Sys Rev Pharm 2020;11(11):596-605
A multifaceted review journal in the field of pharmacy

Formulation and Optimization of Lyophilized Selexipag Nanocrystals to Improve the Saturation Solubility and Dissolution Rate

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Abstract

Selexipag is first in class orally active, selective non-prostanoid prostacyclin receptor agonist with a long half-life. It has low oral bioavailability due to a poor dissolution rate, which is considered a rate-limiting step for drug absorption. Nanocrystals (NCs) formulation is an attractive approach for enhancing the poorly soluble drug's saturation solubility and dissolution rate. They offer the advantages of crystalline state, high drug loading capacity, and simple preparation method. This study aims to prepare and characterize Selexipag NCs, filled in hard gelatin capsule in attempt to improve its solubility and dissolution rate. The effect of stabilizer type and preparation methods on the NC particle size and polydispersity index were evaluated to select the most stable formula. The results revealed that the formula (F7) prepared by the solvent antisolvent precipitation with the ultrasonication method (which contain SLX: Soluplus® at a ratio of 1:2) has the smallest mean particle size (38.5 nm) and appropriate polydispersity index (0.12); hence it was selected as the optimal formula. Simultaneously, the data from powder X-ray diffraction shows no reduction in the crystallinity of drugs. SLX NCs exhibit increased saturation solubility by several folds than pure SLX. The hard gelatin capsule containing the lyophilized powder showed a faster dissolution profile (complete drug release in 15 min) relative to pure SLX (0.1% during the same time).

Keywords: Selexipag, Nanocrystal, nanosuspension, Selexipag nanocrystals.

1. Introduction

Pulmonary arterial hypertension is an intractable life-threatening disease with a very poor prognosis despite many treatment options. PAH is characterized by vasospasm and vascular remodeling, resulting in right ventricular dysfunction and eventually right heart failure and death. Decreased prostacyclin (IP) production is believed to play a particular role in PAH progression [1, 2].

SLX is an orally administered drug used for PAH treatment to delay disease progression and reduce hospitalization risk. It is a selective IP receptor agonist with a long half-life. The activation of the IP receptor by SLX leads to vasodilation and anti-proliferation of the

vascular smooth muscle cell. The advantages of such selectivity include: minimizing off-targeting effects, especially to the gastrointestinal tract, and avoiding the development of tachyphylaxis [3].

SLX is a crystalline powder with various polymorphic forms (form I, II, III). It is a lipophilic molecule (log P is 2.2) and practically insoluble in water (class II, according to BCS). SLX shows a pH-dependent solubility (insoluble at pH 2 to 4 and freely soluble at pH 8). It has low oral bioavailability (49%) due to the poor dissolution profile of SLX [4–6].

Nanocrystals (NC) are crystalline particles of the pure drug in the nanometer range stabilized by polymer or surfactant. NCs platform is an effective strategy for enhancing the saturation solubility and dissolution rate of poorly soluble or insoluble compounds by reducing the particle size, leading to provide high surface area and noticeable improvement in the oral absorption [7].

Drug NCs are typically formulated in liquid dispersion media; hence they are called nanosuspension (NS). Although NS has many unique properties, the physical (sedimentation/ creaming, crystalline state change, and aggregation) and chemical (hydrolysis) stability issue remains the main drawback of using it as a drug delivery system. Thus, to overcome this problem, NS is usually converted into solid-state, further processing into a solid dosage form (tablet and capsule). Lyophilization is an efficient process for the solidification of NS. It involves removing water by sublimation process and then desorption of unfrozen liquid under high vacuum or low temperature. It is essential that after freeze-drying, dry nanoparticles provide rapid re-dispersibility after hydration and keep an initial particle size of the original NS [8–10].

This study aims to prepare and characterize SLXNS to enhance its solubility and dissolution rate. Then lyophilization of the selected formula to dry powder to be filled into a hard gelatin capsule and compared their dissolution profile with a pure drug-loaded capsule.

2. Materials and methods

2.1. Materials

Selexipag (SLX), hydroxypropyl methylcellulose (HPMC E15), Poloxamer-407 (PXM-407) were bought from Hangzhou Hyperchem limited (China). Soluplus® was purchased from BASF (Germany). Sodium hydroxide was provided by Alpha chemika (India). Hard gelatin capsule size 5 was obtained from Capsuline (USA).

2.2. Preparation of SLXNS

2.2.1. Acid-base neutralization method

SLX (10 mg) was entirely dissolved in 3ml of 1N NaOH (pH 12) under sonication for 5 min. Then this solvent phase was added dropwise by a syringe pump at a flow rate of (1ml/ min) into 27 ml of 0.1N HCl (pH 1.2) containing 20 mg of polymeric stabilizer (Soluplus®, PVA, PXM-407, and HPMC E15) under continuous stirring for 1 hr at 500 rpm, as shown in table (1) [11].

2.2.2. Precipitation-ultrasonication method

SLX (10 mg) was dissolved in methanol (3 ml) under sonication for 5 min. This organic solution of SLX was added dropwise at a flow rate of (1ml/min) into 27 ml of an aqueous solution containing 20 mg of polymeric stabilizer (the same type of polymer used in the first method) with the aid of the syringe pump under stirring at 500 rpm and then followed by sonication with probe sonicator at 90% amplitude for 2 min. The period of ultrasound burst was set to 15 seconds with a pause for 15 seconds between two ultrasound bursts. SLXNS was stirred for 1 hr to ensure complete evaporation of the organic solvent, as seen in table (1) [12].

