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Factors Affecting Preparation of Repaglinide Nanosuspension

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ABSTRACT

Limited solubility is a major limiting step in drug delivery systems development. This study aimed to develop a stable nanosuspension of repaglinide to improve its dissolution. The Nano-precipitation method was employed using a variant of stabilizer concentration, co-stabilizers and solvents. Soluplus® (SOL) was used as the primary stabilizer for this research, polyvinyl alcohol (PVA), poloxamer 188 (PXM 188), tween 80 (TW80), and polyvinyl pyrrolidine (PVP k30) were investigated as co-stabilizers. Solvents explored were ethanol, acetone and chloroform. The interplay between these factors was assessed by examining particle size, polydispersity index (PDI), FTIR spectroscopy, scanning electron microscope, and drug crystallinity and thermal changes were evaluated to assess the physicochemical properties of the prepared nanosuspensions. All formulations attempted produced nanosuspension of varying size and drug content. The use of Soluplus® alone at relatively higher content resulted in particles of small diameter, (mean size: 82.96 nm±3.95) and a narrow size distribution (PDI=0.100±0.098) and high drug loading (99%). The in vitro dissolution studies affirmed the value of formulating repaglinide as nanosuspension as evident by improved and complete dissolution compared to drug powder. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) confirmed the amorphous nature of optimal RPG nanoparticle. Careful systematic optimization of formulation parameters, including selecting stabilizers and co-stabilizers have a direct impact on nanosuspension particles size and drug content. Soluplus® also is superior to other co-stabilizer in producing stable nanosuspensions void from aggregation and with high drug loading. The nanosuspension approach successfully improved repaglinide dissolution.

Keywords: Repaglinide, nanosuspension, nano-precipitation, soluplus®, PVP k30, solubilizer, co-solubilize.

INTRODUCTION

Around 40% of newly discovered active pharmaceutical ingredients (APIs) are hydrophobic compounds, and around one-third of the drugs listed in the United States Pharmacopeia (USP) exhibit limited solubility in water. The US Food and Drug Administration (FDA) categorize drugs into four classes based on their aqueous solubility and permeation through biological membranes. These classes are as follows: 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) (Emad,& Abd-Alhammid, 2022; Patravale et al., 2004). The inadequate aqueous solubility is specifically linked to significant problems, such as low or erratic bioavailability, the need for larger doses, and delayed onset of action (Abbas et al., 2017). This became a focus for the pharmaceutical research to enhance the solubility and rate of dissolution of poorly water-soluble drugs. Numerous strategies exist to address the solubility challenges of poorly soluble drugs. These include changing the crystal structure, employing self-emulsification techniques, utilizing solid dispersion methods, enhancing solubility through surfactants, forming salts, adjusting pH levels, engaging in co-crystallization processes, incorporating co-solvents, and employing particle-size reduction technologies (Budiman et al., 2023;

Sakhiya & Borkhataria, 2024; Williams et al., 2013). Reducing particle size is frequently the initial and most straightforward method to improve drug dissolution rates. Solubility of an API is dependent on the resulting particle size. As the particle size decreases, they engage more extensively with the solvent, increasing drug solubility (Kumar et al., 2022; Fadhila et al., 2023). Nanotechnology encompasses scientific and engineering endeavors conducted at the nanoscale, approximately 10^{-9} meters. Nanosuspensions are colloidal dispersions at the submicron level involving API particles in nano-size, stabilized by surfactants. These suspensions typically consist of poorly water-soluble drugs with no matrix substance suspended within the dispersions (Patel & Agrawal, 2011).

The assortment of a convenient technique in preparing nanoparticles depends on the physicochemical features of the utilized stabilizer and the API to be loaded. Repaglinide (RPG) is a chemically distinct compound from sulfonylurea and oral insulin secretagogues. Repaglinide is a treatment of type-2 diabetes mellitus (DM) in adults whose raised blood glucose levels remain insufficiently controlled despite dietary modifications, physical activity, and weight reduction efforts. However, RPG's poor solubility (38 μg/mL at 25°C) and high lipophilic profile (log p=3.97) present challenges for its oral administration, resulting in an absolute bioavailability of only 45–65% (Mandić & Gabelica, 2006). Various studies have attempted to boost the oral bioavailability of RPG by primarily focusing on improving its aqueous dissolution via different approaches, and multiple outcomes have been reported. Some of the attempted approaches are creating RPG-loaded solid lipid nanoparticles using several surfactants/stabilizers (Ebrahimi et al., 2015), preparation and evaluation of RPG nanosuspension using a combination of poloxamer and tween (Jogu, 2020), enhancement of physiochemical properties through adduct formation (Gill & Arora, 2020) and RPG-loaded ethyl cellulose nanoparticles (Lokhande et al., 2013). When administered orally, these approaches were investigated to increase repaglinide dissolution and improve its absorption and bioavailability. In this study, we aimed to boost RPG dissolution through its formulation as a stable nanosuspension using the Nano-precipitation method. The optimum nanosuspension formula is determined by screening different concentration of the solubilizer, Soluplus®, the incorporation of multiple co-stabilizers, and exploiting different solvents.

MATERIALS AND METHODS

Repaglinide (RPG) was acquired from Huainan Lianke Biological Medicine Co. Ltd. in China. Polyvinyl alcohol (PVA) and polyvinyl pyrrolidine k30 (PVP k30), tween 80 (TW80) from China. Poloxamer 407 and 188 (PXM 188) from India. Soluplus® (SOL) from BASF in Germany. All other Solvents and chemicals utilized in the study were of analytical-grade and used without additional refining.

Determination of Repaglinide saturation solubility

RPG saturation solubility evaluation was performed by dissolving excessive amount of the API in a plastic tube, agitated with a water-bath shaker for 48 hours. The API powder underwent continuous agitation with 10 mL in D.W and sodium phosphate buffer (SPHB) 6.8 containing 1% sodium dodecyl sulfate (SDS). After 48 hours, filtration and analysis using UV spectroscopy at its maximum wavelength for each media proceeded. The compound solubility was calculated based on the obtained results (Bashar & Al-Khedairy, 2023). All measurements were conducted in triplicate. Data were stated as mean ± SD.

Preparation of Repaglinide Nanosuspension

RPG nanosuspensions were formulated using the Nano-precipitation method. The solvent phase was prepared by dissolving 1 mg RPG in 1 ml of ethanol. The solvent phase was added drop by drop, using a 1 cc syringe, to a 10 ml aqueous stabilizer solution of the designated stabilizer SOL under different concentrations and a combination of pre-determined ratio of RPG: stabilizer: co-stabilizer, namely: (SOL, SOL-TW80, SOL-PXM188, SOL-PVP, and SOL-PVA) (Abbas et al., 2017). Furthermore, assessment of any possible effect of the solvent on prepared nanoparticles (NPs), acetone and chloroform were utilized to prepare RPG-NPs denoted as (SOLA, SOLC) respectively and compared to with the solvent of choice (ethanol) (Table I). Nanoparticle precipitation transpired swiftly. Following this, the resulting nanosuspensions were stirred on a magnetic stirrer operating at 1000 rpm for 20 minutes to facilitate the evaporation of the organic solvent.

