetting drug particulates, hindering Ostwald ripening, and agglomeration of nanosuspension particulates are the most important advantages of adding pharmaceutical stabilizers [4]. The widely used techniques of stabilization are steric and/or electrostatic stabilization, both ionic surfactants and charged polymers act as electrostatic stabilizers and non-ionic surfactants act as steric stabilizers [5]. Maintenance of stability of nanocrystalline structure is of great significance results from polymer steric stabilization Most widely applied pharmaceutical excipients as polymeric stabilizers are (Soluplus, poloxamer 188, poloxamer 407 and PVP k30) K30). Risperidone is a benzisoxazole atypical antipsychotic, reported being an antagonist at dopamine D_2, serotonin (5-HT2), adrenergic (α_1 and α_2), and histamine (H_1) receptors. It is given orally for the treatment of schizophrenia and other psychoses and in short-term treatment of acute manic or mixed episodes associated with bipolar disorder. Risperidone is effective for treating positive and negative symptoms of schizophrenia owing to its affinity for its loose binding affinity for dopamine D_2 receptors and additional 5-HT antagonism compared to first-generation antipsychotics, which are strong, non-specific dopamine D_2 receptor antagonists[6].

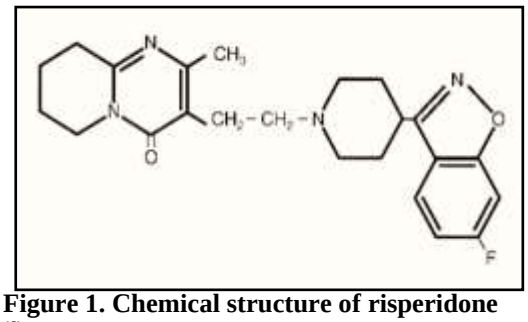

Figure 1. Chemical structure of risperidone [6]

This study aimed to formulate risperidone nanosuspension by the use of solvent-antisolvent technique. An attempt to enhance risperidone solubility and improve dissolution rate.

Materials and Method

Materials

Risperidone, soluplus purchased from BASF/ Switzerland and all other solvents, Di-sodium hydrogen phosphate, Potassium dihydrogen phosphate, and other stabilizers purchased from China.

Preparation of risperidone nanosuspension by liquid solvent- anti-solvent precipitation

2 mg of risperidone was dissolved in 1 mL methanol (acetone or ethanol) which is an organic solvent and sonicated in an ultrasonic bath for 15 min. On the other hand, 5mL of deionized water containing, stabilizers (Soluplus, poloxamer 188, poloxamer 407, and PVP k30) in ratio 1:1 that act as the antisolvent system. The following step is the addition of organic solvent slowly into aqueous solution at rate of (0.5 mL per min) of using a syringe pump, under mechanical agitation at different speeds (300, 1000, or 2000 rpm) with aid of a hotplate magnetic stirrer at room temperature [7].

Then, evaporation of the organic solvent was done after stirring the sample at low stirring speeds for a half-hour at 25ºC temperature to get coveted nanosuspension [8]. Change in solvent and anti-solvent volume and stirring speed was done to formulate different risperidone nanosuspension preparation formulas shown in Table (1).

Determination of saturation solubility

Solubility of saturation is determined by putting an excessive amount of pure risperidone powder determined in 10 mL in various media where 0.1N HCl with 2% w/v Brij 35, water with 2% w/v Brij 35, phosphate buffer pH 6.8 with 2% w/v Brij 35, and methanol were used. Excess amount of drug was added to all of prepared media and kept in an (incubator shaker) at 25 °C, and after three days, prepared solution has been put in a centrifuge at 4000 rpm for duration of 10 min. Then, filtration by filter syringe of 0.45µm and dilution with particular solution of supernatants was done afterward. Absorbance was calculated according to calibration curves and it is determined at a wavelength for each media using UV- spectrophotometer(Carry win UV, Varian, Australia) and solubility [9].

Measurement of particle size and polydispersity index

Size of particle and index of polydispersity were determined by using the Nano Brook 90Plus particle size analyzer which is a dynamic light scattering, act by measuring the intensity of light scattered by the molecules in the sample as a function of time, at a scattering angle of 90º, and a constant temperature of 25 ºC. Dilute suspensions prepared, a short ultrasonication is sometimes auxiliary to separate loosely-held agglomerates. 2 or 3 mL of nanosuspension is required to make the measurements. Disposable, acrylic square cells used for aqueous and alcohol suspensions [10].

Table 1 .Composition of risperidone nanosuspension using different parameters

	Stabilizer	Ratio	Solvent	Ratio	Stirring Speed(rpm)		Stabilizer	Ratio	Solvent	Ratio	Stirring Speed(rpm)
F1	Soluplus	1: 1	methanol	1: 5	1000	F15	PVP k-30	1: 1	Acetone	1: 5	1000
F2	PVP k-30	1: 1	methanol	1: 5	1000	F16	PVP k-30	1: 1	Ethanol	1: 5	1000
F3	Poloxamer1^8	1: 1	methanol	1: 5	1000	F17	Poloxamr188	1: 1	Acetone	1: 5	1000
F4	Poloxamer407	1: 1	methanol	1: 5	1000	F18	Poloxamr188	1: 1	Ethanol	1: 5	1000
F5	Soluplus	1: 1	methanol	1: 10	1000	F19	Poloxamr407	1: 1	Acetone	1: 5	1000
F6	Soluplus	1: 1	methanol	1: 15	1000	F20	Poloxamr407	1: 1	Ethanol	1: 5	1000
F7	PVP k-30	1: 1	methanol	1: 10	1000	F21	Soluplus	1: 1	methanl	1: 5	300
F8	PVP k-30	1: 1	methanol	1: 15	1000	F22	Soluplus	1: 1	methanl	1: 5	2000
F9	Poloxamer188	1: 1	methanol	1: 10	1000	F23	PVP k-30	1: 1	methanl	1: 5	300
F10	Poloxamer188	1: 1	methanol	1: 15	1000	F24	PVP k-30	1: 1	methanol	1: 5	2000
F11	Poloxamer407	1: 1	methanol	1: 10	1000	F25	Poloxamer188	1: 1	methanol	1: 5	300
F12	Poloxamer407	1: 1	methanol	1: 15	1000	F26	Poloxamer188	1: 1	methanol	1: 5	2000
F13	Soluplus	1: 1	Acetone	1: 5	1000	F27	Poloxamer407	1: 1	methanol	1: 5	300
F14	Soluplus	1: 1	Ethanol	1: 5	1000	F28	Poloxamer407	1: 1	methanol	1: 5	2000