Table 1. Composition of SLXNS Formulas

Formulation	SLX (mg)	Stabilizer used	Drug: stabilizer ratio	Preparation method
F1	10	HPMC E15	1:2	acid-base neutralization
F2	10	PVA	1:2	acid-base neutralization
F3	10	Soluplus®	1:2	acid-base neutralization
F4	10	PXM-407	1:2	acid-base neutralization
F5	10	HPMC E15	1:2	Precipitation-ultrasonication
F6	10	PVA	1:2	Precipitation-ultrasonication
F7	10	Soluplus®	1:2	Precipitation-ultrasonication
F8	10	PXM-407	1:2	Precipitation-ultrasonication

2.3. Lyophilization of SLXNCs

The optimal SLXNS formula was converted into dried powder by lyophilization technique. Mannitol was used as a cryoprotectant at a concentration five times the total solid content in the selected formula. Mannitol was added into NS and vigorously shaking until mannitol was completely dissolved. This sample was then kept in a freezer at -20 °C for 24 hrs. The frozen sample was then freeze-dried using a labconnco lyophilizer at -52 °C and a vacuum pressure of 0.2 mBar for 72 hrs to obtain the dried powder [13].

2.4 Characterization of SLXNS

2.4.1 Measurement of the particle size and polydispersity index

The particle size (PS) and polydispersity index **(**PDI) of all formulations were determined by dynamic light scattering technique (DLS) using the ABT-9000 Nanolaser Particle Size Analyzer under the following conditions; temperature 25 ℃, measurement time 100 second, and each sample were read three times without dilution. Therefore all reads represent the mean ±SD [14].

2.4.2. Stability studies

The SLXNS formulas with PS under 100 nm were stored at 4 ℃ for a week. The PS, PDI, and drug concentration change at (0 and day 7) were recorded. Day 0 was the day of preparation [15]. The drug concentration was evaluated by taking an aliquot (1ml) of the SLXNS and diluted with methanol up to 20 ml. Then these samples were analyzed using a UV–visible spectrometer at 297.8nm [16].

2.5. Characterization of solid-state dry powder

2.5.1. Drug content in lyophilized powder

A specific amount of the freeze-dried powder equivalent to 1 mg SLX was accurately weighed then dissolved in 25 ml methanol under sonication for 5 min. The sample was

filtered and analyzed spectrophotometrically for drug content at 297.8 nm. The study was done in triplicate, and the percent drug content was calculated according to equation (1) [17].

Drug content %= (observed drug content / Theoretical drug content) *100 .....Eq. (1).

2.5.2. Redispersibility index) RDI)

The redispersibility for lyophilized powder was determined by redispersing the known weight of the freeze-dried powder in 2.7 ml distilled water with vigorous shaking to obtain the same concentration of drug in aqueous SLX NCs before lyophilization. The PS was measured as described in section 2.4.1. After that, the RDI value was calculated according to equation (2).

RDI= (D /D_0) *100%..... Eq. (2)

The D represents the PS of redispersed NS, and D_0 represents the PS of the pre-freeze-dried NS. The lyophilized SLX powder was well redispersed when the RDI value was close to 100% [18].

2.5.3. Scanning electron microscope

The surface morphology of lyophilized powder and pure SLX was scanned by a field emission scanning electron microscope (FE-SEM). The samples were analyzed at different power of magnification (25Kx and 50Kx). The specimens were directly scattered on double side carbon tapes attached to the FESEM specimen amount. The specimen was sputter-coated with gold by auto-fine coater to obtain uniform coating on the samples [19].

2.5.4. Saturation solubility

The saturation solubility of pure SLX and the freeze-dried powder was determined by adding an excess amount of the powder into 10 ml of dissolution media (0.1 N HCl and phosphate buffer (pH 6.8)). All these solutions were kept in a water bath shaker for 48 hrs, and the temperature was maintained at 37±1 ℃. The supernatant was filtered through a 0.45µm syringe filter. These samples were assayed spectrophotometrically to detect the concentration of SLX [20].

2.5.5. Flowability and compressibility index

The angle of repose, apparent bulk density, and tapped density was determined for the lyophilized SLX powder. The angle of repose (θ) was determined using a stainless-steel funnel with a 10 mm orifice size fixed on a particular height from a horizontal surface. When the cone from the known weight of the lyophilized powder was built, this cone height (h) and the radius (r) base was calculated and applied in equation (3) to measure the angle of repose [21].

$\theta = \tan^{-1}(h/r)$ ..... Eq. (3)

While the bulk density (B_d) and tapped density (T_d) were measured by pouring a known weight (m) of the pre-sieved freeze-dried powder into (250ml) graduated cylinder via a large funnel. This cylinder was subjected to several taping using an E-22 Copley densitometer until no apparent change in volume was observed. The initial apparent volume (V_0) and the tapped volume (V_t) were recorded to calculate B_d and T_d according to equation (4 and 5) in g/ml [21].

$B_d = m/V_0$ ..... Eq. (4)

$T_d = m/V_t$ ..... Eq. (5)

The compressibility index is used to determine lyophilized powder's flow properties. It is calculated according to the equation (6). The smaller Carre's index (CI) value was had, the better the flow properties [22].

CI= {Td – B_d /T_d} *100..... Eq. (6)

2.5.6. Powder X-ray diffraction (PXRD)

The crystallinity state of pure SLX and selected lyophilized formula were analyzed using a powder X-ray diffractometer (Shimadzu-6000, Japan). The sample was scanned over a range of 5° to 60° (2θ), the operating voltage 40Kv, and a current of 30mA [23].