Table I. Composition of repaglinide nanoparticles

No	Formula*	Soluplus® (mg)	PVPK30 (mg)	PVA (mg)	Tween80 (mg)	poloxamer188 (mg)	Solvent**
1	SOL1mg	10					E
2	SOL2mg	20					E
3	SOL3mg	30					E
4	SOL4mg	40					E
5	SOL-PVPK30	30	15				E
6	SOL-PVA	30		15			E
7	SOL-TW80	30			15		E
8	SOL-PXM188	30				15	E
9	SOL3mg	30					A
10	SOL3mg	30					C

*All formulas contain 1 mg RPG; **E= ethanol, A=acetone, C= chloroform.

The desired particle size (mean diameter <200 nm) and size distribution (PDI<0.3) (Abd-Alhammid, 2022) were attained by adjusting the type of co-stabilizer while maintaining a constant ratio of RPG to SOL polymer (Table I).

To reach our optimized formula, Soluplus® (SOL) as a standalone stabilizer and combined with four different co-stabilizers at a pre-specified concentration were used to prepare repaglinide nanosuspensions. Particle size analysis observes the effect of mixing two stabilizers in contrast with using Soluplus® alone and the impact of changing the solvent. The optimal nanoparticle formula selection involved considering particle size, polydispersity index (PDI), and drug loading.

Characterization of Repaglinide Nanosuspension

Determination of Particle Size and polydispersity index (PDI)

Particle size and PDI of nanoparticles were measured using dynamic light scattering technique (DLS) (Malvern Zeta Sizer, Spectris Company, UK). The data obtained were conveyed as mean ± standard deviation (SD) based on triplicate measurements unless otherwise stated (Alwan & Rajab, 2021).

Drug Loading

Nanosuspension samples, each containing 400 mcg of RPG, were forwarded for drug loading analysis. The measurements were conducted using the Amicon® Ultra-4 Centrifugal Filter with a molecular weight cutoff (MWCO) of 10 kDa (Toma, & Abdulrassol, 2021). Accordingly, RPG nanosuspensions were ultracentrifuged at 4000 rpm for 30 min. The concentrate of RPG NPs, after a suitable dilution with methanol, RPG concentration was calculated by measuring the absorbance at a wavelength of 240 nm using a UV-visible spectrophotometer. Drug loading calculations utilized equation (1), and the resulting data were presented as mean ± SD from three independent measures.

$$\text{Drug loading \%} = \frac{(\text{measured RPG concentration})}{(\text{theoretical RPG concentration})} \times 100 \ldots\ldots\ldots \text{(eq1)}$$

***In-Vitro* Dissolution Study**

The dissolution of nanosuspension samples equivalent to 2 mg of RPG, was operated using a USP Type II dissolution test apparatus (Lab India DS- apparatus). Amount of RPG in the nanosuspension samples were adjusting by adjusting nanosuspension volume used according to paired content measurements results. Phosphate buffer, pH 6.8, and 1% SDS (200 ml) were utilized as the dissolution media (Fouad et al., 2023). Samples were conserved at 37 ± 0.5 °C, stirred at 100 rpm. A 0.1 µm filter was attached to the sampling syringe for the duration of the dissolution studies to avoid contamination of samples that may affect readings. Samples of 3 ml were gathered at 5, 10, 15, 30, 45, and 60 min intervals. After each collection, the dissolution medium was substituted with a fresh one of equal volume. The concentration of the dissolved RPG was determined using a Shimadzu UVmini-1240 spectrophotometer at a wavelength of 282 nm(Mandić & Gabelica, 2006). Pure RPG samples were treated similarly. The dissolution experiments and subsequent sample analysis were conducted in three independent measurements.

Fourier Transform Infrared Spectroscopy

An FTIR spectrometer (FTIR-8300 Shimadzu, Japan) was used to record the FTIR spectrum of pure RPG and nanoparticles of designed formulas (as nanosuspension). (FTIR-8300 Shimadzu, Japan), scanning the wave numbers that ranged from 4000 to 400 cm^{-1}. The FTIR analysis was accorded for detection any apparent interaction or complexation between repaglinide and the excipients used in the nanoparticle formulation (Malik & Al-Khedairy, 2023).

Scanning Electron Microscopy (SEM)

Surface morphology of the procured RPG nanosuspensions of the optimized formulae was examined by SEM (INSPECT-F50- FEI Netherlands) conducted with a secondary detector at varying acceleration voltages and magnifications. Nanosuspension samples were prepared by depositing a liquid nanosuspension on the double-sided carbon tape and drying it at room temperature before coating it with gold (Thamer & Abood, 2021).

Lyophilization of repaglinide NPs

Nanosuspension formulas were freeze-dried using a Labconco freeze dryer (USA) after adding 1% w/w mannitol as a cryoprotectant (Areej W. Alhagiesa & Ghareeb, 2021). The sample was deep-frozen in a refrigerator for 24h before undergoing Lyophilization using a vacuum freeze dryer through water sublimation. The duration of this process was 24h. The resulting lyophilized nano-particles were utilized for compatibility studies.

Powder X-ray Diffraction

This procedure included evaluation of the crystal lattice of the pure RPG and lyophilize of optimum RPG nanosuspension using powder X-ray diffraction (DX2700BH, China). Measurements were carried out using a Cu Kα filter at a voltage of 40 kV and a current of 30 mA. The scanning was conducted at a 2θ range of 5 to 80°, utilizing a step size of 1.5406 Å (Jassim & Hussein, 2014).

Differential Scanning Calorimetry (DSC)

The study of thermal changes of pure RPG and lyophilize of RPG optimal formula were evaluated using automatic thermal analyzer equipment (setram.Evol31, France). Each sample (5 mg) was heated at a rate of 5°C per minute throughout a temperature range of 0°C to 30°C in an aluminum pan that was hermetically airtight sealed. The analysis was conducted under atmospheric flow conditions (Dawood et al., 2018).

Statistical analysis

The results for particle size were recorded as average values based on the device settings, while most other data were presented as mean samples ± standard deviation (SD). These results were statistically analyzed according to one-way analysis of variance (ANOVA) using Graph-Pad Prism 20 software, at which significant results were of ($p<0.05$) and non-significant ($p>0.05$).

