



# Development of spray-dried amorphous solid dispersions of tadalafil using glycyrrhizin for enhanced dissolution and aphrodisiac activity in male rats



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## ABSTRACT

Tadalafil (TDL) is a phosphodiesterase-5 inhibitor (PDE5I), indicated for erectile dysfunction (ED). However, TDL exhibits poor aqueous solubility and dissolution rate, which may limit its application. This study aims to prepare amorphous solid dispersion (ASD) by spray-drying, using glycyrrhizin—a natural drug carrier. Particle and physicochemical characterizations were performed by particle size, polydispersity index measurement, yield, drug content estimation, Fourier Transformed Infrared (FTIR) spectroscopy, Differential scanning calorimetry (DSC), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and dissolution study. In order to evaluate the aphrodisiac activity of the prepared ASD, sexual behavior study was performed in male rats. It is further considered for the stability study. Our results revealed that TDL-GLZ spray-dried dispersion was a successful drug-carrier binary mixture. XRD and SEM showed that ASD of TDL with GLZ presented in the amorphous state and dented-spherical shape, unlike the drug indicating crystalline and spiked shaped. The optimized ASD3 formulation with particle size (1.92 μm), PDI (0.32), yield (97.78%) and drug content (85.00%) showed 4.07 folds' increase in dissolution rate compared to pure TDL. The results obtained from the *in vivo* study exhibit significantly improved aphrodisiac activity with ASD3. The stability study revealed that the prepared ASD3 did not show any remarkable changes in the dissolution and drug content for 1 month storage at room temperature.

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## 1. Introduction

Tadalafil (TDL) is a regulatory approved selective phosphodiesterase-5 inhibitor, chemically it is pyrazino[1,2:1,6]p

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yrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-, (6R,12aR). According to Biopharmaceutical Classification System (BCS), it's classified into BCS-class II drug, practically water-insoluble with low dissolution rate and absorption leads to poor bioavailability (Baek and Cho, 2016). Active pharmaceutical ingredient (API) in solid state plays a vital role in the success of formulation development. Polymorphism may be the major cause of the poor bioavailability of TDL. It acts by reversible competitive inhibition of phosphodiesterase-5 (PDE5), an enzyme that in-turn inactivate the cyclic guanosine monophosphate (cGMP) (Kim et al., 2018). The recommended dose of TDL is 2.5–20 mg, represents a long duration of action (36 h), and has relatively less toxicity associated with vision abnormalities (Park et al., 2018). Being PDE5 inhibitor TDL has been indicated for

erectile dysfunction (ED) and also for pulmonary hypertension (Karabakan et al., 2017, Yang et al., 2019). The efficacy and safety of PDE5 for the treatment of ED have been reported and well-documented (Giuliano and Varanese, 2002). Prevalence of ED is profound in the adult age group and acts as an indicator of cardiovascular diseases (Buvat et al., 2014). Patients with diabetes mellitus are the anchorage of ED (Fahmy and Aljaeid, 2018). Agents used for the treatment of ED showed the least drug adherence due to less efficacy and patient compliance.

More than 40% of new molecular entities (NMEs) approved by FDA aborted in the drug development process and failed before reaching the clinical trials and market due to a lack of optimal biopharmaceutical properties (Su et al., 2019; Gupta et al., 2013). Currently, 90% of the new chemical entities (NCEs) discovered, revealed to be from BCS Class-II and IV. Several methods have been employed by formulation scientists in order to increase the aqueous solubility, dissolution rate and bioavailability of the therapeutically active compounds (Karen et al., 2019; Rahman et al., 2020). Based on the drug properties, excipients characteristics and the nature of anticipated dosage form solubility enhancement technologies will be selected. Broadly, water-insoluble and poorly soluble drugs can be developed into a modified crystalline solid, lipid formulations, inclusion complexation, self-emulsifying drug delivery systems (SEDDS), amorphous- solid dispersions (Gupta et al., 2013).

Exploring the solid dispersion (SD) technologies brought cost-effective, feasible and scalable results for the pharmaceutical industries, with the emergence of commercial drug product of SDs. Solid dispersion is defined as a composition of hydrophobic drug dispersed with at least one hydrophilic carrier. The resultant product will have greater aqueous solubility, improved dissolution rate, better wettability with reduced particle-agglomeration ensuring enhanced bioavailability. SD can be prepared by various techniques such as melt-dispersions, solvent evaporation, hot-melt extrusion, and spray-drying (Tran et al., 2019). In spray-drying, the mixture of drug-carrier/polymer(s) and drug suspended in common solvent(s) systems. The drug-carrier solution atomized in the inert gas-supplied controlled heating chamber, within millisecond liquid gets transformed to a spherical solid with narrow size distribution. Spray-drying is industrial adopted technology for thermolabile compounds and scalable production. End product characteristics can be modified by optimizing the process parameters (De Mohac et al., 2020). In the present investigation, solid dispersion prepared by spray-drying demonstrate to maintain an amorphous state. Literature disseminate that the amorphous form of the crystalline drug has up to thousand-fold enhanced solubility (Wijjani et al., 2020). Amorphous solid dispersions (ASDs) showed improved dissolution rate due to disordered structure compared to the crystalline state and higher Gibbs free energy (Ziaee et al., 2017). The dissolution rate is directly proportional to the effective surface area of the drug particle and it is increased with decreasing the particle size, as referred by Noyes and Whitney equation and Nernst-Brunner theory (De Mohac et al., 2020; Noyes and Whitney, 1897.). Scientists exploiting different natural/synthetic drug-carrier, production processes and stabilization strategies to develop an efficient drug delivery system. In recent past, natural drug carriers are focused simultaneously alongside solubility enhancement technologies on several purposes, including faster dissolution rates, and consequently, enhanced bioavailability (Polyakov, 2011).