2.6. Filling of SLX lyophilized powder into a hard gelatin capsule

After knowing the formulation tapped density and the anticipated dose, the lyophilized powder equivalent to 1 mg SLX was packed manually into the hard gelatin capsule of a suitable size.

2.7. *In vitro* dissolution study

The *in vitro* dissolution test was conducted in USP dissolution apparatus type II for freeze-dried loaded capsule and pure SLX-loaded capsule. The experiment was performed in 300 ml of 0.1N HCl with 1% Brij-35 (to maintain sink condition) and phosphate buffer pH 6.8. The media's temperature was kept at 37 ±0.5 ℃ while the paddle's stirrer rate is 50 rpm. The capsule was placed in a sinker to prevent floating then put in the dissolution media. Samples of 5 ml were withdrawn at a predefined time interval and replaced by freshly prepared media. The samples were filtered through a .045 µm syringe filter. A UV- visible spectrophotometer was used to analyze the drug content in the filtrate [24].

The similarity factor (*f2*) was used to compare the difference in dissolution between the lyophilized powder of SLXNCs and pure drug, according to equation (7).

$f_2 = 50 * \log\{100 * [1 + (1/n) * \sum (Rt - Tt)^2]^{-0.5}\}$ ..... Eq. (7).

R_t and T_t are the amounts of dissolved drug from reference and test formulation at time t, respectively, and n is the number of time points [25].

2.8. Statistical analysis

Data were expressed as the mean ± stander deviation (SD), which was analyzed by one–way analysis of variance (ANOVA) and t-test student. The results were considered statistically significant if $p < 0.05$, highly significant if $p < 0.01$, and nonsignificant if $p > 0.05$.

3. Results and discussion

3.1. Characterization of SLXNS

3.1.1. PS and PDI

The average PS and PDI results for all the prepared SLXNS formulas were displayed in table (2).

PDI was an essential parameter that gives an overview of the variation within PS distribution and NS's physical stability. When the PDI value of the sample range from 0.1-0.25 indicates narrow size distribution. In contrast, when PDI value >0.5, the sample is considered to have a broad size distribution [26].

3.1.1.1. Effect of stabilizer

Four types of stabilizers, which are (HPMC E15, PVA, Soluplus®, PXM-407), were used to prepare the formulas (F1-F4) to study their effect on the PS of SLXNS. The acid-base neutralization technique was used to prepare all these formulas at a drug: stabilizer ratio 1:2.

The result revealed a significant difference in the PS ($P<0.05$) reduction between the four types of stabilizers. The formula that stabilized by Soluplus® showed the smallest average PS (PS 62.8 nm)), while NS stabilized by

other polymers (HPMC E15, PVA, PXM-407) have higher average PS in the range 219-511 nm as seen in figure (2). This phenomenon may be related to the excellent surface affinity between the stabilizing agent (Soluplus®) and newly formed nanoparticles compared to other polymers. Soluplus® is an amphiphilic graft copolymer with a hydrophilic part (polyethylene glycol backbone) and a lipophilic part (vinyl caprolactam/vinyl acetate side chains). The hydrophobic part of the polymer will be adsorbed onto the hydrophobic drug surface. In contrast, the hydrophilic portion may be extended outward into the aqueous phase, reducing the interfacial tension formed between the newly formed nanoparticles, providing full surface coverage and steric stabilization to NS hence inhibiting agglomeration and particle growth [27].

The PDI values of these formulations were in the range from (0.01- 0.1). This result indicates that SLXNS had narrow PS distribution.

Table 2. The PS and PDI of the Various Formulation of SLXNS.

Formula no.	Stabilizer used	Drug: stabilizer: ratio	PS±S.D	PDI
F1	HPMC E15	1:2	511±19	0.01
F2	PVA	1:2	502.6±8	0.02
F3	Soluplus®	1:2	62.8±4.6	0.058
F4	PXM407	1:2	291±10.3	0.1
F5	HPMC E15	1:2	761.3±52.5	0.02
F6	PVA	1:2	550.3±2.3	0.01
F7	Soluplus®	1:2	38.5±4.3	0.12
F8	PXM-407	1:2	197±0	0.211