RESULTS AND DISCUSSION

This research examined the probability of improving RPG dissolution via nanoparticle technology. The outcome of saturation solubility of repaglinide in (SPHB) (pH 6.8) with 1% w/v SDS was revealed to be (91.66 ± 10) mcg/ml and in D.W (37.67±2.5), labelling RPG as a practically insoluble API as stated in US Pharmacopeia. Repaglinide is an API of Class II drugs that has low aqueous solubility and good absorption through the gastrointestinal tract due to the elevated lipophilicity and consequently permeability (Mandić & Gabelica, 2006). This study focused on employing SOL alone at varying concentrations, explored addition of an extra stabilizer to boost RPG dissolution via nanoparticle formulation. Particle size for formulations prepared in this study was within the Nano-size range (143.23nm ± 30 - 61nm ± 2.6), with PDI that varied from (0.086 ± 0.013 to 0.257 ± 0.135), indicating narrow size distribution. The overall size, PDI, and drug content outcomes highlighted some formulations with more substantial potential than others did.

Effect of Soluplus® concentration on particle size

Formulating the drug as small-sized nanoparticles can increase its effective surface area, thereby improving its dissolution. The PDI indicates the uniformity of particle size distribution throughout the nanosuspension. A PDI value ranging between 0.1 and 0.25 indicates a narrow size distribution. In contrast, a PDI 0.5 indicates a broad size distribution (Ali & Abd-Alhammid, 2019; Hamed & Hussein, 2020). For particles prepared using SOL alone, elevations in the stabilizer concentration resulted in smaller particle size and better size distribution (Figure 1. A-B). SOL3 had desirable particle size and narrow size distribution (82.96 ± 3.95 nm, 0.100 ± 0.098), respectively, SOL4 only showed a marginal, almost a trivial change in these measures (81.66 ± 6.50 nm, 0.108 ± 0.093).

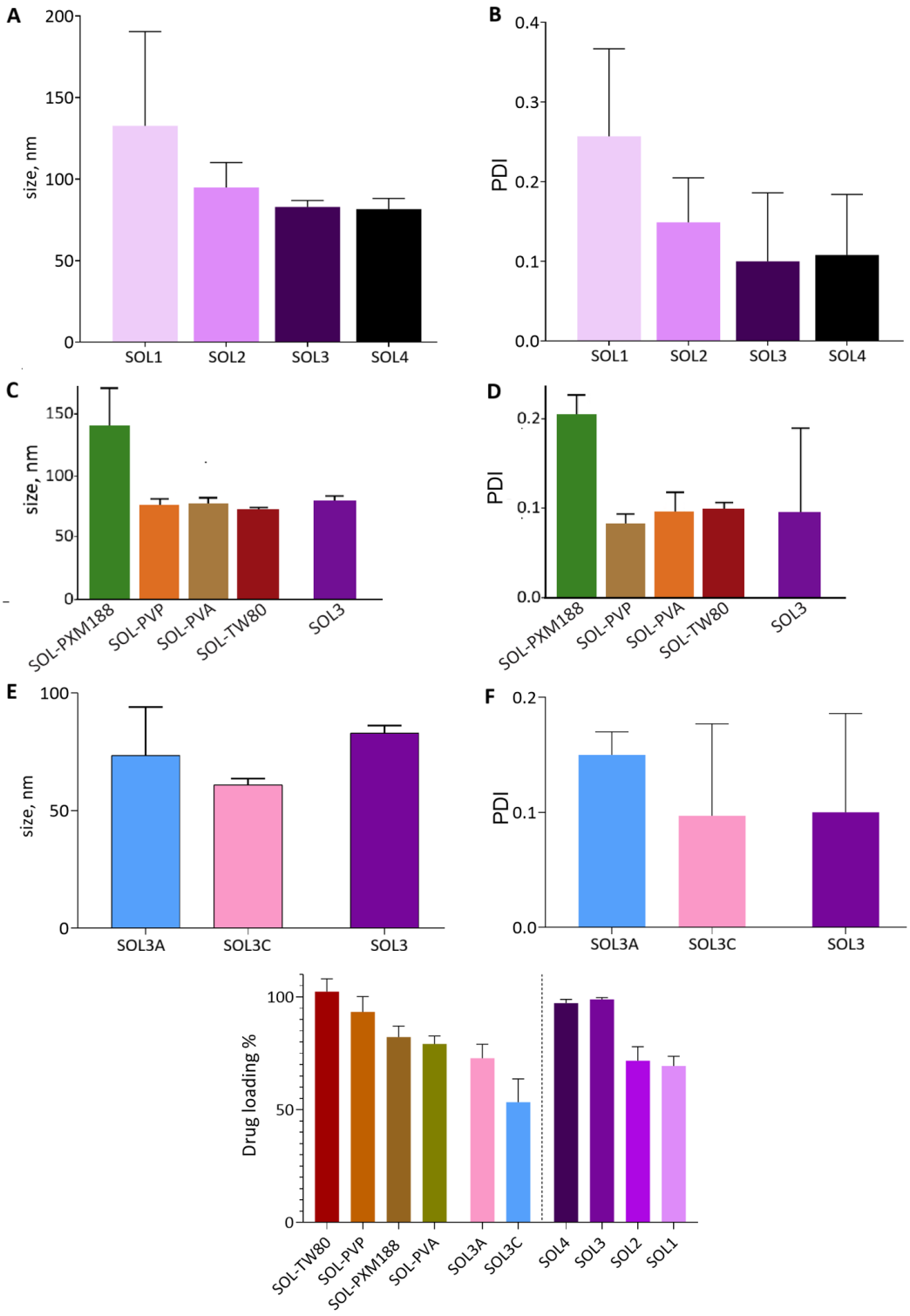

Figure 1. RPG NP characterization on the left representation particle size and PDI; A-B. Represents the effect of concentration. C-D. Compare data on utilizing one vs. two stabilizers. E-F. Effect of different solvents. Data presented as mean± STD, n=3. Lower panel presents drug loading in RPG NPs. Data are presented as mean ±STD, n=3.

