Nanosuspensions of Selexipag: Formulation, Characterization, and in vitro Evaluation

Rusul M. Alwan* and Nawal A. Rajab**

* Ministry of Health and Environment, Najaf Health Department, Najaf, Iraq.
**Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Selexipag (SLP) is an orally selective long-acting prostacyclin receptor agonist indicated for pulmonary arterial hypertension treatment. It is practically insoluble in water (class II, according to BCS). This work aims to prepare and optimize Selexipag nanosuspensions (SLPNS) to enhance the saturation solubility and in vitro dissolution rate. The solvent antisolvent precipitation method was used for the production of NS, and the effect of formulation parameters (stabilizer type, drug: stabilizer ratio, and use of co-stabilizer) and process parameter (stirring speed) on the particle size (P.S) and polydispersity index (PDI) were studied. The result revealed that the P.S of all prepared SLPNS formulation was in the nanometer range, except for the formulas that stabilized by Poloxamer. The optimal SLPNS (F15), which is stabilized by Soluplus® (SLP: stabilizer ratio 1:2) and prepared at a stirring speed of 1000 rpm, showed the smallest P.S and appropriate PDI, which are 47 nm and 0.073. The formula F5 exhibits 136 folds, an increase in the saturation solubility, and an enhancement in the dissolution rate at a stirring speed of 1000 rpm, showed the smallest P.S and appropriate PDI, which are 47 nm and 0.073. The formula F5 exhibits 136 folds, an increase in the saturation solubility, and an enhancement in the dissolution rate.

Keywords: Selexipag nanosuspension, Solvent antisolvent precipitation method.

Introduction

It was reported that about 40% of new chemical entities are hydrophobic compounds, and one-third of drugs documented by United States Pharmacopeia (USP) are poorly water-soluble. Poor aqueous solubility may trigger some issues: such as low bioavailability caused by uncontrolled precipitation & extensive variation in the absorption profile (4).

Nanosuspensions (NS) is defined as a unique submicron colloidal dispersion of nanosized drug particles that need to be stabilized by suitable stabilizers (polymer and/or surfactant) (3). Currently, NS has been considered as an attractive approach for enhancing the bioavailability of insoluble drugs owing to increase their saturation solubility and dissolution rate (5). In general, NS can be prepared either by top-down or bottom-up methods. The top-down processes involve a breakdown of large solid particles into nanosized particles, while bottom-up approaches involve precipitation of dissolved drug molecules into nanoparticles (6).

---

1Corresponding author E-mail: rusul90alwan@yahoo.com
Received:22 /7/ 2020
Accepted:24 /10/ 2020
Although the top-down processes are favored in the pharmaceutical industry, the bottom-up processes have the advantage of providing a homogenous dispersion system (narrow particle size distribution) with lower energy input (5).

The liquid solvent anti-solvent precipitation method is a type of bottom-up technique that is most commonly used to prepare NS due to its simplicity and cost-effectiveness. The basic principle of this method is based on modifying the compound solubility by mixing a water-miscible organic solvent containing the drug with the antisolvent (in which the drug is insoluble) in the presence of the stabilizer; precipitation of nanoparticles will occur instantaneously. During precipitation, the stabilizer will be absorbed on to the surface of the newly formed nanoparticles to prevent further growth of the particles (6,7).

Selexipag (SLP) is a selective, orally active, long-acting prostacyclin (IP) receptor agonist that has been used for long term treatment of pulmonary arterial hypertension to delay disease progression and reduce the risk of hospitalization (7). In contrast, all other licensed drugs that target the prostacyclin pathway have a short half-life. Most of them need to be administered by an intravenous or subcutaneous infusion or by the inhalation route (8).

After oral administration, SLP is rapidly hydrolyzed into the active metabolite ACT-333679 (approximately 37 fold more potent than SLP) following absorption. The oral bioavailability of SLP is approximately 50% (9).

SLP is a non-hygroscopic powder which appears as a pale yellow crystalline powder. It is practically insoluble in water (<1mg/ml) and exhibits pH-dependent solubility. According to the biopharmaceutical classification system, it is considered a class II drug (low solubility, high permeability) (10,11).