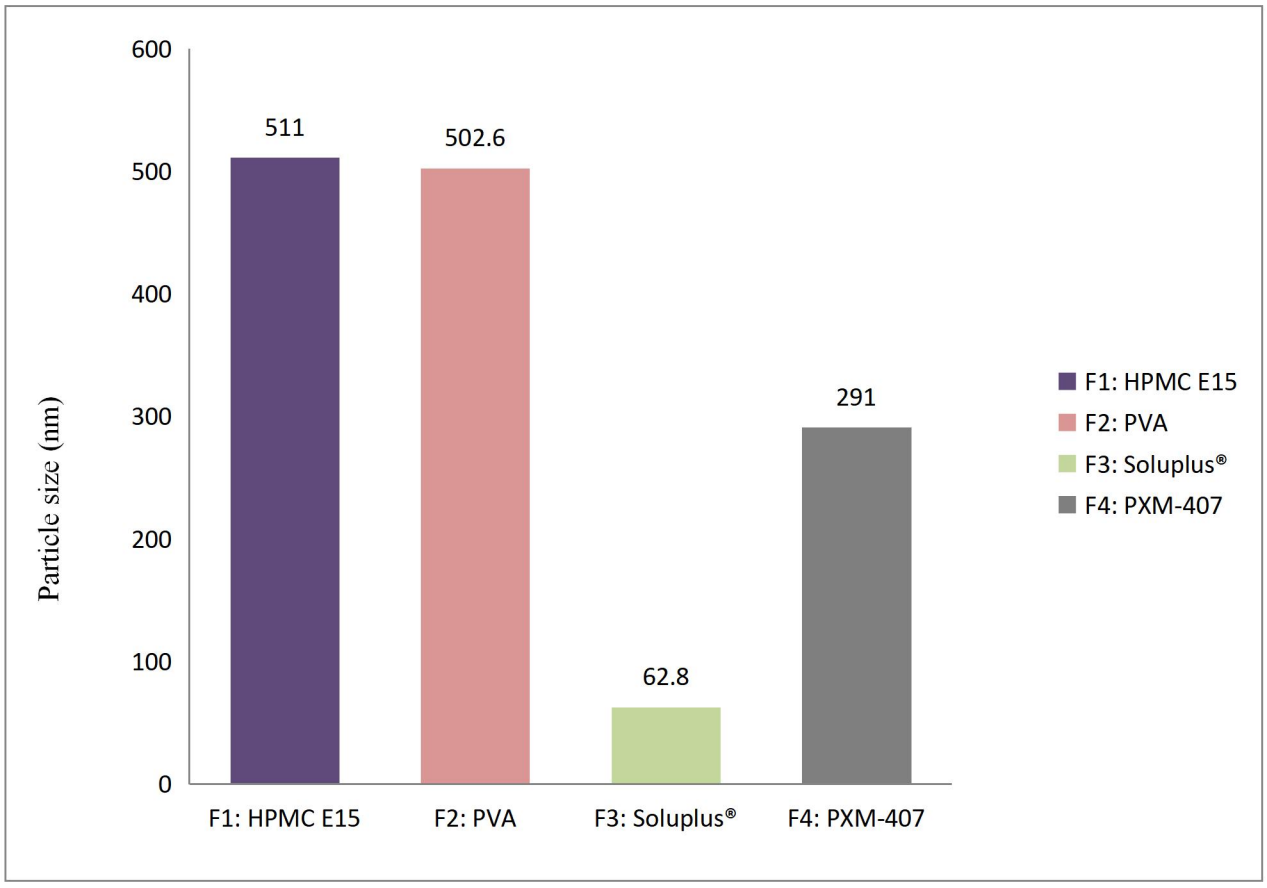

Figure 2. Effect of stabilizer type on the PS of the prepared SLXNS.

3.1.1.2. Preparation method effect

The precipitation-ultrasonication process was used to prepare the formulas (F5-F8). Ultrasonication was regarded as an important means to decrease the induction time between establishing supersaturation and nucleation. These formulas were compared with the formulas (F1-F4) prepared by the acid-base neutralization technique to investigate the sonication process's effect on PS of SLXNS. All the formulas were prepared at a constant drug: stabilizer ratio (1:2) and at a stirring speed of 500 rpm.

The formulas F7 (38.5 nm) and F8 (197 nm) that stabilized with Soluplus® and PXM 470 show a significant reduction in PS ($p<0.05$) when compared with F3 (62.8 nm) and F4 (291 nm) as depicted in figure (3). The principal mechanism of ultrasonication was dependent on the formation of bubbles (cavitation), followed by a collapse that provides high energy to the system. This energy can break the intermolecular interaction found between large particles of SLX, leading to a reduction in the PS [28].

While the PS of formulas F5 (761.3 nm) and F6 (550.3 nm)

that stabilized by HPMC E15 and PVA, respectively, seems to increase significantly ($p<0.05$) in comparison with formulas F1 (511nm) and F2 (502.6 nm), which prepared by the acid-base neutralization method as seen in figure (4). This result could be explained by realizing the high viscosity of samples containing the HPMC and PVA may lead to retard the transmission of ultrasonic vibration and diffusion between the multiphase during precipitation, which results in the formation of large particles [28].

The PDI values of these formulations (F5-F8) were in the range from (0.01-0.2), which are in the acceptable range.

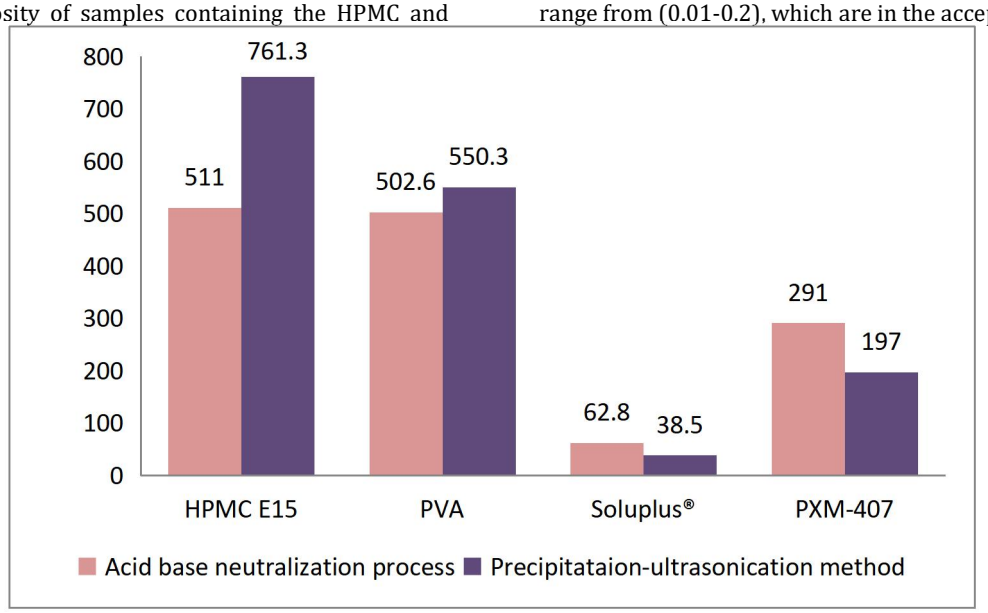

Figure 4. Effect of preparation method on the PS of SLXNS.