Consequently, we speculated that a further increase in SOL would not significantly change particle size. Soluplus® concentration was found to significantly affect formulated particle size and PDI. SOL is an amphiphilic, water-soluble graft-copolymer composed of both hydrophilic and lipophilic components. It inhibits drug precipitation by restraining drug nucleation and crystal growth (Attia et al., 2023). Additionally, Soluplus® can decrease the interfacial tension of nanosuspension particle surfaces (Mohammed & Alhammid, 2024) by providing water-surfactant interactions, which results in smaller particle sizes, demonstrating the effectiveness of higher polymer amounts (Thamer & Abood, 2021). A hydrophobic interaction between RPG and Soluplus® occurs due to the presence of amphiphilic groups, such as polyethylene glycol (hydrophilic) and vinyl caprolactam/vinyl acetate (hydrophobic) (Gumaste et al., 2016; Attia et al., 2023). These groups enhance surface activity and reduce interfacial tension, manifesting complete surface coverage and steric stabilization of the nanosuspension. As a result, as polymer concentration increases, the particle size decreases, and the monodispersed system is structured, as evidenced by the smaller particle size and PDI values (Emad & Abd-Alhammid, 2022).

Effect of adding a co-stabilizer on particle size

Upon the addition of co-stabilizers PVPK30, PVP, and tween80, diminutive reduction in particle size was obtained. However, PXM188 resulted in larger particles than all other formulations attempted (Figure 1. C-D). The repercussions of a co-stabilizer addition to the primary stabilizer can create a mix of steric and electrostatic stabilization mechanisms, formulating a nanosuspension depending on the type of added surfactant/stabilizer. SOL3 was chosen to be incorporated with Tween 80, PVPK30, PVA, and PXM188. Tween 80, a nonionic surfactant, helps stabilize the formulation and maintains uniform particle distribution (Ali & Abd-Alhammid, 2019; Hamed & A. Hussein, 2020). Although its addition gave a stable monodispersed nano-system with an excellent nanoparticle loading capacity, it was not notably different from SOL3 nanosuspensions. Polymeric nonionic stabilizers (PVP K30, PXM188) have amphiphilic segments that improve the wetting of RPG nanoparticles. This means that the nanoparticles are better dispersed in the solvent, which helps maintain a uniform suspension and prevents aggregation. When incorporated into nanosuspensions, non-ionic stabilizers adhere to drug particle surfaces through a strongly interacting anchor component while the solvated tail extends into the surrounding medium (Kulshreshtha et al., 2009). Hence, steric stabilization occurs due to adsorbed polymer chains extending into the dispersion medium, providing a repulsive force that keeps particles separated. The amphiphilic nature of these polymers, having both hydrophilic and hydrophobic segments, enhances this effect by improving the dispersion of nanoparticles in the medium (Müller et al., 2001). While the PVPK30-SOL3 combination imposed no significant change to SOL3 results, an interesting observation upon SOL3-PXM188 creation was particle size, and PDI increased compared to SOL3 results. Hypotheses for these results were either solubilization of RPG, which resulted in particle aggregation caused by Ostwald ripening (Attia et al., 2023; Weng et al., 2020), or an increase in the viscosity of the dispersion medium, making it an ineffective combination that cannot stabilize the system (Hamed & A. Hussein, 2020). Poloxamers are block copolymers known for forming gels at certain concentrations. At the same time, Soluplus®, as mentioned previously, is a graft co-polymer and can increase the viscosity due to its polymeric nature. Elevated viscosity can impede the free movement of nanoparticles (Müller et al., 2001) and reduce the kinetic energy of the particles, preventing them from remaining adequately dispersed and making it difficult to maintain a stable suspension. The stabilization provided by adding PVA to SOL3 is explained by its ability to form hydrogen bonds with drug particles, facilitated by the abundance of hydroxyl groups in its chemical structure. Additionally, PVA efficiently adsorbs onto drug particle surfaces, contributing to steric stabilization of the system. This adsorption leads to the formation of a stable thermodynamic barrier surrounding the particle surface, effectively impeding particle growth.

Solvents effect of on particle size

Acetone and chloroform were utilized as an alternative to ethanol as the organic solvents of RPG in the study to assess the effect of solvent choice on our optimized nanosuspension-stability, (SOLA) and (SOLC), respectively. They produced small sized particles (73.5nm±20.5 to 61nm±2.6) nm and had a narrow polydispersity index of (0.14±0.028 to 0.097±0.086) (Figure 1.E-F).

Ethanol, acetone, and chloroform are all organic solvents with varying features. These features affect the formulation of nanosuspensions and the produced particle size PDI, and loading of API molecules. Factors such as the solvent polarity, surface tension, miscibility with anti-solvent and solubility of RPG can affect the formed nanoparticles. Ethanol is a polar solvent, an excellent solvent of RPG, and is highly miscible with the anti-solvent (water). Depending on the choice of used polymer, it produced stable, monodispersed RPG nanosuspensions with appropriate particle size, PDI and loading capabilities (Khoza et al., 2012; Müller et al., 2001). This and its availability and safety profile made it the solvent of choice. Acetone has intermediate polarity and miscibility with (water) the anti-solvent. Acetone was employed as an alternative to ethanol in reproducing SOL3, resulting in a smaller nanoparticle size. Chloroform is less polar than acetone, leading to the formation of larger nanoparticles. Although RPG has good solubility in both of them, their safety profiles, toxic fumes, and poor loading of RPG molecules led us to proceed with our research with ethanol (Bose et al., 2013).

Formualtion parameters effect of drug loading

Drug loading in the resulted nanosuspensions must be considered as an element in optimization of RPG nanoparticles. Drug loading for created formulas in this study spanning the range from (53.5% ± 10.2 to 102.4%±5.68) (Figure 1). Drug loading for SOL3 formula was most convenient among the single stabilizer nanoparticles. It was noticed that the loading capacity of the SOL particles statistically different increased upon elevating SOL concentrations SOL1 loaded only (69.4%±5.2) of RPG, while SOL3 loaded (98.9% ±0.95). For co-stabilizer formulas, SOL-Tween80 nanoparticles had the highest drug content among other co-stabilizers used, in addition, SOLA and SOLC gave a disappointing loading capacities in comparison to its analogue SOL3 (Figure 1).

Nanosuspension mediated In vitro dissolution

Based on the above outcomes, three formulations were furtherly evaluated for the in-vitro dissolution of RPG. The in-vitro dissolution study showed improved repaglinide dissolution in the nanosuspension formulations, where complete dissolution was recorded within 30 min for the nanosuspensions compared to a maximum of 74.3% ± 4.6% of pure RPG by the end of the 60-minute study (Figure 2). Potential improvement in RPG dissolution in dosage forms prepared with the nanosuspension compared to pure RPG. Several factors contribute to this enhanced dissolution. The smaller particle size significantly increases the specific surface area of the particles. Additionally, the reduction in particle size decreases the diffusion layer thickness surrounding the drug particles, thereby increasing the concentration gradient (Li et al., 2020; Patnaik et al., 2016; Pignatello & Corsaro, 2019). Soluplus® decreases the interfacial tension on the surface of nanosuspension particles by providing a water–surfactant interaction, which contributes to the smaller particle size. The hydrophobic interaction between RPG and Soluplus®, where the hydrophilic segment extends into the aqueous phase, also explains these results. This interaction reduces interfacial tensions, resulting in complete surface coverage and steric repulsion. This marked enhancement can be attributed to the excellent wettability and micellar solubilization propertiesof Soluplus® (Gadadare et al., 2015; Pignatello & Corsaro, 2019). SOL3-TW80, SOL3-PVPK30, and SOL3 similarity in terms of particle size and PDI and loading capacity is reflected when comparing the results of in-vitro release, no significant difference in release profiles was observed; regardless, in SOL3-TW80, Soluplus® formed a steric barrier around the drug particles, while Tween 80 provided electrostatic stabilization. PVPK30 amphiphilic segments that suggest steric stabilization act in a similar manner to SOL moiety (Müller et al., 2001). This dual stabilization prevents particle growth and aggregation, maintaining a stable nanosuspension with consistent release characteristics compared to the pure RPG release profile (Kocbek et al., 2006).