This study aims to prepare and characterize SLPNS prepared by liquid solvent anti-solvent precipitation process in an attempt to improve the saturation solubility and dissolution rate of SLP.

**Materials**

Selexipag (SLP), polyvinyl alcohol (PVA), hydroxypropyl methylcellulose (HPMC E15), and Poloxamer-407 (PX-407) were purchased from Hangzhou hyper chemical limited (China). Soluplus® was obtained from BASF (Germany). Twin-80 was provided by Alpha chemika (India). All other solvents and chemicals used were of analytic grade and utilized without further purification.

**Method**

**Preparation of SLPNS**

The SLPNS was prepared by the liquid solvent anti-solvent precipitation method. Briefly, 10 mg of SLP was dissolved in 3 ml methanol. After that, this solvent phase was added dropwise at a flow rate (1ml/ min) by the aid of a syringe pump into 27 ml of an aqueous solution (water containing polymer alone or in combination with co-stabilizer) under a continuous stirrer for 1 hr at a certain speed (12).

Different types of stabilizers, which are HPMC E15, PVA, Soluplus®, and PX-407, were used at different concentrations, either alone or in combination with twin-80, as shown in Table (1).

The effect of formulation parameters (stabilizer type, SLP: polymer ratio, and co-stabilizer) and process parameter (stirring speed) on the P.S and PDI of SLP NS were investigated to select the most stable formula.

In order to increase the stability of the final product and perform some evaluation tests, the freshly prepared SLPNS was immediately lyophilized in the presence of cryoprotectant (mannitol) to prevent the formation of the collapsing cake at a concentration of 0.5% w/v. The dispersion was pre-frozen in a freeze at -20 °C for 24 hr, followed by lyophilization for 72 hr at -52 °C and vacuum pressure of 0.2 mbar using Labocconc lyophilizer (13).

### Table 1. Composition of SLPNS formulation

| Formula symbol | SLP (mg) | PVA (mg) | HPMC E15 (mg) | Soluplus® (mg) | PX-407 (mg) | twin-80 (ml) | Stirrer speed |
|----------------|---------|---------|----------------|----------------|-------------|--------------|--------------|
| F1             | 10      | 10      | --             | --             | --          | --           | 500          |
| F2             | 10      | 20      | --             | --             | --          | --           | 500          |
| F3             | 10      | --      | 10             | --             | --          | --           | 500          |
| F4             | 10      | --      | 20             | --             | --          | --           | 500          |
| F5             | 10      | --      | --             | 10             | --          | --           | 500          |
| F6             | 10      | --      | --             | 20             | --          | --           | 500          |
| F7             | 10      | --      | --             | --             | 10          | --           | 500          |
| F8             | 10      | --      | --             | --             | 20          | --           | 500          |
| F9             | 10      | 10      | --             | --             | 0.01        | --           | 500          |
| F10            | 10      | --      | 10             | --             | 0.01        | --           | 500          |
| F11            | 10      | --      | --             | 10             | 0.01        | --           | 500          |
| F12            | 10      | --      | --             | --             | 10          | 0.01         | 500          |
| F13            | 10      | 20      | --             | --             | --          | --           | 1000         |
| F14            | 10      | --      | 20             | --             | --          | --           | 1000         |
| F15            | 10      | --      | --             | 20             | --          | --           | 1000         |
| F16            | 10      | --      | --             | --             | 20          | --           | 1000         |
Characterization of SLPNS
P.S and PDI
Dynamic light scattering technique was used to measure the P.S and PDI of SLPNSs at 25 \(^\circ\)C in a detection angle of 90\(^\circ\) using the ABT-9000 Nano Laser Particle Size Analyzer. All samples were measured without dilution and in triplicate. The results were expressed by a mean and standard deviation (SD)\(^{(14)}\).