Determination of entrapment efficiency of a drug (EE) in Nanosuspension

Nanosuspensions of different ratios were centrifuged at about 5000rpm for 20 min. at 4°C using a cooling ultracentrifuge. The amount of free drug was detected by calculating the absorbance of an appropriately diluted sample of supernatant at 280 nm using a UV-spectrophotometer (Carry win UV, Varian, Australia). For each formula, the experiment was repeated three times and the average was calculated. (EE) could be calculated by the following equation:

E.E%= (the total drug in a formula – free drug) *100 / total drug in formula .... (1)

In vitro dissolution of risperidone nanosuspensions

Dissolution study of prepared nanosuspension was done under sink condition using paddle-type II according to USP pharmacopeia. Briefly, freshly an accurately weighed plain drug (2mg), and prepared nanosuspensions of (F1, F2, F5, F6, F13, and F21) formulas were placed in a pretreated dialysis bag (dialysis membrane); soaked in dissolution media overnight before use; and fitted with a paddle then dispersed in 900mL of 0.1N HCl with 2% w/v Brij35 solution as dissolution medium. The paddle has been built up to be rotated at 100 rpm maintained at 37 ± 0.5°C. An aliquot of (5mL) samples was withdrawn from the receiver compartment at predetermined time intervals (5, 10, 15, 20, 30, 45, 60,120) min and replenished with the equivalent volume of fresh dissolution medium to preserve the constant volume. And then, samples were analyzed for risperidone content spectrophotometrically after proper dilution using UV-spectrophotometer scanning at 200-400nm. The experiment repeated in triplicate for each formulation [11].

Zeta potential evaluation of nanosuspension

Zeta-potential evaluated by the use of zetasizer (Zetasizer Nano ZS, Malvern instrument, Worcestershire, UK) [12]. The characteristics of surface charge were studied to assess the stability of the prepared nanosuspension and lyophilized nanoparticle formulas. The minimum limit needed for electrostatic stabilization of nanosuspension is ± 30 mv and for steric stabilization of ± 20 mv [13]

Freeze drying of nanosuspension

Freeze drying was used to convert the optimum formula to dry powder, later for further evaluation. Mannitol used as a cryoprotectant at 3% w/v. About 400mL of optimized formula was prepared and freeze-dried to yield a dry powder for evaluation. Four flasks froze in a deep freezer at -20°C for 24 hr. The frozen flasks were attached to the vacuum port of the device, then four flasks each containing 100mL of nanosuspension, instrument operated till dry powder yielded. Sublimation of solvent from frozen samples took 48 -72hr [14].

Effect of stirring speed on the particle size of risperidone nanosuspension

Three different speeds were used to show their effect on the formulation of risperidone Nanosuspension. Speeds of 300 and 2000 rpm used in the formulas (F21-F28) were used to study this effect

Effect of solvent/antisolvent ratio change on the particle size of risperidone nanosuspension

Study the effect of change in solvent/anti-solvent ratio in nanosuspension where the change in volume of deionized water (anti-solvent) from 50 mL to 100 mL and 150 mL. Formulas (F5-F12) were used [15].

Effect of polymer type when using different solvents on the particle size of risperidone nanosuspension

By changing the solvent (methanol)in formulas (F1-F5) to acetone and ethanol. Formulas (F13-F20) were used [16].

Characterization of lyophilized powder

Saturation solubility of lyophilized powder

Three dissolving solvents water, 0.1 N HCl with pH 1.2, and phosphate buffer pH 6.8 were used to investigate the saturation solubility of lyophilized formula (F13). An excess amount of drug was added to each test tube containing the above-mentioned solvents then were shaken for at least 48hr in a water bath shaker at 25 °C. Then filter with a conventional filter paper before reading for each sample. Sample test tubes were centrifuged at 6000 rpm for 15 min. The absorbance of the supernatant was recorded and a calibration curve was used to determine the amount of drug dissolved in the specific volume of each dissolving media [17].

The in-vitro dissolution profile of lyophilized risperidone nanoparticle

In-vitro dissolution test of risperidone lyophilized powder was estimated using (paddle assembly) type II dissolution test apparatus. Accurately weighed lyophilized powder equivalent to 2 mg of risperidone was placed on a pretreated dialysis bag and fitted to the paddle which rotates at 100 rpm at 37 °C± 0.5 in 900ml of dissolution medium 0.1N HCl. An aliquot of 5-milliliter samples was withdrawn from the receiver compartment at predetermined time intervals and refilled the

equal volume of fresh dissolution medium to conserve the constant volume. The same procedure was applied on lyophilized powder with the same conditions at 100 rpm rotation of paddle containing phosphate buffer (pH 6.8). Then samples were filtered by 0.45µm filter syringe and assayed spectrophotometrically by UV–spectrophotometer. Results obtained were compared using similarity factors and the experiment was repeated in triplicate [18].

Differential scanning calorimetry (DSC)

DSC can be used to evaluate the crystalline state of drug especially when converted to a lyophilized powder. 8-10 mg sample of the pure risperidone and lyophilized powder of the selected formulations heated at a scanning rate of 10°C/min. This done under dry nitrogen flow (100 mL/min) between 30 and 300° against an empty aluminum pan as a reference using DSC- Shimadzu 60 with TDA trend line software [19].

X-ray powder diffraction (XRPD) analysis

Patterns for pure risperidone and nanoparticles were analyzed using an XRD-6000, Shimadzu-Japan. The continuous scan range of 5-80 degree. 40 (kV) was for the operating voltage, 30mA for the current, scan step size of 0.050° (2θ), and scan step time of 60 sec [20].

Scanning electron microscopy (SEM) study

The morphology of pure drug and lyophilized formula were examined by (VEGA3Tuscan) by direct deposition of powder on double-sided carbon tape and coated with gold at 1K, 2K, 5K, and 500x magnification then coated with gold [21].

Stability study

In specific capsules, the selected formula powder was filled and stored at three various 40°C, 50°C, and 60°C temperatures degrees for 90 days, at an interval of 14 days, samples were dissolved in 100 ml methanol. And then, the drug concentration was measured spectrophotometrically at λ max considering this solvent as blank. K at 25°C was predicted from the Arrhenius plot and the date of expiration was determined [22].

Statistical analysis

The results experiments were expressed as a mean triplicate sample ± standard deviation (SD) and the analysis was done with the help of one-way analysis of variance (ANOVA) using SPSS software at which the results would be significant when $p < 0.05$, and the results would be non-significant if $p > 0.05$ [23]. Difference factor (f_1), similarity factors (f_2) were also calculated to compare dissolution profiles, using DD Solver program. The difference factor (f_1) measures the percent error between two curves over all time points. The similarity factor (f_2) was introduced by Moore and Flanner [24].