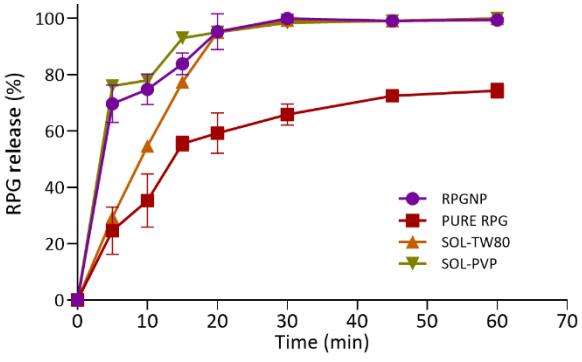

Figure (2). In vitro dissolution of repaglinide nano-suspensions vs. pure drug phosphate buffer pH 6.8, SDS 1%. Data are presented as mean ± STD, n = 3

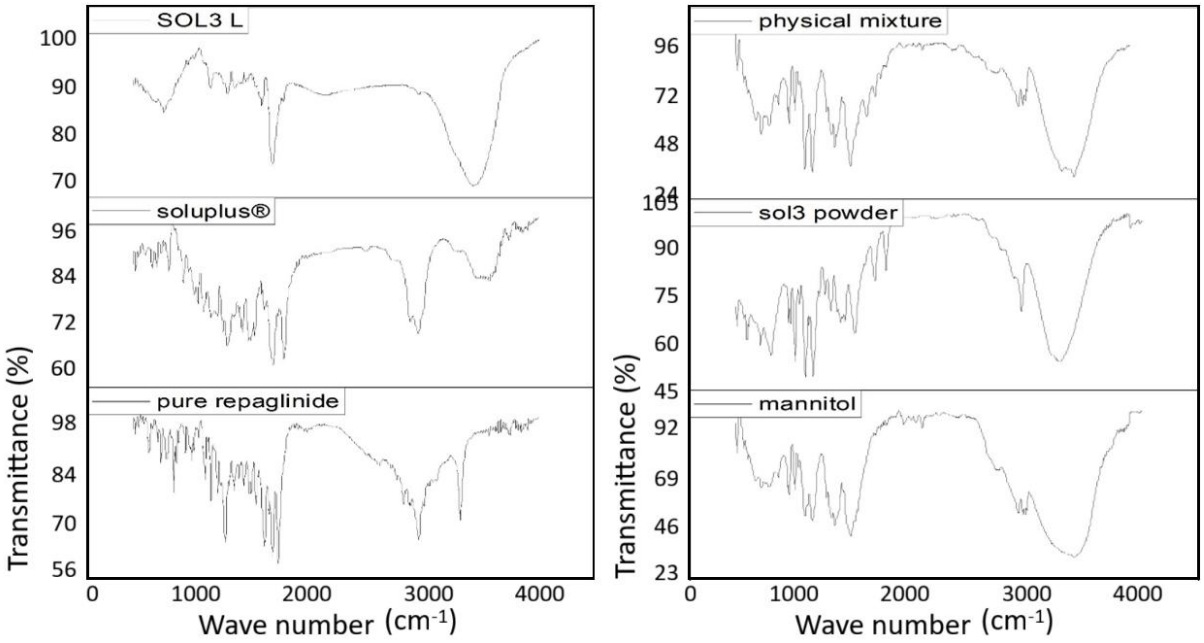

Figure 3. FTIR of RPG nanosuspension, freeze dried NPs, associated physical mixture and neat drug and additives

FTIR spectroscopy of RPG nanoparticle

The recording and comparison between of FTIR spectrum of the nanoparticles the pure repaglinide spectrum investigated possible drug-polymer interactions. The pure RPG spectrum exhibited peaks at 3306 cm^{-1} (NH stretching), 2931.8 cm^{-1} (CH stretching), and 1685 cm^{-1} (C=O stretching). Additionally, bands at 1037 cm^{-1} and 1219 cm^{-1} were observed, related to C–O stretching in the phenyl alkyl ether structure. The bands at 1566 cm^{-1} and 1635 cm^{-1} correspond to aromatic C=C and N–H bending, respectively. In the nanoparticle spectrum, broadband is observed in place of the band at 3306 cm^{-1}, corresponding to the NH stretching. The wavelength range in which this peak appears corresponds to the literature values for an alkyl stretch (C-H stretch) and C-O stretch, respectively; these outcomes with the bond formed between the carboxylic moiety of repaglinide and the hydrogen bond-forming atom present in the -CO of Soluplus® (Figure 3). SOL3, SOL3-PVPK30, and SOL3-TW80 exhibited changes with the addition of new peaks, C-H stretching vibrations, and C-O stretching vibrations. The presence of the poly-oxy ethylene chain in Tween 80 is reflected in the spectrum. C=O stretching vibrations, N-H stretching vibrations, and C-H stretching vibrations belonging to PVPK30 (Gill & Arora, 2020; Yang et al., 2016).

Surface morphology of RPG nanoparticles

Surface morphology scanning was conducted for formulated nanoparticles to ascertain proper nanoparticle formation (Figure 4). SEM images of repaglinide particles exhibited a refined morphology with smoother edges and a spherical structure, further confirming successful nanoparticle formulation.

DSC/XRD analysis of RPG nanoparticle

PXRD diffraction analysis illustrated that while the nanoparticle formulation contributed to the loss of RPG crystalline peaks, physical mixtures maintained it (Figure 5). The DSC thermograms, (Figure 5) support the findings of the PXRD analysis, which displays the thermogram of pure RPG, which exhibited a sharp melting peak at 138°C, corresponding to the crystalline lattice of RPG. In contrast, this peak was absent in the thermogram of the nanoparticles.