The drug content in SLPNS
All formulas that have P.S under 100 nm were subjected to the drug content estimation study by diluting 1 ml of SLPNS with methanol into 20 ml. Each sample was assayed by UV-Visible spectroscopy at 297.8 nm for at least three times to measure the actual drug content based on the following equation\(^{(15)}\):

\[
\text{Drug content} \% = \frac{\text{observed drug content}}{\text{Theoretical drug content}} \times 100 \quad \text{...(Eq. no.1)}
\]

In vitro dissolution study
The dissolution test for SLPNS formulas that have P.S under 100 nm was performed using USP apparatus type II (paddle type). The volume of NS equivalent to 1 mg SLP was placed in a pretreated dialysis bag (MWCO 12.14 KD). The dialysis bag was fixed into the paddle and immersed into the dissolution media (300 ml phosphate buffer pH 6.8 in the presence of 1% Brij-35 to maintain the sink condition). The temperature was kept at 37±0.5 \(^\circ\)C, while the speed of the paddle was 50 rpm. An aliquot of 5 ml was withdrawn at a scheduled time interval (5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min) and replaced by the freshly prepared dissolution media. The SLP amount was assayed spectrophotometrically at specified \(\lambda\) max for this media, which is 305 nm. All the measurements were done in triplicate\(^{(16)}\).

The similarity factor \(f_2\) was used to measure the similarity in the percent of drug dissolution between two curves according to the following equation:

\[
f_2 = 50 \times \log \left\{ 1 + \frac{1}{n} \sum_{t=1}^{n} |R_t - T_t|^2 \right\}^{-0.5} \times 100
\]

Where \(n\) represents the number of sampling points, while \(R_t\) and \(T_t\) is the average percentage of dissolved drug in the reference and test sample at time \(t\), the FDA guideline suggests that two dissolution profiles can be considered dissimilar if \(f_2\) is less than 50\(^{(17)}\).

Saturation solubility study
The shaking flask method was utilized to determine the saturation solubility. An excess amount of pure SLPX, lyophilized NS (of the optimal formula), was added to 10 ml of D.W and subjected to shaking at 25 \(^\circ\)C for 48h using a water bath shaker. The supernatant was filtered through a 0.45\(\mu\)m syringe filter and analyzed by the UV-visible spectrophotometer\(^{(18)}\).

Fourier Transformed Infrared Spectroscopy (FTIR)
The FTIR spectra of pure SLP, Soluplus®, mannitol, and freeze-drying powder of the selected formula were recorded to investigate the interaction between active pharmaceutical ingredient and excipient using FTIR spectrometer (FTIR-8300 Shimadzu, Japan) by compressing the sample into a disc using KBr. The sample was analyzed through a wavelength region between 4000- 400 cm\(^{-1}\)\(^{(19)}\).

Statistical analysis
The results in this experiment were expressed as the mean ± (SD). One way analysis of variance (ANOVA) and t-test student was used to analyze the difference between the samples at the level of significance (p<0.05).

Result and Discussion
P.S and PDI analysis
The mean P.Ss and PDI values are shown in Table (2). A PDI value < 0.3 indicates a narrow size distribution, while PDI >0.3 is considered a broad size distribution\(^{(20)}\).

The mean P.S of all the prepared formulas was in the range of 47 nm- 3835 nm. These results indicate that it is possible to formulate SLPNS with small P.S by controlling the critical formulation and process parameters.

The PDI values of formulas ranged from 0.005-0.073, which indicates that these formulas have a narrow P.S distribution: except the formulas F4 and F9, which have a PDI value of 0.5 and 0.4, respectively, so this system is considered to have broad size distribution.
Adsorption, they cannot provide a stable thermodynamic barrier that lead to steric stabilization of the system by can effectively adsorb onto drug particle surfaces. These polymers are abundance with hydroxyl group. These polymers hydroxyl group while the chemical side chains). The adsorption of Soluplus® onto drug particles decreases the interfacial tension of the surface particles, thus providing steric hindrance, thus prevent particle aggregation and growth.

The formulas that stabilized by Soluplus® has the smallest P.S (p<0.05) followed by NS prepared with HPMC E15, PVA, and PX-407, as illustrated in figure (1). This can be attributed to the natural chemical composition and superior surface activity and wettability of Soluplus®. Soluplus® is an amphiphatic graft copolymer contains a hydrophilic part (polyethylene glycol backbone) and a lipophilic part (vinyl caprolactam/ vinyl acetate side chain). The adsorption of Soluplus® onto drug particles decreases the interfacial tension of the surface particles, thus providing steric hindrance to prevent the aggregation of the newly formed nanoparticles (22).