Results and Discussion

Determination of risperidone saturation solubility

The solubility study was performed in four different media, 0.1N HCl, phosphate buffer pH 6.8 methanol, and distilled water. Risperidone is a weak base with pKa value of 8.76 and expected to be more soluble in an acidic medium of about 1.3 mg/mL. In contrast, risperidone solubility in phosphate buffer was very low with a solubility value of 0.61mg/mL. risperidone was soluble in organic solvent(methanol) in 6.1mg/mL. On the other hand, the water solubility of risperidone was the lowest at about 0.019 mg /mL which considered practically insoluble.

Characterization of the prepared risperidone nanosuspension

Determination of the particle size and polydispersity index

The estimated average particle size was in the range of (40.9 nm – 1286 nm) and were illustrated in Table (2). On the contrary, the PDI which measures the size distribution of the nanoparticles; were ranged from (0.1-0.62) depending on formulation variables, (0.1) indicating good uniformity, a monodisperse system in the particle size distribution of nanoparticles while (0.62) indicated polydisperse system.

Effect of stirring speed of the anti-solvent system

When the stirring was 300 rpm the process failed to nanosizing the stabilizer around the drug due to the stuffiest energy required to achieve the mixing. Besides that, high stirring speed (2000 rpm) not always favored due to high agitation with the formation of foams that separate drug particles from the vehicle medium which act as an obstacle in the preparation process, thus an optimum speed (1000 rpm)is required to have optimum drug particles within the acceptable range [25].

Effect of solvent/antisolvent ratio

The results illustrated in Figure (2). It can be seen that when the ratio increases between the solvent and water increase particle size due to decrease the quality of mixing of media because of the decrease in specific stirrer speed to the solution that need to nucleation causing growth formation and increase flocculation [26]. Increase in water volume decrease stabilizer in water leading to decrease stabilization and increase particle size. Formulas from F5to F12 were significant differences ($p<0.05$) in particle size and PDI of these formulas.

Table 2. Particle size, polydispersity index, and E.E. of formulas from 28 formula data are expressed as Mean ± SD, p <0.05. Standard deviation (SD) (mean±SD) n=3.

	Particle Size(nm)	PDI	E.E,	Zeta potential		Particle Size(nm)	PDI	E.E%	Zeta potential
F1	51.2	0.173	97.2±0.2	-14.59	F15	335	0.35	96.26±0.2	-20
F2	77.8	0.264	92± 0.2	-16.24	F16	160.3	0.34	95±0.1	-19.2
F3	233.5	0.353	90±0.21	-12.76	F17	260	0.3	92±0.14	-18.5
F4	245.5	0.465	87± 0.2	-11.39	F18	450	0.36	89±0.2	-16.7
F5	134.3	0.26	88±0.05	-10.2	F19	502	0.3	88±0.3	-18.9
F6	92.8	0.19	92±0.05	-11.55	F٢٠	492	0.327	84±0.15	-11.55
F7	162	0.518	85±0.05	-12	F21	140	0.4	90.1±0.2	-16
F8	216.9	0.37	90±0.05	-10.3	F22	210	0.38	87±0.1	-11.4
F9	602	0.48	88± 0.2	-9.1	F23	371	1.8	92±0.19	-10
F10	867	0.31	94±0.2	-8.9	F24	449	0.36	88±0.05	-9.2
F11	271	0.6	84±0.2	-15.1	F25	308.5	0.3	93±0.2	-6.9
F12	333	0.6	88±0.2	-16	F26	811	0.14	82±0.2	-15.7
F١٣	40.9	0.1	98±0.2	-18.8	F27	815	0.3	94±0.3	-16
F14	92.15	0.178	94.2±0.2	-17	F28	1286	0.4	84±0.3	-17.8

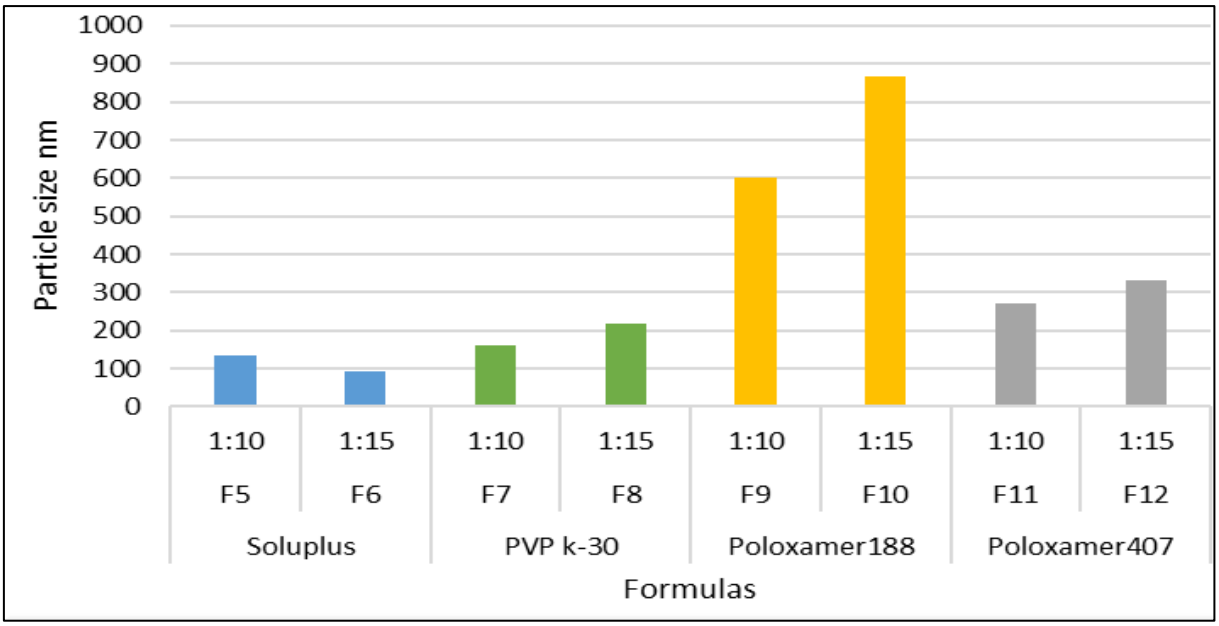

Figure 2. The effect of solvent/antisolvent on the particle size features on the prepared risperidone nanosuspension