The PXRD pattern of pure repaglinide exhibited characteristic high-energy diffraction peaks at two theta values between 9° and 40°, indicating its crystalline structure (Ghadhban & Ahmed, 2024). The differences observed between pure repaglinide and repaglinide nanoparticles primarily lie in peak intensities. The variations in the relative intensities of their peaks may be attributed to reduced crystallinity in the nanoparticles.

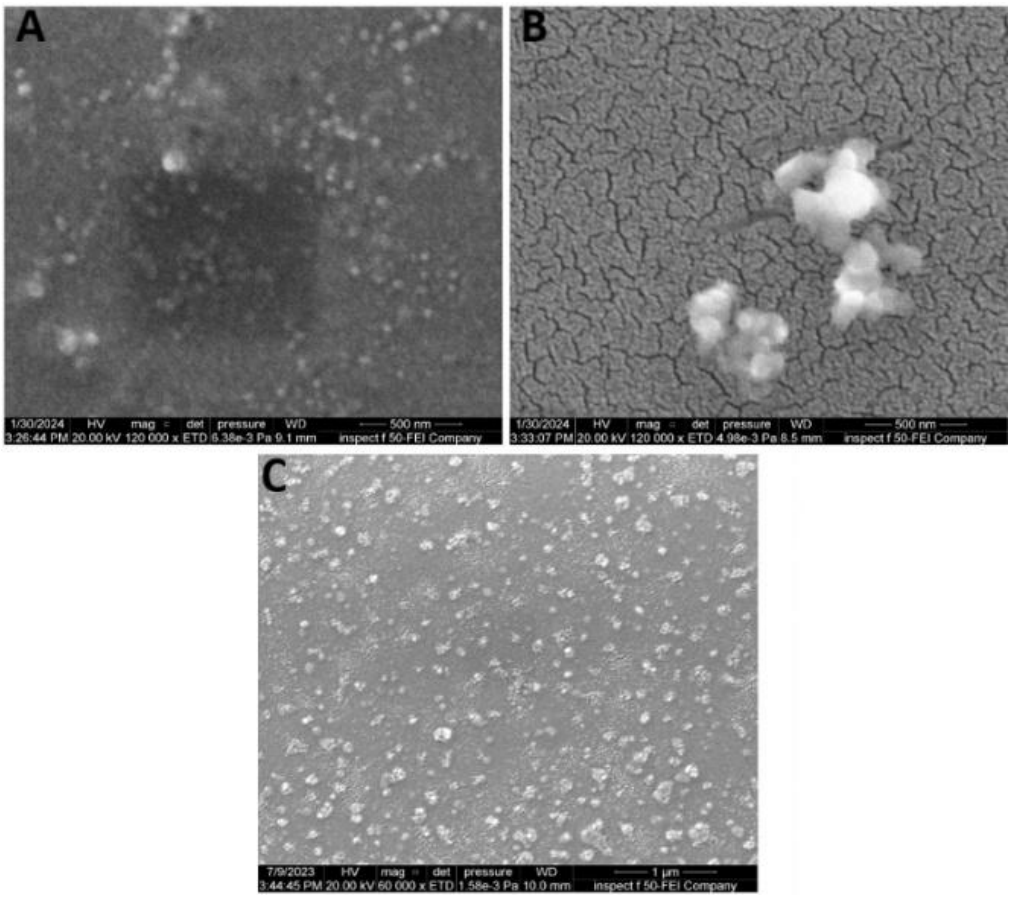

Figure 4. SEM imaging of RPG NPs. A. SOL-TW80. B.SOL-PVPK30. C.SOL3.

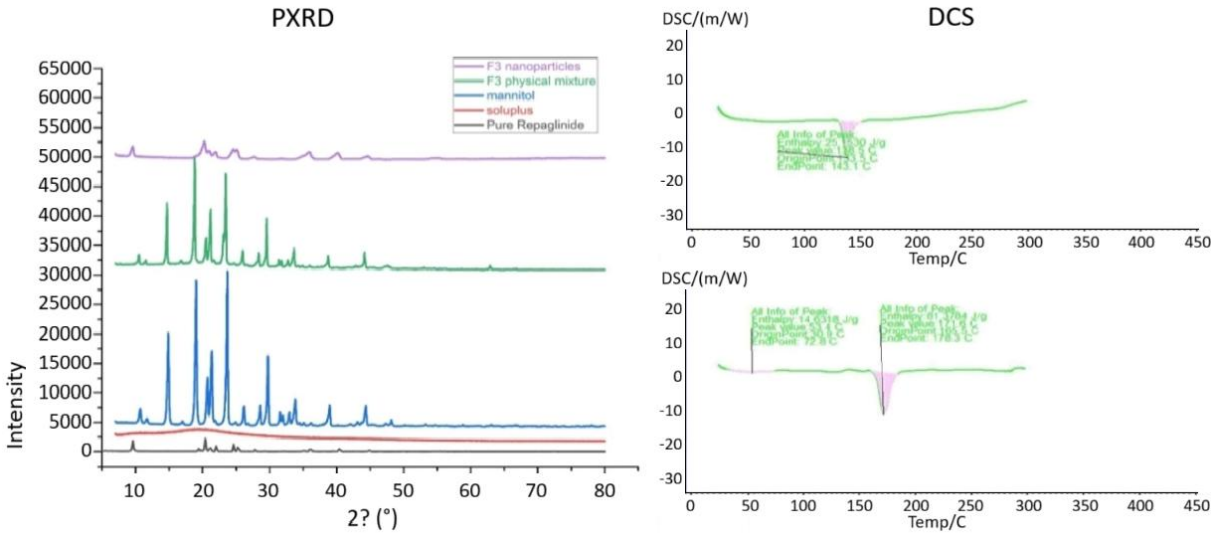

Figure (5): Validation of drug conversion to amorphous status via PXRD (left) of lyophilized RPG NPs and their composition and DSC (right) Top. pure RPG and bottom. SOL3 RPG NPs.

Additionally, mannitol used during the freeze-drying process exhibited high-energy diffractions at two theta values between 9° and 45°, masking the characteristic diffraction peaks of repaglinide in the freeze-dried formulation. This absence indicates a probable SOL-mediated amorphous transformation of the previously crystalline RPG particles. DSC thermograms agreed with these results.