3.1.2. Stability study

The SLXNS formulas F3 and F7, which has a PS under 100 nm, were stored for 7 days at 4 ℃ to select the most stable formula. The result revealed that the F7 was sufficiently stable because the PS, PDI, and SLX concentrations remain constant during the test period. While all these parameters were significantly changed for the formula F3, as shown in table (3).

Table 3. PS, PDI, SLX Concentration of SLX NCs at 0 and 7 Days.

Formula no.	PS	PDI	drug concentration	PS	PDI	drug concentration
	At day 0			At day 7		
F3	62.8± 4.6	0.05	334.5± 0.09	132 ± 0.012	0.08	307.3± 0.12
F7	38.5± 4.3	0.12	359.1± 0.3	41.3 ± 4.4	0.13	358.6 ± 0.46

3.2. Lyophiliaztion of SLXNCs

The optimal formula was lyophilized to do another characterization for the freeze-dried powder and processing into a solid oral dosage form. Simultaneously, a cryoprotectant (mannitol) was added to prevent nanoparticle aggregation and maintain the freeze-dried powder's re-dispersibility as separate particles [29, 30].

The formula (F7), which stabilized by Soluplus® and prepared by the precipitation-ultrasonication process, was selected to be dried by lyophilization technique since it exhibits the smallest PS and sufficient physical stability. The resultant lyophilized powder appears as off-white fluffy powder, as shown in figure (5)

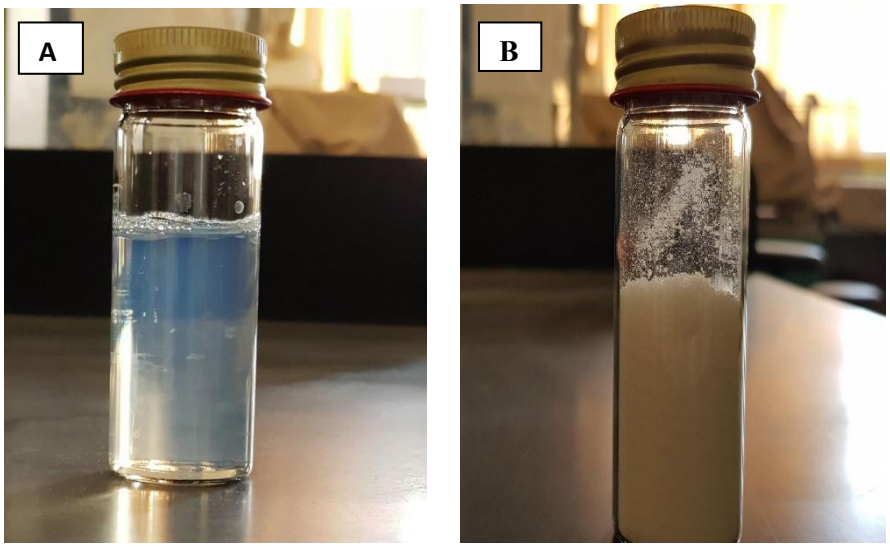

Figure 5. Formula F7: A- SLXNS before lyophilization. B- lyophilized NCs

3.3. Characterization of solid-state dry powder

3.3.1. Drug content of the lyophilized powder

The drug content in the freeze-dried powder of the selected formula (F7) was found to be around 95.5%.

3.3.2. Redispersibility index

After redispersing 19 mg of the freeze-dried powder (which contains an equivalent amount of 1 mg SLX) in 2.7 ml water, PS &PDI of this suspension was measured to evaluate the re-dispersibility of the freeze-dried powder based on RDI value as shown in table(4).

Table 4. The PS and PDI Value of F7 Before (D_0) and After (D1) Lyophilization and their RDI

	PS	PDI	RDI
D_0	38.5±4.3	0.12±0.024	86.2
D1	33.2±3.2	0.1±0.02	

RDI value was nearly 100%, indicating that the lyophilized SLX powder can be reconstituted entirely to the original PS of the NS after rehydration [31].

3.3.3. SEM

FE-SEM analysis was performed to check any change in surface morphology of SLX in lyophilized powder compared to pure drug. The FE-SEM images of pure SLX showed irregularly shaped crystal with a rough surface, as seen in figure 6 (A and B). In contrast, SLX in lyophilized powder appeared to have an entirely different surface morphology, which appears as flake shaped crystal with a smoother surface, as seen in figure 7 (A and B). This result indicates that the SLX in the lyophilized powder remains in the crystalline state, which imparts physical stability.