Effect of polymer type on particle size when using different solvents

Acetone and ethanol were used in the preparation of nanosuspension in the formulas (F13-F20) and compared to the formulas prepared with methanol (F1, F2, F3, and F4). For acetone, as shown in Figure (3), the ratio of the drug to the stabilizer was fixed to a 1:1 ratio to study the effect of the type of the solvent. Formula F13 in which acetone was used as a solvent and soluplus as a stabilizer showed the smallest particle size among all formulations with other stabilizers within an optimum range of 40 nm and a PDI was 0.101 which indicate a good uniform particle size distribution. For formulas, F15 and F17 showed almost similar results of particle size 335 nm and 260 nm and a PDI of 0.34 and 0.32 which indicate almost homogenous particle size distribution. While F19 formula showed a bigger particle size of 502 nm and PDI of 0.308 which is similar to other formulas. This due to the strength of solvents polarity, where the acetone considered less polar and best miscible with water than methanol or ethanol, So risperidone highly solubility in acetone [27]. Compared with other solvents (methanol, ethanol) that gave a good miscibility degree to a solvent carry the drug and facilitate diffusion when mixed by dripping with antisolvent media that contain a stabilizer leading to the formation of highly stabilizing nanosuspension by reducing the surface energy of the fine particles and effective energy barrier to prevent agglomeration and crystal growth. Besides, there is a fact that the soluplus is soluble up to (50%, 45%, and 25%) than water in acetone methanol and

ethanol consecutively, where the process of precipitation method and before the solvent was evaporated the solvent could be contributed by made complete dissolved miscible component whether it was the drug or the stabilizer [28]

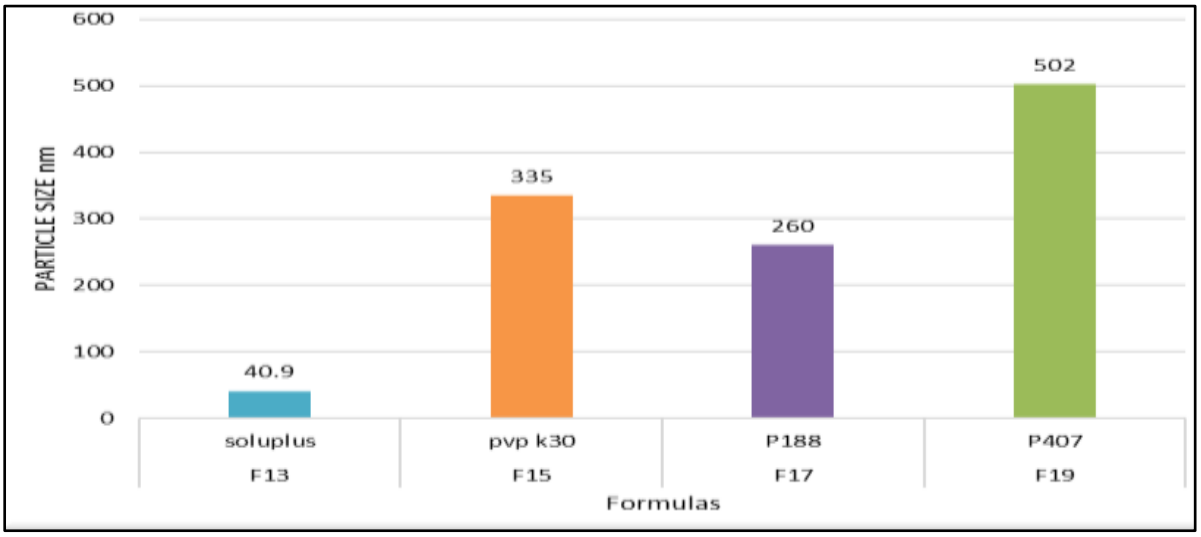

Figure 3. Effect of polymer type when using acetone on particle size for F13, F15, F17, and F19

For methanol as a solvent result in Figure (4) showed that formulas F1 and F2 showed particle sizes of 51 nm and 77.8 nm respectively but the F2 formula showed more uniform particle size distribution with PDI of 0.17. Formulas F3 and F4 presented a similar pattern of particle size and their distribution form of 233.5 nm and 245.5 nm respectively, and a PDI was 0.353 and 0.465 respectively. These results indicate similar superiority in using both stabilizers soluplus and PVP k30.

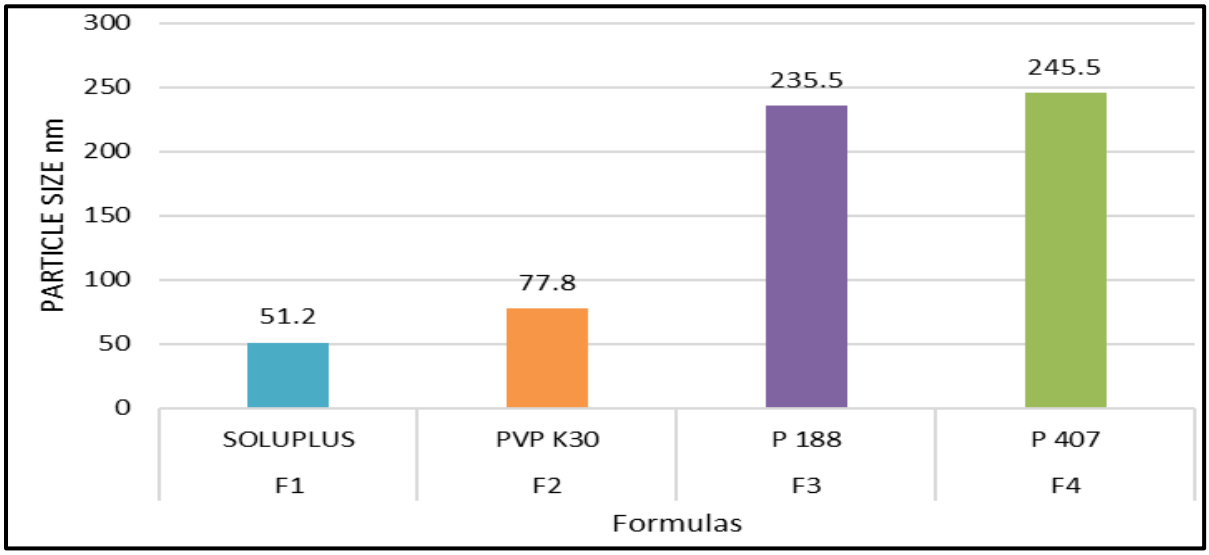

Figure 4. Effect of polymer type when using methanol on the particle size of F1, F2, F3, and F4

For ethanol as a solvent, results are shown in Figure (5). F14 formula where soluplus used showed the smallest significant particle size of 92 nm ($P<0.05$) and provided a uniform particle size distribution within the formulation with a PDI of 0.101. While F16 formula where PVP K30 was used presented particle size 160.3 nm with almost a uniform homogenous size distribution with PDI of 0.34. Formulas F18 and F20 showed about the same results when used poloxamer 188 and poloxamer 407 with a particle size of 450 nm with PDI of 0.36 and 490 nm with PDI of 0.32 respectively.