CONCLUSION

The present study revealed that it is possible to develop a stable nanosuspension of RPG to boost its dissolution profile. Achievement of an optimized nanosuspension with the desired physiochemical features through augmentation of formulation parameters; including the use of a single vs. combined stabilizers. The integration of SOL as the primary stabilizer and combining co-stabilizers such as (PVA, PVPK30, TW80, and PXM188) can effectively prevent particle aggregation and boost long-term stability of the produced nanosuspension with no additional benefit to using SOL alone. Polarity of a solvent used in nanoprecipitation techniques is of such importance that although it maintained particle size reduction, recovery of the loaded RPG was unsatisfactory. In vitro dissolution study exposed the significant boost of RPG release when compared to its original pure powder form. Overall, this study underlined potential of nanoprecipitation to procure a stable nanosuspension as a promising approach for enhancing dissolution and in turn solubility of poor-water soluble, such as RPG.

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CONFLICT OF INTEREST

The authors declare no conflict of interest".

REFERENCES

Abbas, H. K., Wais, F. M. H., & Abood, A. N. (2017). Preparation and evaluation of ketoprofen nanosuspension using solvent evaporation technique. *Iraqi Journal of Pharmaceutical Sciences*, 41–55. https://doi.org/10.31351/vol26iss2pp41-55

Ali, A. H., & Abd-Alhammid, S. N. (2019). Enhancement of solubility and improvement of dissolution rate of atorvastatin calcium prepared as nanosuspension. *Iraqi Journal of Pharmaceutical Sciences*, *28*(2), 46–57. https://doi.org/10.31351/vol28iss2pp46-57

Alwan, R. M., & A. Rajab, N. (2021). Nanosuspensions of Selexipag: Formulation, Characterization, and in vitro Evaluation. *Iraqi Journal of Pharmaceutical Sciences* , *30*(1), 144-153. https://doi.org/10.31351/vol30iss1pp144-153

Areej, W. A., & Ghareeb, A. M. M. (2021). Formulation and characterization of nimodipine nanoparticles for the enhancement of solubility and dissolution rate. *Iraqi Journal of Pharmaceutical Sciences*, *32*(Suppl.), 244–253. https://doi.org/10.31351/vol32isssuppl.pp244-253

Attia, M. S., Elshahat, A., Hamdy, A., Fathi, A. M., Emad-Eldin, M., Ghazy, F.-E. S.,...Ibrahim, T. M. (2023). Soluplus® as a solubilizing excipient for poorly water-soluble drugs: Recent advances in formulation strategies and pharmaceutical product features. *Journal of Drug Delivery Science and Technology*, *84*, 104519. https://doi.org/https://doi.org/10.1016/j.jddst.2023.104519

Bashar, K. K. G., & Al-Khedairy, E. (2023). Solubility and dissolution enhancement of atorvastatin calcium using phospholipid solid dispersion technique. *Iraqi Journal of Pharmaceutical Sciences*, *32*(Suppl.), 244–253. https://doi.org/10.31351/vol32issSuppl.pp244-253

Bose, S., Du, Y., Takhistov, P., & Michniak-Kohn, B. (2013). Formulation optimization and topical delivery of quercetin from solid lipid-based nanosystems. *International Journal of Pharmaceutics*, *441*(1–2), 56–66. https://doi.org/10.1016/j.ijpharm.2012.12.013

Budiman, A., Lailasari, E., Nurani, N. V., Yunita, E. N., Anastasya, G., Aulia, R. N., Lestari, I. N., Subra, L., & Aulifa, D. L. (2023). Ternary Solid Dispersions: A Review of the Preparation, Characterization, Mechanism of Drug Release, and Physical Stability. *Pharmaceutics*, *15*(8), 2116. https://doi.org/10.3390/pharmaceutics15082116

Dawood, N., Abdal-Hammid, S., & Hussein, A. (2018). Formulation and characterization of

lafutidine nanosuspension for oral drug delivery system. *International Journal of Applied Pharmaceutics*, *10*(2), 20. https://doi.org/10.22159/ijap.2018v10i2.23075

Ebrahimi, H. A., Javadzadeh, Y., Hamidi, M., & Jalali, M. B. (2015). Repaglinide-loaded solid lipid nanoparticles: Effect of using different surfactants/stabilizers on physicochemical properties of nanoparticles. *DARU Journal of Pharmaceutical Sciences*, *23*(1). https://doi.org/10.1186/s40199-015-0128-3

Emad, H., & Abd-Alhammid, S. N. (2022). Improvement of the solubility and dissolution characteristics of risperidone via nanosuspension formulations. *Iraqi Journal of Pharmaceutical Sciences*, *31*(1), 43–56. https://doi.org/10.31351/vol31iss1pp43-56

Fadhila, M., Wahyuni, R., Halim, A., & Proklawati, H. (2023). Effectiveness of Dry Grinding and Wet Grinding Methods on Physicochemical Properties, Solubility, and Dissolution Rate of Nimodipine-HPMC Nanoparticles. *Indonesian Journal of Pharmacy*, *34*(4), 567–573. https://doi.org/10.22146/ijp.7267

Fouad, S. A., Teaima, M. H., Gebril, M. I., Abd Allah, F. I., El-Nabarawi, M. A., & Elhabal, S. F. (2023). Formulation of novel niosomal repaglinide chewable tablets using coprocessed excipients: *In vitro* characterization, optimization, and enhanced hypoglycemic activity in rats. *Drug Delivery*, *30*(1), 2181747. https://doi.org/10.1080/10717544.2023.2181747

Gadadare, R., Mandpe, L., & Pokharkar, V. (2015). Ultra rapidly dissolving repaglinide nanosized crystals prepared via bottom-up and top-down approach: Influence of food on pharmacokinetics behavior. *AAPS PharmSciTech*, *16*(4), 787–799. https://doi.org/10.1208/s12249-014-0267-8

Ghadhban, H. Y., & Ahmed, K. K. (2024). Nanosuspension-Based Repaglinide Fast-Dissolving Buccal Film for Dissolution Enhancement. *AAPS PharmSciTech*, *25*(6). https://doi.org/10.1208/s12249-024-02868-w

Gill, S., & Arora, P. (2020). Improving physicochemical properties of repaglinide through pharmaceutical adduct formation. *Journal of Pharmaceutical Technology, Research and Management*, *8*(1), 31–37. https://doi.org/10.15415/jptrm.2020.81005

Gumaste, S. G., Gupta, S. S., & Serajuddin, A. T. M. (2016). Investigation of polymer–surfactant and polymer–drug–surfactant miscibility for solid dispersion. *The AAPS Journal*, *18*(5), 1131–1143. https://doi.org/10.1208/s12248-016-9939-5

Hamed, H. E., & Hussein, A. A. (2020). Preparation, *in vitro* and *ex vivo* evaluation of mirtazapine nanosuspension and nanoparticles incorporated in orodispersible tablets. *Iraqi Journal of Pharmaceutical Sciences*, *29*(1), 62–75. https://doi.org/10.31351/vol29iss1pp62-75