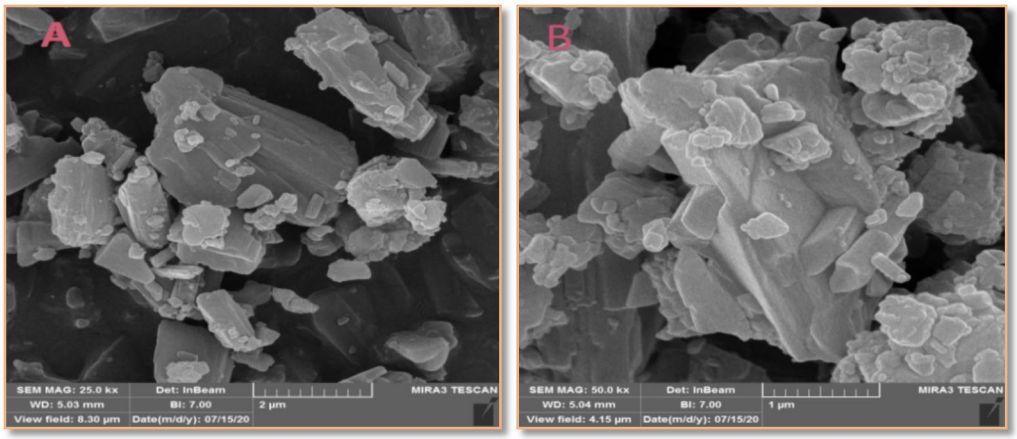

Figure 6. FE-SEM images of pure SLX at A- 25 Kx and B- 50 Kx magnification.

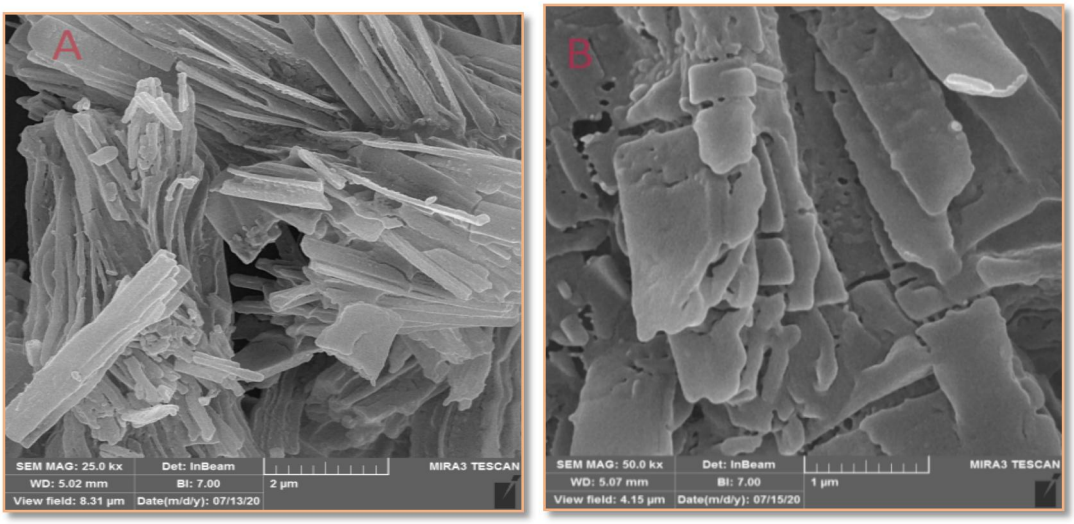

Figure 7. FE-SEM images of SLX in lyophilized powder at A- 25 Kx and B- 50Kx magnification.

3.3.4. Saturation solubility

The saturation solubility of pure SLX and lyophilized SLXNCs powder was performed in 0.1N HCl and phosphate buffer (pH 6.8) to determine the enhancement in saturation solubility of SLX after the formation of lyophilized powder than that of pure SLX. The results revealed significant enhancement in the freeze-dried powder's saturation solubility by several folds in these media, as shown in table (5).

The higher solubility of lyophilized SLX powder could be explained based on the Ostwald-Freundlich equation. The reduction of the drug's PS to nanoscale leads to an increase in the surface area, which results in enhancement of the SLX solubility. Another reason may be attributed to amphiphilic stabilizer (Soluplus®), a type of polymer that can provide extra solubilization

effect on the poorly water-soluble drug [15].

Table 5. The Saturation Solubility of Pure SLX and Lyophilized Powder in Different Media.

Dissolving media	Pure SLX saturation solubility (µg/ml)	Lyophilized SLXNCs powder saturation solubility (µg/ml)	Solubility increment fold
Phosphate buffer (pH6.8)	15.04 ± 0.33	3447± 0.009	229
0.1N HCl	3.48± 0.05	139.2± 0.009	40

3.3.5. Flowability and compressibility index

The bulk density for lyophilized SLXNCs powder is 0.136g/ml, while the tapped density for this powder is 0.147 g/ml.

Moreover, the Carre,s index (CI) and angle of repose values are 7.4 and 29.60, respectively, which indicates that this lyophilized powder showed excellent flowability [22].

3.3.6. PXRD

The PXRD Study was performed to assay the crystalline state of SLX in lyophilized powder. The XRD pattern of pure SLX and lyophilized SLX powder was displayed in figures 8 (A and B). The diffraction pattern of pure SLX showed sharp and intense peaks at 2θ of 9.5, 16.8, 20.5, and 23.3, which indicate the crystallinity of pure drug [31]. While the PXRD pattern of SLX in lyophilized powder showed sharp and higher intensity peaks that indicate that SLX is still present in a crystalline state with high quality, so impart long term stability. This result agrees with the study done by Ali et al., which reported that the XRPD pattern of nano-atropine sulfate showed higher intensity peaks compared to a pure drug [32].