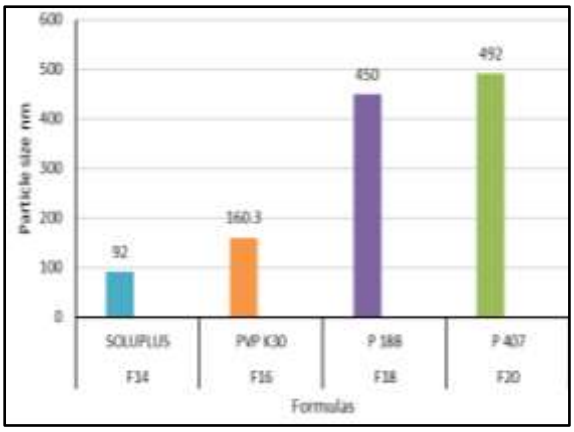

Figure 5. Effect of polymer type when using ethanol on particle size for the formulations F14, F16, F18, and F20

***In – vitro* drug release study**

The dissolution profile of the prepared risperidone nanosuspension formulas is illustrated in Figure (6). The results showed a significant increase in the dissolution rate of the risperidone nanosuspension formulas which reach 100% release within 45 min when compared to the pure drug that reached 56 % release within the same time frame. Also, the F13 formula presented the fastest complete release within 35 min, while pure drug showed the slowest incomplete release profile within the specified time.

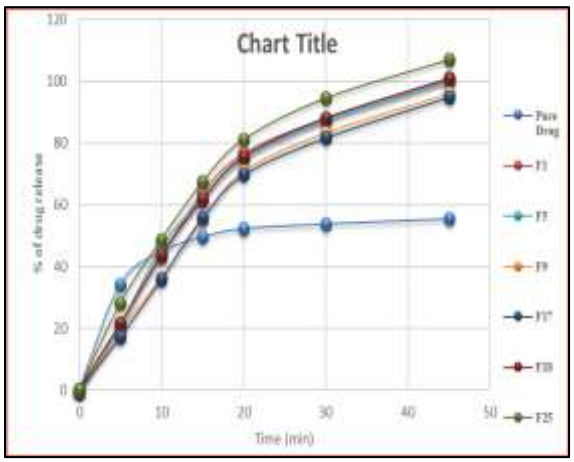

Figure 6. The release profile of risperidone nanosuspension in 0.1N HCl

To validate these findings, the fit factors have been used and expressed by two factors; f_1 the difference factor, and f_2 the similarity factor. For two dissolution profiles to be considered similar and bioequivalent, f_1 should be between 0 to 15 whereas, f_2 should be in the range of 50 to 100.

The similarity between F13 of the best release profile with the pure drug release profile showed the similarity value (f_2) was 23.7 which is less than 50 which indicates a significant improvement in the release behavior. Also, the difference factor value was 84.2 which confirmed the previous similarity test results and conclude the greater and faster release pattern of the best formula F13.

F13 formula was selected to investigate the best release profile in two different mediums 0.1N HCl and 6.8 phosphates and the comparison was shown in Figure (7). It can be seen that the F13 formula showed a faster release profile in 0.1N HCl than phosphate buffer pH 6.8. In terms of validation, the similarity and difference factors were calculated and provided that, the similarity factor obtained was equal to 26.7 and the difference factor was equal to 84.4. According to these data results, 0.1N HCl is considered the best medium for drug release of the selected F13 formula. This comparison in similarity and difference factor were significantly improved.

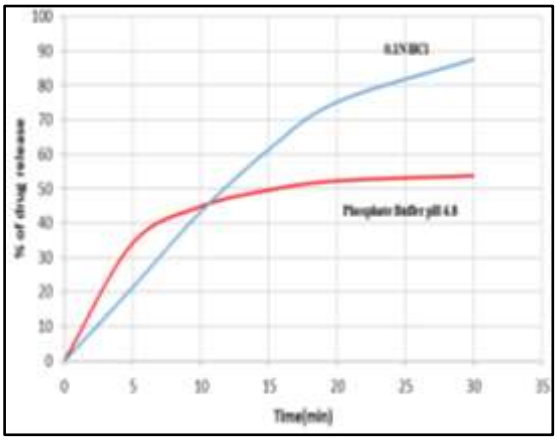

Figure 7. The release profile of F13 nanoparticle in phosphate buffer pH 6.8 and 0.1N HCl

Selection of the best formula

According to the previous results of all required tests of particle size determination, polydispersity index, entrapment efficiency and *in-vitro* drug release it can be concluded that the F13 nanosuspension formula was the best formula with the smallest particle size of 40.9 nm and PDI value of 0.101 and zeta potential -18 showed highest drug entrapment efficiency of about 98.7 which will be subjected for further characterization after lyophilization and converted to nanoparticle by determining zeta potential, powder x-ray properties, drug excipient compatibility, in vitro drug release after lyophilization by freeze-drying process

Solidification of risperidone nanosuspension by freeze-drying

The selected optimized formula F13 was freeze-dried to obtain the powder form of the prepared nanosuspension, enhanced amorphization, and subjected for further studies of solubility and *in-vitro* drug release.

Characterization of the lyophilized selected formula

Self-dispersibility of freeze-dried powder in aqueous medium

The selected F13 lyophilized powder was tested for self-dispersibility in aqueous media. The

powder was completely dispersed within seconds in the aqueous media. The dispersed formula was tested for the particle size and polydispersity index and the results showed that the particle size was 60.5 nm which is higher than the particle size of the same formula when it was nanosuspension and the PDI of the lyophilized powder was 0.115 which is also higher which was 0.101

The Zeta potential of the lyophilized F13 formula showed -18.96 mv which is similar to the value obtained before lyophilization which indicates the robust formulation by the action of the selected polymer with risperidone drug particles and last durable after freeze-drying.

These results indicate that the lyophilized F13 formula provided an accepted effective property of the nanosuspension formula which makes it potential for further study.

Solubility study of the selected lyophilized formula

Solubility study done at three different media; water, 0.1N HCl, and phosphate buffer pH 6.8(all with 2% w/v Brij 35). The results illustrated in Figure (8) and showed risperidone water solubility has significantly increased by about 105 times when compared with the one before lyophilization. Risperidone showed higher solubility in 0.1NHCl that results in higher and faster drug release when compared to the pure drug about 13.5 folds these were responsible for the significantly enhanced ($p<0.05$) saturation solubility.