Optimization of SLPNS using different types of stabilizers and at various concentrations.

The selection of suitable stabilizers and concentration is one of the most critical parameters that affect the P.S and stability of NS(21).

The effect of type and concentration of polymer on SLPNS formulations are seen in formulas (F1- F8).

Table 2. P.S and PDI of the different formulation.

| Formula no. | Stabilizer used | Drug: stabilizer: co-surfactant ratio | Stirrer speed | P.S±SD | PDI |
|-------------|-----------------|--------------------------------------|--------------|--------|-----|
| F1          | PVA             | 1:1                                  | 500          | 541.6±6.3 | 0.009 |
| F2          | PVA             | 1:2                                  | 500          | 439.6±26.5 | 0.016 |
| F3          | HPMC E15        | 1:1                                  | 500          | 467±13.8  | 0.02  |
| F4          | HPMC E15        | 1:2                                  | 500          | 181.6±2.3 | 0.5   |
| F5          | Soluplus®       | 1:1                                  | 500          | 74.6±2.6  | 0.015 |
| F6          | Soluplus®       | 1:2                                  | 500          | 73.2±4.8  | 0.009 |
| F7          | PX-407          | 1:1                                  | 500          | 2737.6±23 | 0.007 |
| F8          | PX-407          | 1:2                                  | 500          | 3835.3±21 | 0.005 |
| F9          | PVA: tween80    | 1:1:1                                | 500          | 279±0     | 0.4   |
| F10         | HPMC E15: tween80 | 1:1:1                               | 500          | 453.6±16.7 | 0.012 |
| F11         | Soluplus®: tween80 | 1:1:1                               | 500          | 83.2±6    | 0.019 |
| F12         | PX-407: tween80 | 1:1:1                                | 500          | 4356±40   | 0.007 |
| F13         | PVA             | 1:2                                  | 1000         | 632±24.2  | 0.029 |
| F14         | HPMC E15        | 1:2                                  | 1000         | 603±12.12 | 0.02  |
| F15         | Soluplus®       | 1:2                                  | 1000         | 47±9.2    | 0.073 |
| F16         | PX-407          | 1:2                                  | 1000         | 3656.6±11 | 0.007 |

Further, the stabilization of SLPNS by HPMC E15 or PVA could be explained by the formation of a hydrogen bond between SLP particle and one of these polymers (HPMC E15 comprised a high degree of substitution of the methoxy and hydroxy group while the chemical structure of PVA is abundance with hydroxyl group). These polymers can effectively adsorb onto drug particle surfaces that lead to steric stabilization of the system by providing a stable thermodynamic barrier surrounded the particle surface, which retarded particle growth(23,24).

PX-407 is an amphiphilic, linear tri-block copolymer comprised of the hydrophobic central segment of polypropylene oxide (PPO) and two hydrophilic side segments of polyethylene oxide(PEO). The hydrophobic PPO segment has driven the adsorption of polymer onto the drug particle surface. At the same time, the hydrophilic PEO chains surround the particles and provide steric hindrance, thus prevent particle aggregation and growth(25).

Despite this advantage and unique nature of PX-407, but is ineffective in producing SLPNS even at high concentrations. Their adsorption onto drug particle surface depends on physisorption; if hydrophobic- hydrophilic forces are not strong enough for surface adsorption, they cannot overcome the attractive force between two-particles that become dominant due to depletion of stabilizer between the gap of particles(25,26).

It was observed that with an increasing concentration of PVA and HPMC E15. There was a dramatic reduction in P.S (p<0.05). The P.S of the formulas F1 and F3 (which contain drug: polymer ratio of 1:1) was 541.6 nm and 467 nm, respectively, compared to 439.6 nm and 181.6 nm for F2 and F4, which contain drug: polymer ratio of 1:2. This result could be explained based on that with increasing stabilizer concentration, the surface tension will decrease, which facilitates the adsorption of stabilizer on the surface of the newly formed nanoparticle so prevent particle growth and recrystallization by providing steric stabilization. Otherwise, at low concentration, the stabilizer was ineffective in providing sufficient stability to the system(27).