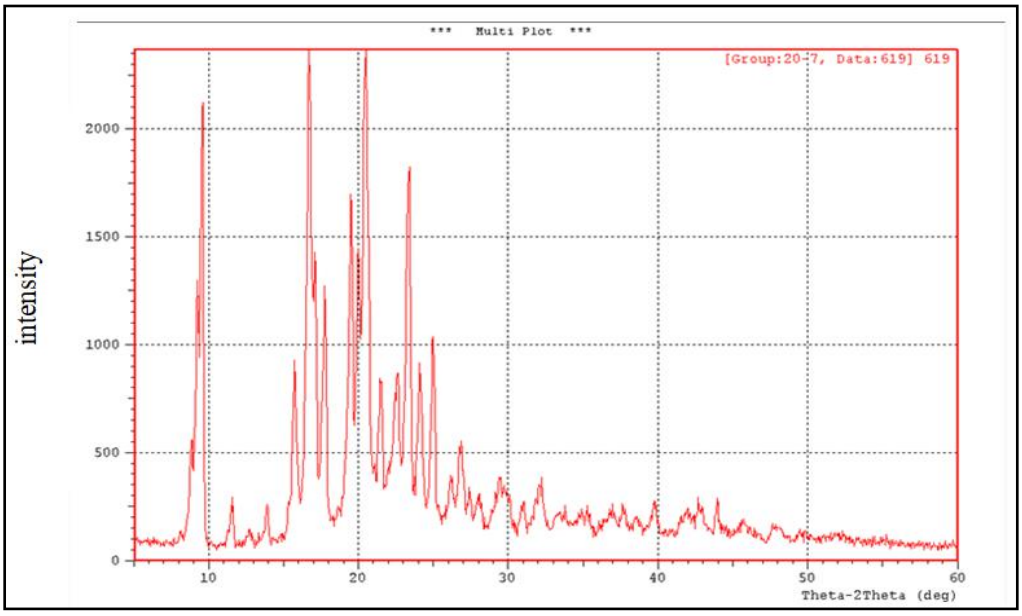

Figure 8. A. XRPD of pure SLX.

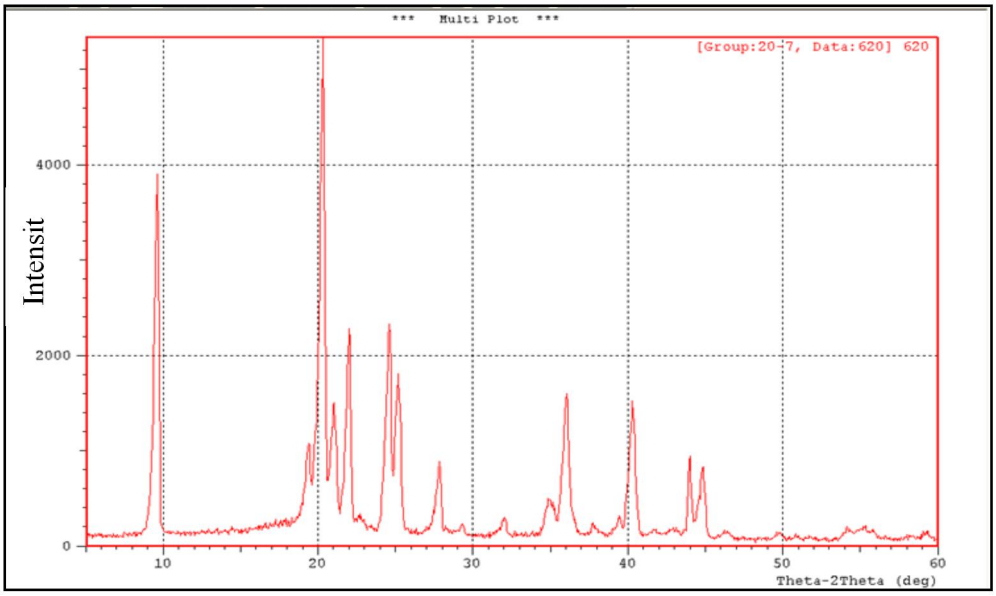

Figure 8. B. XRPD of SLX in lyophilized powder.

3.4. Filling of SLX lyophilized powder into a hard gelatin capsule

19 mg SLX lyophilized powder (containing SLX equivalent to 1 mg) was filled into size five hard gelatin capsules.

3.5. *In vitro* dissolution study

The dissolution behaviors of SLX from the freeze-dried powder-loaded capsules and pure drug-loaded capsules in 0.1N HCl (pH 1.2) contains 1% Brij-35 and phosphate buffer (pH 6.8), are shown in figures (9 and 10), respectively. In both dissolution media, the freeze-dried-loaded capsules' dissolution rate was superior compared to the pure powder-loaded capsules with complete dissolution during the test period.