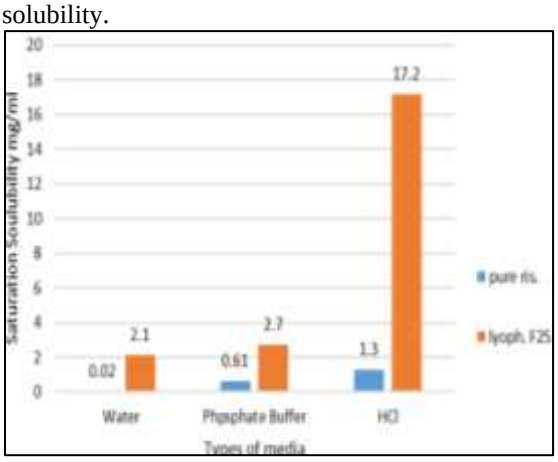

Figure 8. Solubility of lyophilized F13 in distilled water, phosphate buffer pH 6.8 and 0.1N 0.1N HCl

In – vitro drug release study of the lyophilized selected formula

The dissolution study of the selected lyophilized F13 formula was performed in 0.1N HCl with 2% w/v Brij35 and compared with the pure drug and the results presented in Figure (9). It can be seen that lyophilized F13 formula nanosuspension showed a very characteristic enhancement in drug release when compared to the pure drug with faster and complete drug release within 30 min when compared to the pure drug.

This advancement in drug release was confirmed by using the similarity factor and showed 27.4 (f_2<50) and the different factor was 83.6 (f_1>50) which is explained due to better dissolution characteristics which in turn theoretically will boost the dissolution and absorption rate of risperidone nanosuspension and eventually its bioavailability.

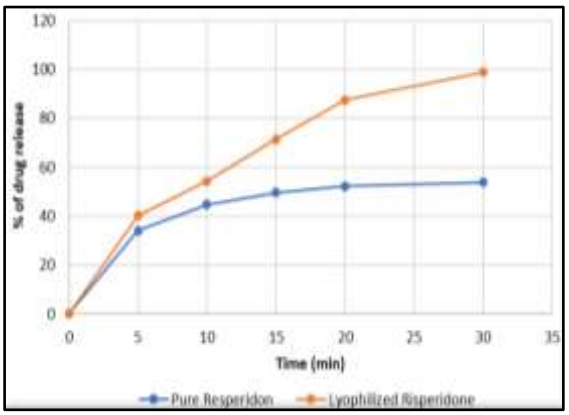

Figure 9. Drug release percentage of the lyophilized risperidone

Differential scanning calorimetry (DSC)

Lyophilized F13 was selected to investigate the crystalline purity after lyophilization and the result is presented in Figure (10). The Figure shows a sharp peak at 170°C, and it is similar as reported and indicates the purity of the drug [29]. On the other hand, an endothermic broad peak at 162°C is shown for the lyophilized risperidone. Broad endothermic peak is including lower in melting points, broadening, shifting, and reducing in intensities supported the suggestion of a reduction in the crystalline state of risperidone optimized formula with a significant increment in the amorphous state that formed in lyophilization [30].

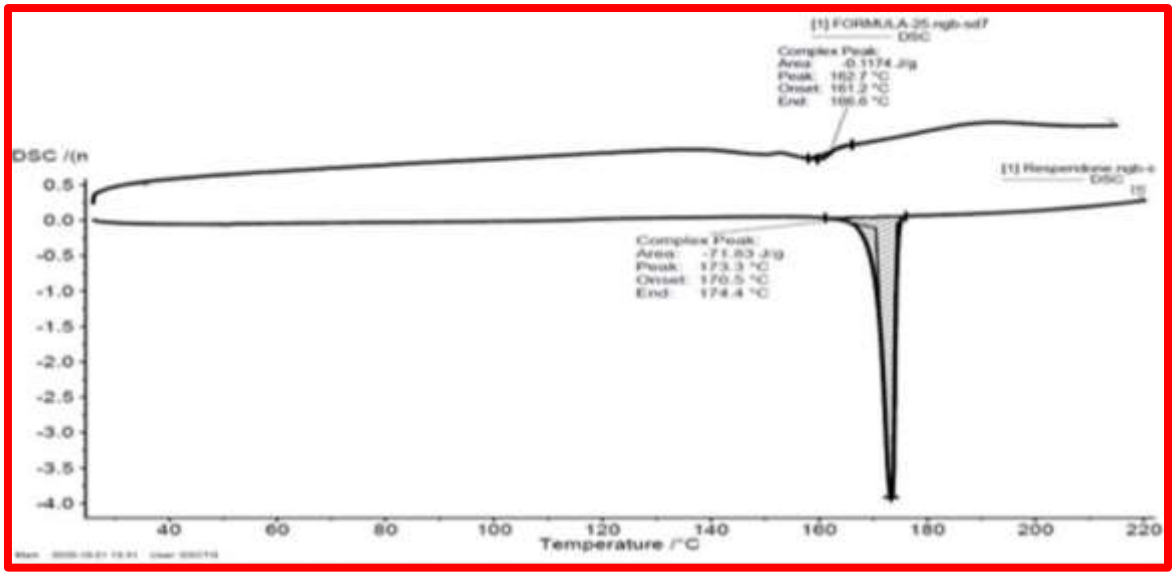

Figure 10. DSC of pure risperidone and the lyophilized F13

X-ray powder diffraction (XRPD)

The diffractograms of pure drug and lyophilized F13 were shown in Figures (11) and (12), respectively. It can be seen that there is a significant change in the X-ray pattern after the preparation of the nanosuspension formulation. The pure drug showed high intense peaks at 14°, 15°, 19°, 21°, 22°, 23°, 24°, 33°, and 34° that indicates a high degree of crystallinity of the pure risperidone.

Although, after the preparation of the lyophilized powder, leaser intensity peaks are observed which indicates a significant change in the crystallinity nature of the drug to be more amorphous. It has been noted that the emergence of new peaks at 31°, 39°, and 43° are related to the mannitol that has been added to the lyophilized powder [31]. These changes indicate higher stability of the prepared lyophilized powder with the enhancement of water wettability and dissolution profile [32].