While the P.S of formula F6 (73.2 nm) stabilized by Soluplus® and prepared at 1:2 drug: polymer ratio was not significantly reduced.
compared with the formula F5 (74.6 nm) that prepared at a drug: polymer ratio of 1:1. This result indicates that once the stabilizer concentration is efficiently covering newly generated surfaces, a further increase in stabilizer concentration did not significantly affect the P.S and PDI\(^{(28)}\).

**Figure 1.** Effect of polymer types and concentration on the P.S of NS formulation.

**Effect of the addition of co-stabilizer on the P.S**

The liquid solvent antisolvent precipitation method was used to formulate SLPNS in the presence of stabilizer alone (PVA, HPMC E15, Soluplus®, and PX-407) and in combination with co-stabilizer (tween-80, which is a nonionic surfactant that sterically stabilized the system) to study the effect of polymer-surfactant interaction on the P.S as shown in Figure (2).

The P.S of the formula F2 (439.6 nm) that contains PVA as the primary stabilizer was significantly reduced (p<0.05) with the addition of co-stabilizer (tween-80) as seen in formula F9 (P.S is 297 nm). This means there is an efficient surface affinity for this combination (PVA and tween-80), which could form a stable thermodynamic barrier at the drug particle surface interface, thus delay particle growth\(^{(29)}\).

While the P.S of the formula F4 (181.6 nm), F6 (73.2 nm), and F8 (3835.3 nm) that stabilized by HPMC E15, Soluplus® and PX-407 respectively increased with the addition of tween-80 as shown with F10 (453.6 nm), F11 (83.7 nm), and F12 (4356 nm). This result indicates that this combination was inappropriate for SLPNS. Co-stabilizer addition might retard the interaction between the drug and stabilizer, increasing the P.S of nanosuspended particles\(^{(29)}\).

**Figure 2.** Effect of the addition of co-stabilizer on the P.S of NS formulation.

**Effect of the stirring speed on the P.S**

Two different stirrer speeds, 500 and 1000 rpm were used to prepare the formulas (F2, F4, F6, F8, and F13- F16, respectively) to study the stirring rate's impact on the P.S of SLPNS as shown in Figure 3. All these formulas were prepared at a drug: stabilizer ratio of 1:2.

The P.S of the formula F15 (47 nm) and F16 (3656 nm), which were stabilized by Soluplus® and PX-407 respectively, and prepared at 1000 rpm was significantly reduced (p<0.05) compared to formula F6 (73.2 nm) and F8 (3835.3 nm) that stabilized by the same polymer but at lower stirring speed (500rpm). The high, stirring rate will create high shear stress on the particles, which leads to the breakdown of these particles into smaller ones. In contrast, the P.S of formulas F13 (632nm) and F14 (603) that stabilized by PVA and HPMC E15 respectively was significantly increase (p>0.05) compared to the formulas F2 (439.6 nm) and F4 (181.6 nm) that stabilized by the same polymer but at a lower stirrer speed of 500 rpm. Although the high stirring speed ensures rapid nucleation and breakdown of large particles, this robust mechanical stirrer may provide the system with excessive energy that may overcome the barrier created by the stabilizer and initiates the collision between particles causing increased the P.S of the formulation\(^{(30,31)}\).

**Figure 3.** The effect of stirring speed on the P.S of NS formulation.
Percent of drug content in SLPNS

The drug content in the NS formulas (which stabilized by Soluplus® as a primary stabilizer) that have P.S under 100 nm was found to range from 91.1- 94.59%, as illustrated in Table (3). The high drug loading was one of NS’s advantages because this drug delivery system is free from any carrier system(32).

Table 3. Percent Drug Content of SLPNS with Soluplus®

| Formula | Percent of drug content |
|---------|-------------------------|
| F5      | 93.4± 0.27              |
| F6      | 94.59± 0.1              |
| F11     | 91.16± 0.7              |
| F15     | 91.69± 0.4              |

In vitro dissolution study of SLPNS

The in vitro dissolution test was performed for the pure drug and NS formulas that have P.S under 100 nm in phosphate buffer (pH 6.8) to simulate the in vivo release in the intestine, as shown in Figure 4 (33).