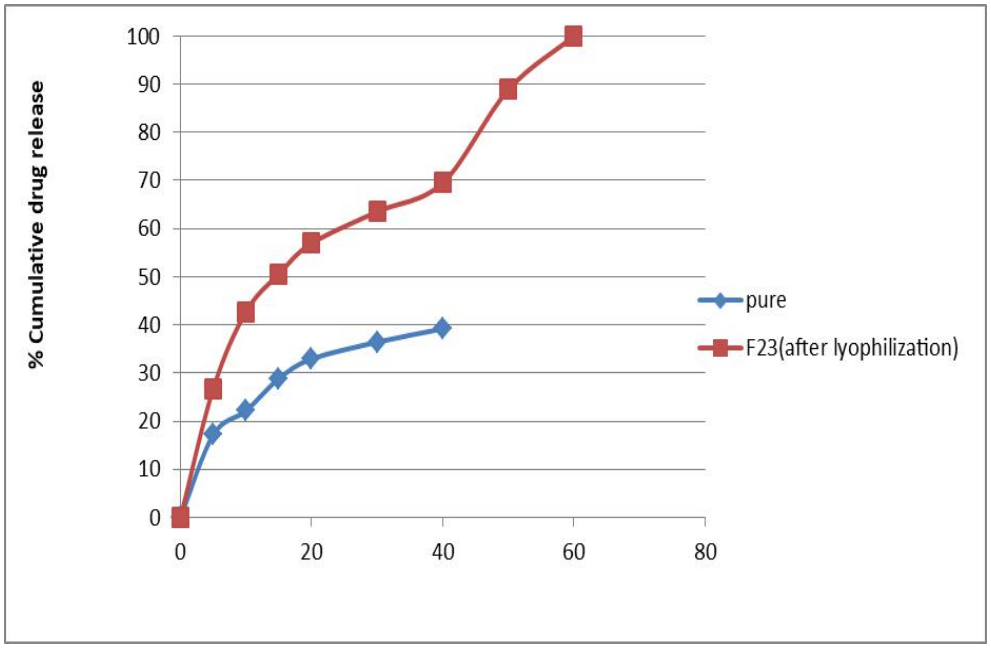

Figure 9. The dissolution profile of capsules loaded with SLX freeze-dried and pure drug in 0.1 N HCl in the presence of 1%Brij at 37±0.5 ℃.

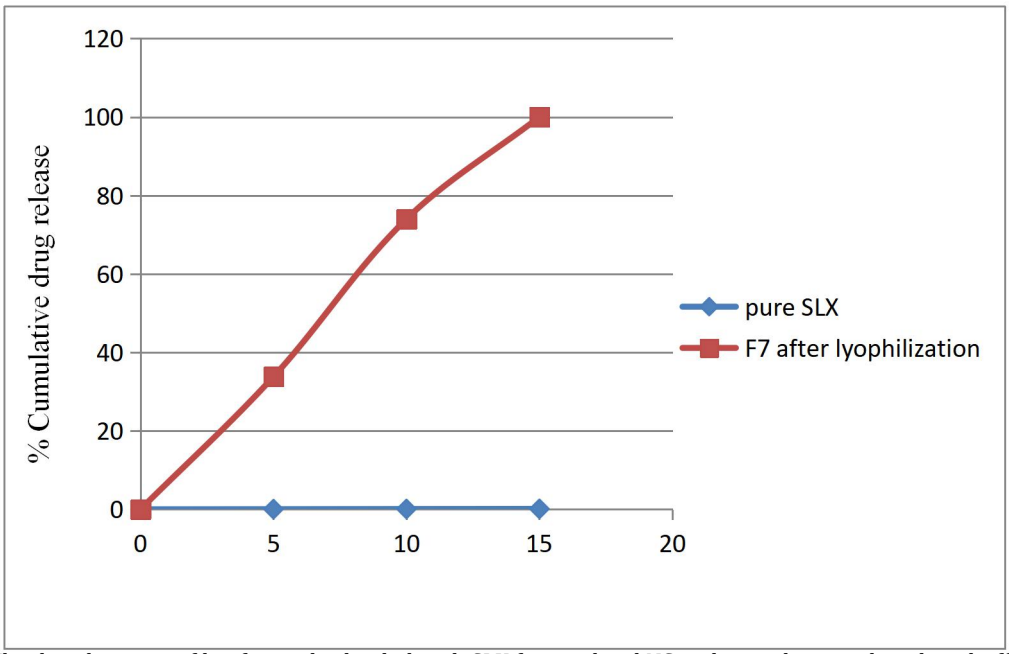

Figure 10. The dissolution profile of capsules loaded with SLX freeze-dried NS and pure drug in phosphate buffer pH 6.8 at 37±0.5 ℃.

The similarity factor *f2* was used to compare the capsules' dissolution profiles filled with SLX lyophilized powder and capsules filled with the pure drug (reference). The results revealed that the *f2* values were less than 50 in dissolution media, as seen in table (6), indicating that these profiles were dissimilar.

Table. 6. *f2* of the SLX Freeze-Dried Powder- Loaded Capsule in Different Dissolution Media.

***f2* in 0.1N HCl**	***f2* in phosphate buffer**
26.15	9.56

This enhancement in the dissolution rate can be explained based on the following reasons. According to

the Noyes Whitney equation, the reduced PS of SLX (the presence of mannitol as a cryoprotectant contributes to avoiding the formation of collapse cake and maintaining the particles in the nanosize range) provide higher surface area, which results in an excessive enhancement of the dissolution velocity. Also, the increase in the surface wetting by Soluplus® may contribute to enhance the dissolution rate [33].

4. Conclusions

SLXNCs were successfully prepared using the solvent antisolvent precipitation with the ultrasonication method. The optimal formula (F7), which was prepared by this method and stabilized by Soluplus® in drug: stabilizer ratio of 1:2, showed the smallest particle size, appropriate PDI, and sufficient physical stability. While the freeze-dried SLXNCs powder exhibit a dramatic increase in the saturation solubility and dissolution rate than a pure drug.

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