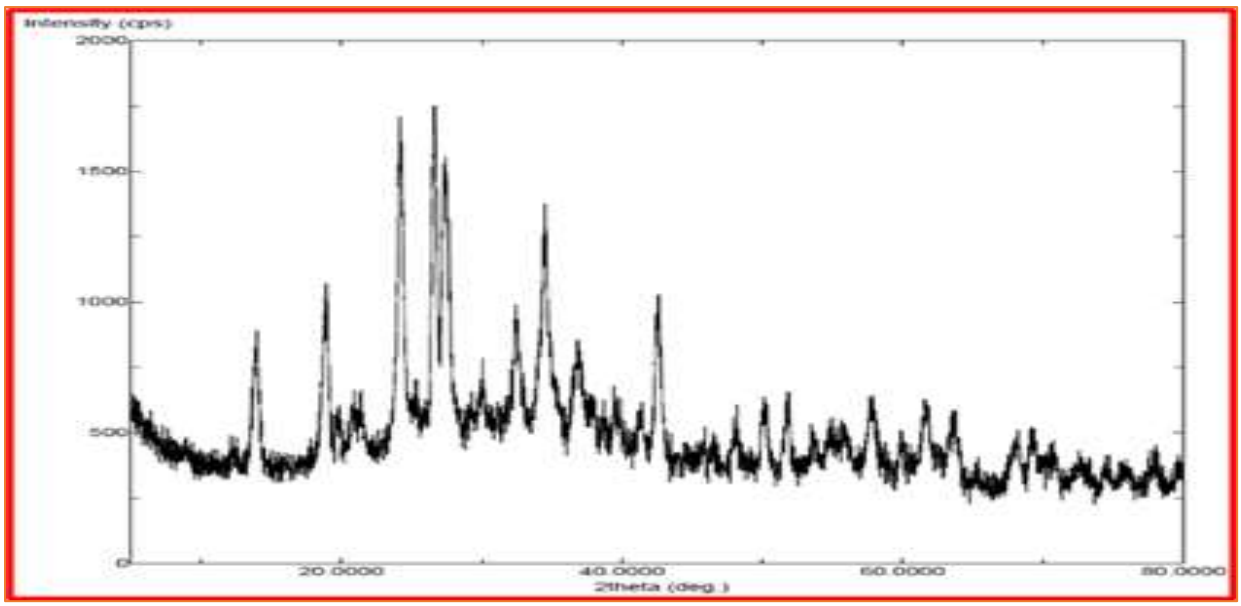

Figure 11. XRD diffraction of pure risperidone

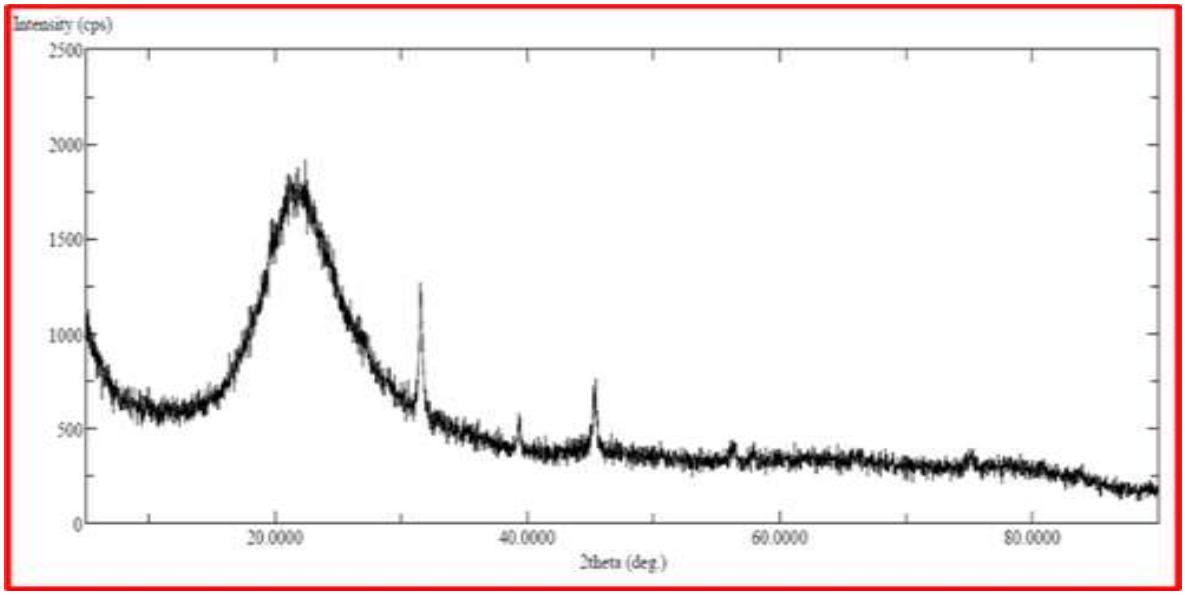

Figure 12. XRD diffraction of the lyophilized F13

Scanning electron microscopy (SEM)

The SEM pictures are shown in Figures (13) and (14). The SEM of the pure powder showed coarse agglomerates of risperidone crystals that packed together forming larger particles. Lyophilized F13 showed a homogenous dispersion of small flake irregular particles within a nanosized range contributed to cryoprotectants employed in nanoparticles where the freeze-drying process could influence the morphology. The surface area of nanoparticles affects dissolution. The larger surface area could increase the dissolution rate of nanoparticles [33].