Figure 4. The dissolution profile of the pure drug and SLPNS formulas in phosphate buffer pH 6.8 at 37± 0.5 ℃.

A comparison between the dissolution profiles of SLP NS formulas and pure drug (which was used as a reference) was made using the similarity factor, $f_2$. The similarity factor value falls between 0 and 100, and two dissolution profiles are considered to be similar when $f_2$ is greater than 50(17). While the result of the comparison between the NS formulas and the pure drug was less than 50, as seen in Table (4), this indicates there is a dissimilarity between the dissolution profile of SLPNS and pure SLP powder.

Table 4. Similarity Factor $f_2$ for SLPNS Formula

| Formula name | $f_2$ in phosphate buffer pH6.8 |
|--------------|---------------------------------|
| F5           | 26.08                           |
| F6           | 24.04                           |
| F11          | 19.19                           |
| F15          | 17.81                           |

F15 shows superior in vitro performance (complete drug dissolved within 60 minutes) compared to other NS formulas. So it was selected as the optimal formula.

The enhancement in the SLPNS dissolution rate could be explained based on the Noyes Whitney equation that states with reducing the P.S, especially to the nanoscale range, the surface area will be increased, which results in enhancement of dissolution velocity. The amphiphilic stabilizer (Soluplus®) could also improve the surface wettability of the poorly water-soluble drug, thus leading to a faster release of drugs through NS formulation when compared to pure SLX powder(34).

Determination of saturation solubility

Saturation solubility of pure SLP in D.W was found to be 4.2±0.07 mg/ml while in the case of lyophilized NS (for the optimal formula F15), it was found to be 574.4±0.03 mg/ml. Around 136 folds increased in saturation solubility was observed in SLP lyophilized NS.

The higher solubility of SLP in lyophilized NS could be explained based on the Ostwald-Freundlich equation. The surface area will be increased by reducing the P.S of the drug to the nanoscale, enhancing the SLP 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(2,35).

Fourier transforms infrared spectroscopy (FTIR)

FT-IR spectra for pure SLP, Soluplus®, mannitol, and lyophilized SLP powder of the selected formula (F15) were carried out to investigate any possible interaction among different components, as seen in Figures (5-8).

The characteristic bands of structural moieties of SLP are shown in table (5) (36).

Table 5. The FT-IR absorption bands of SLP.

| Band assignment | Wavenumber (cm$^{-1}$) |
|-----------------|-----------------------|
| N-H stretching  | 3360-3053 (as multiple bands) |
| aromatic stretching C-H | 3024 |
| aliphatic stretching C-H | 2988 cm$^{-1}$ - 2872 |
| C=O stretching  | 1724                  |
| C=C ring stretch | 1556, 1477, 1350      |
| C=N stretching  | 1477                  |
| SO2 stretching  | 1350                  |
| C-N stretching  | 1240                  |
| C-O-C stretching| 1124                  |

While the FTIR spectrum of Soluplus® displays the characteristic peak of a hydroxyl group at 3437 cm$^{-1}$ (O-H stretching). Peaks at 1741 cm$^{-1}$ and 1633 cm$^{-1}$ are attributed to C=O stretching of ester group and tertiary amide group, respectively.
In the lyophilized powder, an intermolecular hydrogen bond may be formed between the Carbonyl group of SLP and hydroxyl group of Soluplus®, which results in a shift of the characteristic peaks. The peak assigned to C=O stretching was shifted from 1724 cm\(^{-1}\) to 1735 cm\(^{-1}\) and became less intense\(^{32}\). Also, the N-H peak may be overlapped with the O-H peak of mannitol, forming a band at 3280 cm\(^{-1}\).\(^{37}\)

![Figure 5. Fourier transforms infrared spectroscopy (FTIR) of pure SLP.](image)

![Figure 6. Fourier Transforms Infrared Spectroscopy (FTIR) of Soluplus®.](image)
Conclusion
SLPNS exhibited improvement in the dissolution rate of poorly soluble SLP, which may be attributed to increasing the surface area due to decreasing the P.S to the nanometer range.