Jogu, C. (2020). Preparation and evaluation of repaglinide nanosuspensions. *International Journal of Novel Trends in Pharmaceutical Sciences*, *9*(1), 6–11. https://doi.org/10.26452/ijntps.v9i1.1166

Khoza, P. B., Moloto, M. J., & Sikhwivhilu, L. M. (2012). The effect of solvents (acetone, water, and ethanol) on the morphological and optical properties of ZnO nanoparticles prepared by microwave. *Journal of Nanotechnology*, *2012*, 1–6. https://doi.org/10.1155/2012/195106

Kocbek, P., Baumgartner, S., & Kristl, J. (2006). Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. *International Journal of Pharmaceutics*, *312*(1), 179–186. https://doi.org/10.1016/j.ijpharm.2006.01.008

Kulshreshtha, A. K., Singh, O. N., & Wall, G. M. (2010). *Pharmaceutical Suspensions: From Formulation Development to Manufacturing*. https://doi.org/10.1007/978-1-4419-1087-5

Kumar, R., Thakur, A. K., Chaudhari, P., & Banerjee, N. (2022). Particle Size Reduction Techniques of Pharmaceutical Compounds for the Enhancement of Their Dissolution Rate and Bioavailability. *Journal of Pharmaceutical Innovation*, *17*(2), 333-352. https://doi.org/10.1007/s12247-020-09530-5

Li, G., Lu, Y., Fan, Y., Ning, Q., & Li, W. (2020). Enhanced oral bioavailability of magnolol via mixed micelles and nanosuspensions based on Soluplus®-Poloxamer 188. *Drug Delivery*, *27*(1), 1010–1017. https://doi.org/10.1080/10717544.2020.1785582

Lokhande, A. B., Mishra, S., Kulkarni, R. D., & Naik, J. B. (2013). Preparation and characterization of repaglinide-loaded ethylcellulose nanoparticles by solvent diffusion technique using a high-pressure homogenizer. *Journal of Pharmacy Research*, *7*(5), 421–426. https://doi.org/10.1016/j.jopr.2013.04.049

Mandić, Z., & Gabelica, V. (2006). Ionization, lipophilicity, and solubility properties of repaglinide. *Journal of Pharmaceutical and Biomedical Analysis*, *41*(3), 866–871. https://doi.org/10.1016/j.jpba.2006.01.056

Malik, B., & Eman B. H. Al-Khedairy. (2023). Formulation and in vitro /in vivo Evaluation of Silymarin Solid Dispersion- Based Topical Gel for Wound Healing. *Iraqi Journal of Pharmaceutical Sciences* , *32*(Suppl.), 42-53. https://doi.org/10.31351/vol32issSuppl.pp42-53

Mohammed, A. A., & Abd-Alhammid, S. N. (2024). Formulation and characterization of clozapine nanosuspension as a sublingual film. *Al-Rafidain Journal of Medical Sciences*, *6*(2), 82–88. https://doi.org/10.54133/ajms.v6i2.772

Müller, R. H., Jacobs, C., & Kayser, O. (2001). Nanosuspensions as particulate drug formulations in therapy: Rationale for development and future expectations. *Advanced Drug Delivery Reviews*, *47*(1), 3–19. https://doi.org/10.1016/S0169-409X(00)00118-6

Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Nanostructured lipid matrices for improved microencapsulation of drugs. *International Journal of Pharmaceutics*, *242*(1–2), 121–128. https://doi.org/10.1016/S0378-5173(02)00180-1

Patnaik, S., Chunduri, L. A. A., Akilesh, M. S., Bhagavatham, S. S., & Kamisetti, V. (2016). Enhanced dissolution characteristics of piroxicam–Soluplus® nanosuspensions. *Journal of Experimental Nanoscience*, *11*(12), 916–929. http://dx.doi.org/10.1080/17458080.2016.1178402

Patravale, V. B., Date, A. A., & Kulkarni, R. M. (2004). Nanosuspensions: A promising drug delivery strategy. *Journal of Pharmacy and Pharmacology*, *56*(7), 827–840. https://doi.org/10.1211/0022357023691

Pignatello, R., & Corsaro, R. (2019). Polymeric nanomicelles of Soluplus® as a strategy for enhancing the solubility, bioavailability, and efficacy of poorly soluble active compounds. *Current Nanomedicine*, *9*(1), 9–21. https://doi.org/10.2174/2468187309666190314152451

Rajab, R. M. A., & N. A. (2021). Nanosuspensions of selexipag: Formulation, characterization, and *in vitro* evaluation. *Iraqi Journal of Pharmaceutical Sciences*, *30*, 144–153. https://doi.org/10.31351/vol30iss1pp144-153

Sakhiya, D. C., & Borkhataria, C. H. (2024). A review on advancement of cocrystallization approach and a brief on screening, formulation and characterization of the same. *Heliyon*, *10*(7), e29057. https://doi.org/10.1016/j.heliyon.2024.e29057

Thamer, A. K., & Abood, A. N. (2021). Preparation and *in vitro* characterization of aceclofenac nanosuspension (ACNS) for enhancement of percutaneous absorption using hydrogel dosage form. *Iraqi Journal of Pharmaceutical Sciences*, *30*(2), 86–98. https://doi.org/10.31351/vol30iss2pp86-98

Toma, N., & Abdulrasool, A. A. (2021). Preparation and evaluation of microneedles-mediated transdermal delivery of montelukast sodium nanoparticles. *International Journal of Drug Delivery Technology*, *11*, 1075-1082. https://doi.org/10.25258/ijddt.11.3.74

Patel, V. R., & Agrawal, Y. K. (2011). Nanosuspension: An approach to enhance solubility of drugs. *Journal of advanced pharmaceutical technology & research*, *2*(2), 81–87. https://doi.org/10.4103/2231-4040.82950

Weng, J., Tong, H. H. Y., & Chow, S. F. (2020). *In vitro* release study of polymeric drug nanoparticles: Development and validation of a novel method. *Pharmaceutics*, *12*(8). https://doi.org/10.3390/pharmaceutics12080732

Williams, H. D., Trevaskis, N. L., Charman, S. A., Shanker, R. M., Charman, W. N., Pouton, C. W.,…Christopoulos, A. (2013). Strategies to

Address Low Drug Solubility in Discovery and Development. *Pharmacological Reviews*, *65*(1), 315-499. https://doi.org/10.1124/pr.112.005660

Yang, X.-D., Li, W.-S., Tian, Y.-J., Liu, C.-G., Gao, D.-H., & Ma, H.-L. (2016). Dissolution rate enhancement of repaglinide by solid dispersion. *Tropical Journal of Pharmaceutical Research*, *15*(6), 1133–1140. https://doi.org/10.4314/tjpr.v15i6.2