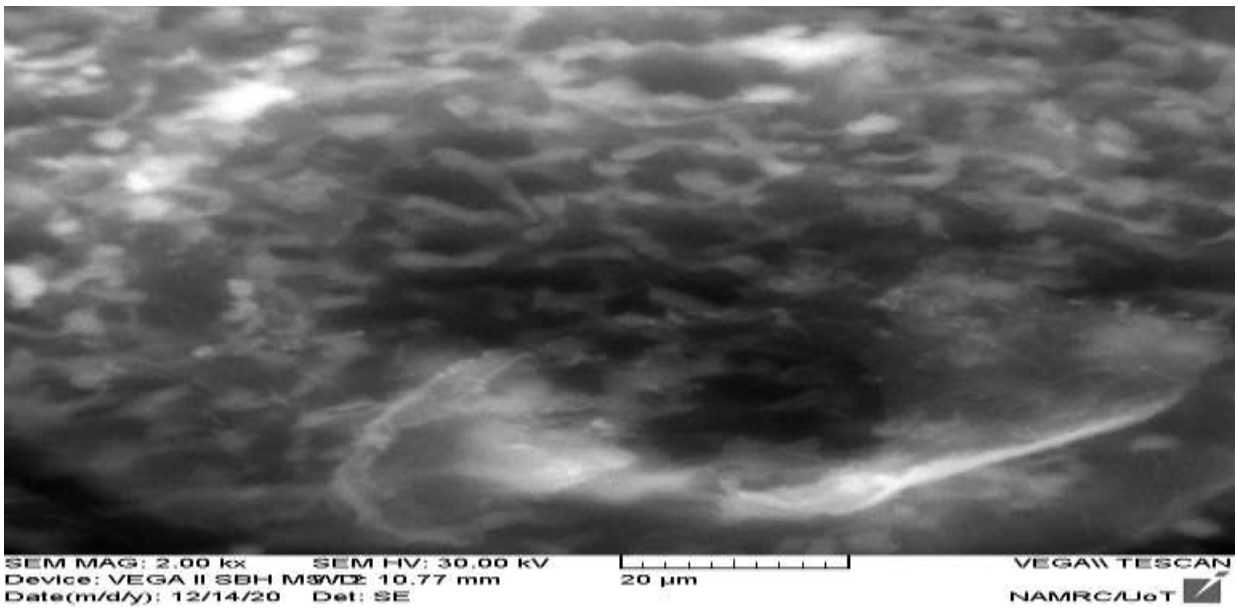

Figure 13. SEM of risperidone pure powder

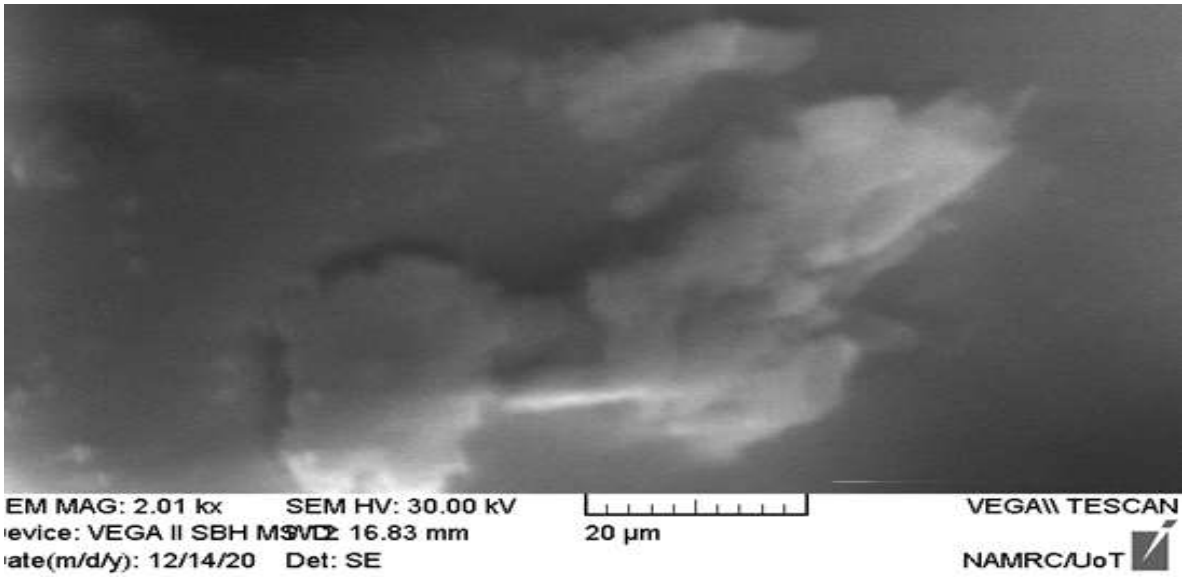

Figure 14. SEM of lyophilized selected F13

Stability study of the selected F13 lyophilized powder

The prepared risperidone lyophilized powder was investigated for stability study at three accelerated temperatures 40°C, 50°C, and 60°C for 16 consecutive weeks. The F13 lyophilized powder was subjected to the study and the results are illustrated in Figure (15).

Table 3.The degradation constant of the selected formula f13 lyophilized powder

The Temperature in ºC	K(week^{-1})
40 ºC	**1.84×10^{-3}**
50 ºC	**3.454× 10-3**
60 ºC	**7.83× 10-3**

expiration date for risperidone in F13 lyophilized powder determined through the application of the following equation:

t90% remaining = 0.105 / K25

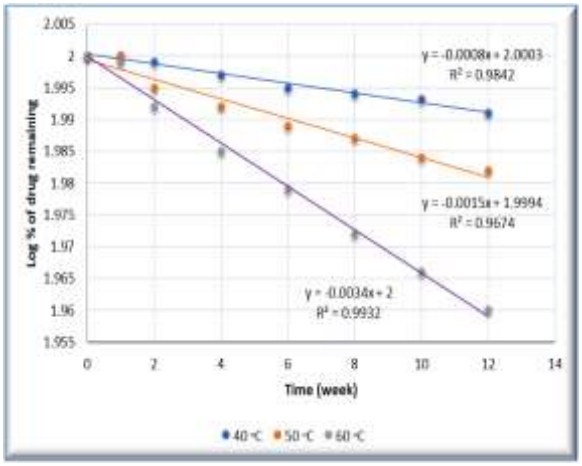

Figure 15. Accelerated degradation of the selected F13 lyophilized powder at 40 ˚C, 50 ˚C, and 60 ˚C

The degradation constant was calculated through constructing Arrhenius plot as shown in Figure (16). T90% was determined according to the previously mentioned equation and found to be 205.09 weeks that equal to 3.9 years.

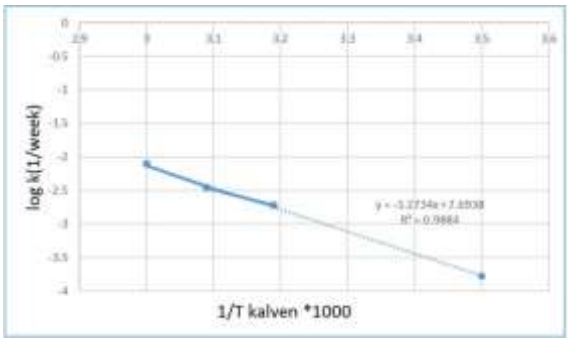

Figure 16. Arrhenius plot of the prepared risperidone in the selected F13 lyophilized powder for estimation of the expiration date

Conclusions

The solvent antisolvent-precipitation method is effective, easy to operate, and less cost. Nanosuspension is a promising approach to improve the solubility, dissolution rate. Risperidone nanoparticle was successfully prepared using soluplus as a stabilizer at ratios (1:1) using acetone as a solvent that gave a higher *in-vitro* release profiles compared to pure drug powder with an expiration date of about to 4 years for self-dispersible dry nanoparticles. The conclusion was that the formulation of risperidone nanosuspension can improve solubility and dissolution and might improve bioavailability.

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