Reference
1. Zhang J, Xie Z, Zhang N, Zhong J. Nanosuspension drug delivery system: preparation, characterization, postproduction processing, dosage form, and application. Nanostructures for Drug Delivery. Elsevier Inc.; 2017. 413–443.
2. Wang H, Xiao Y, Wang H, Sang Z, Han X, Ren S, et al. Development of daidzein nanosuspensions: preparation, characterization, in vitro evaluation, and pharmacokinetic analysis. Int J Pharm. 2019;566:67–76.
3. Calleja P, Irache JM, Zandueta C, Martínez-Oharriz C, Espuelas S. A combination of nanosystems for the delivery of cancer chemoimmunotherapeutic combinations: 1-
Methyltryptophan nanocrystals and paclitaxel nanosuspensions. Pharmacol. Res. 2017;126:77–83.

4. Guo L, Kang L, Liu X, Lin X, Di D, Wu Y, et al. A novel nanosuspension of androgapholide: preparation, characterization and passive liver target evaluation in rats. Eur J Pharm Sci. 2017;104:13–22.

5. Alshweiat A, Katona G, Csóka I, Ambrus R. Design and characterization of loratadine nanosuspension prepared by ultrasonic-assisted precipitation. Eur J Pharm Sci. 2018;122:94–104.

6. Zhao X, Wang W, Zu Y, Zhang Y, Li Y, Sun W, et al. Preparation and characterization of betulin nanoparticles for oral hypoglycemic drug by antisolvent precipitation. Drug Deliv. 2014;21(6):467–479.

7. Duggan ST, Keam SJ, Burness CB. Selexipag: a review in pulmonary arterial hypertension. Am J Cardiovasc Drugs. 2017;17(1):73–80.

8. Scott LJ. Selexipag: first global approval. Drugs. 2016;76(3):413–418.

9. Kaufmann P, Hurst N, Astruc B, Dingemanse J. Absolute oral bioavailability of Selexipag, a novel oral prostacyclin IP receptor agonist. Eur J Clin Pharmacol. 2016;73(2):151–156.

10. Australian Government department of TGA. Australian public assessment report for selexipag. TGA. 2016.

11. European Medicines Agency. Uptravi, International non-proprietary name: Selexipag. Vol. 44. 2016.

12. Shariare MH, Sharmin S, Jahan I, Reza HM, Mohsin K. The impact of process parameters on carrier free paracetamol nanosuspension prepared using different stabilizers by antisolvent precipitation method. J Drug Deliv Sci Technol. 2018;43:122–128.

13. Liu D, Xu H, Tian B, Yuan K, Pan H, Ma S, et al. Fabrication of carvedilol nanosuspensions through the anti-solvent precipitation-ultrasonication method for the improvement of dissolution rate and oral bioavailability. AAPS PharmSciTech. 2012;13(1):295–304.

14. Santos AM, Meneguin AB, Fonseca-Santos B, Souza MCP, Ferreira LM, Sábio RM, et al. The role of stabilizers and mechanical processes on physico-chemical and anti-inflammatory properties of methotrexate nanosuspensions. J Drug Deliv Sci Technol. 2020;57:101638p.

15. Qureshi MJ, Phin FF, Patro S. Enhanced solubility and dissolution rate of clopidogrel by nanosuspension: formulation via high pressure homogenization technique and optimization using box behken design response surface methodology. J Appl Pharm Sci. 2017;7(2):106–113.

16. Jassem NA, Rajab NA. Formulation and in vitro evaluation of azilsartan medoxomil nanosuspension. Int J Pharm Pharm Sci. 2017;9(7):110–119.

17. Zuo J, Gao Y, Bou-Chacra N, Löbenberg R. Evaluation of the DDSolver software applications. Biomed Res Int. 2014;2014:1–9.

18. Oktay AN, Karakucuk A, Ilbasmis-Tamer S, Celebi N. Dermal flurbiprofen nanosuspensions: optimization with design of experiment approach and in vitro evaluation. Eur J Pharm Sci. 2018;122:254–263.

19. Na YG, Pham TMA, Byeon JJ, Kim MK, Han MG, Baek JS, et al. Development and evaluation of TPGS/PVA-based nanosuspension for enhancing dissolution and oral bioavailability of ticagrelor. Int J Pharm. 2020;581:119287.

20. Wang R, Yang M, Li G, Wang X, Zhang Z, Qiao H, et al. Paclitaxel–betulinic acid hybrid nanosuspensions for enhanced anti-breast cancer activity. Colloids Surfaces B Biointerfaces. 2019;174:270–279.

21. Ali AH, Abd-Alhammid SNA-A. Enhancement of solubility and improvement of dissolution rate of atorvastatin calcium prepared as nanosuspension. Iraqi J Pharm Sci. 2019;28(2):46–57.

22. Yang H, Teng F, Wang P, Tian B, Lin X, Hu X, et al. Investigation of a nanosuspension stabilized by Soluplus® to improve bioavailability. Int J Pharm. 2014;477(1–2):88–95.

23. Mishra B, Sahoo J, Dixit PK. Enhanced bioavailability of cinmarizine nanosuspensions by particle size engineering: optimization and physicochemical investigations. Mater Sci Eng C. 2016;63:62–69.

24. Tuomela A, Liu P, Puranen J, Rönkkö S, Laaksonen T, Kalesnykas G, et al. Brinzolamide nanocrystal formulations for ophthalmic delivery: reduction of elevated intraocular pressure in vivo. Int J Pharm. 2014;467(1–2):34–41.

25. Tuomela A, Hirvonen J, Peltonen L. Stabilizing agents for drug nanocrystals: effect on bioavailability. Pharm MDPI AG. 2016;8(2):1–16.

26. Jassim ZE, Hussein AA. Formulation and evaluation of Clopidogrel tablet incorporating drug nanoparticles. MSC.thesis. The College of Pharmacy/University of Baghdad; 2014.

27. Hussein AA, Alhammid SNA. Enhancement of solubility and dissolution rate improvement of Atorvastatin calcium using nanosuspension technique. MSC.thesis. The College of Pharmacy/University of Baghdad in; 2019.

28. Sharma S, Verma A, Pandey G, Mittapelly N, Mishra PR. Investigating the role of Pluronic-g-Cationic polyelectrolyte as functional stabilizer for nanocrystals: Impact on Paclitaxel oral
bioavailability and tumor growth. Acta Biomater. 2015;26:169–183.
29. Rajab NA, Jassem NA. A design and in vitro evaluation of Azilsartan medoxomil as a self-dispersible dry nanosuspension. Der Pharm Sin. 2018;9(1):12–32.
30. Rao MRP, Bajaj A. Study of effect of variables on particle size of telmisartan nanosuspensions using Box-Behnken design. Drug Res. 2014;64(12):663–667.
31. Rashid AM, Abd-Alhammid SN. Formulation and characterization of itraconazole as nanosuspension dosage form for enhancement of solubility. Iraqi J Pharm Sci. 2019;28(2):124–133.
32. Shekhawat P, Pokharkar V. Risk assessment and QbD based optimization of an Eprosartan mesylate nanosuspension: in-vitro characterization, PAMPA and in-vivo assessment. Int J Pharm. 2019;567:118415.
33. Center for drug evaluation and research. Application number: 207947 orig 1s00 chemistry review(s). 2015.
34. Kilor V, Sapkal N, Daud A, Humne S, Gupta T. Development of stable nanosuspension loaded oral films of glimepiride with improved bioavailability. Int J Appl Pharm. 2017;9(2):28–33.
35. Singh MK, Pooja D, Ravuri HG, Gunukula A, Kulhari H, Sistla R. Fabrication of surfactant-stabilized nanosuspension of naringenin to surpass its poor physiochemical properties and low oral bioavailability. Phytomedicine. 2017;40:48–54.
36. Rao DR. Pharmaceutical composition comprising amorphous Selexipag. World Intellectual Property Organization International Bureau. WO 2018/078383 Al, 2018.
37. Patel PJ, Gajera BY, Dave RH. A quality-by-design study to develop Nifedipine nanosuspension: examining the relative impact of formulation variables, wet media milling process parameters and excipient variability on drug product quality attributes. Drug Development and Industrial Pharmacy. 2018 ;44 (12): 1942–1952 .