Glycyrrhizin (GLZ)-a triterpenoid saponin is obtained as a major chemical constituent from the Licorice root, *Glycyrrhiza glabra*. Glycyrrhizin reported numerous pharmacological activities; antiviral (Covid-19), anti-carcinogenic, anti-inflammatory, antiarrhythmic (GLZ-nifedipine) and anti-oxidant (Wang et al., 2016). In addition to these, GLZ has an ability to increase the solubility

of the hydrophobic drugs by micellar solubilization (Polyakov, 2011). It's an amphiphilic substance encompassed of both hydrophilic (glucuronic acid) and lipophilic (glycyrrhetic acid) fragments. The amphiphilic property of GLZ demonstrates micelle formation in lower pH, optimal critical micellar concentration for GLZ in water was found to be 1 mM, higher content of GLZ leads to aggregates and solubilization of hydrophobic drugs. GLZ moieties self-associated and form the complex structures, rod-like micelles at a lower concentration, whereas higher concentration leads to fibrillary network. Studies on GLZ discovered that the drug entrapped as guest molecule into GLZ micelles (Wang et al., 2016). Effective surface area accessibility to solvent and H-bond donor/acceptor sites are larger for GLZ, contributing a molecular interaction of a drug with the carrier (Selyutina et al., 2016). Increase in the bioavailability of hydrophobic drugs with GLZ is suggested to be by the larger micellar-aggregates. Besides, permeation enhancement of drug also reported by GLZ, suggested mechanism influencing the intactness of cell membrane include; extraction of membrane cholesterol by saponin moiety of GLZ, which affects the fluidity of the cell membrane (Selyutina et al., 2016). Thus GLZ was found to act as a novel drug carrier, drug stabilizer, bioavailability, and therapeutic activity enhancer.

The goal of the current investigation was to prepare and characterize the amorphous solid dispersion of TDL using GLZ to enhance the dissolution rate and therapeutic efficacy. The optimized formulation was further evaluated for *in vivo* sexual behavior and stability testing.

## 2. Materials and methods

Materials sources: Tadalafil (TDL) and Glycyrrhizin, Ammonium Salt (GLZ) were obtained as gift samples from Riyadh Pharma and Jazeera Pharmaceuticals (JPI), Riyadh, respectively. Acetone, acetic acid and other chemicals used were of analytical grades, purchased from Sigma Aldrich, Germany.

### 2.1. Preparation of amorphous solid dispersion by spray drying technique

TDL and GLZ combination was atomized in spray dryer "(Mini Spray Dryer Büchi B-290, Switzerland)". Three different amorphous solid dispersions were prepared in the weight ratio of TFL: GLZ as 1:0.5 (ASD1); 1:1 (ASD2) and 1:2 (ASD3). A binary solvent mixture composed of; acetone and acetic acid (1%) in (70:30, v/v) was used. The dispersion (100 mL) was prepared by dispersing drug; carrier ratios in the binary solvent to get solid content of 1.5w/v, 2w/v and 3w/v for three batches of ASD1, ASD2 AND ASD3, respectively. The prepared solution was sonicated for half-hour followed by feeding to the spray dryer by a peristaltic pump and sprayed through a 0.7 mm nozzle with the help of flow of compressed air. Flow of heated air in glass drying chamber provided rapid evaporation of solvent, resulted into dried particles. The dried particles were separated through cyclone separator and finally collected in collector. Spray dryer was operated at inlet temperature, 80 °C and outlet temperature of 60° C; aspiration level 100%; atomization gas flow of 25% and pump speed was 3 mL/min. The outlet temperature was monitored and maintained near the boiling point of the solvents used, by adjusting the inlet temperature. Immediately after spray drying the powder was collected separately and packed in glass scintillation vials, stored at ambient temperature before their physicochemical characterizations and therapeutic testing (Anwer et al., 2020; Sree Harsha et al., 2020).

## 2.2. Particle size and polydispersity index (PDI) measurements

To measure the particle size and dispersion uniformity, the sample was dispersed in distilled water (1:200) and sonicated. The particle characterization carried at 25 °C by using “Microtrac particle size analyzer (Microtrac S3500)” worked on the principle of Dynamic Light Scattering and Laser Diffraction. Means of particle size, PDI and their standard deviations were calculated by measuring each sample in triplicate (Czyz et al., 2020).

## 2.3. Process yield and drug content estimation

Amorphous solid dispersions obtained from the spray drying were weight (Weighing Analytical Balance Mettler Toledo, Shanghai Yoke Instrument Co., Ltd., Shanghai, China) and amount subtracted from the total weight of the drug-carrier (TDL: GLZ). Yield the percentage was calculated by Eq. (1).

$$\text{Yield}(\%) = \frac{\text{Weight of amorphous solid dispersion}}{\text{Initial weight of TDL and GLZ ratio}} \times 100 \quad (1)$$

The total drug content in the sample was estimated by dissolving a predetermined amount of solid dispersion in ethanol. The mixture was sonicated for 1 h, then pre-filtered solution was analyzed by using a UV-spectrophotometer at  $\lambda_{\text{max}}$ -max 284 nm with a light source of a deuterium lamp (Jasco V-630 Made in Japan). The drug content was calculated by using regression analysis (Khan et al., 2014).

## 2.4. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectrums of pure drug TDL, and prepared spray-dried amorphous solid dispersions of TDL in GLZ (ASD1-ASD3) were recorded by using FTIR spectrometer (Model-V-5300, JASCO and Japan). The samples under investigation were triturated with anhydrous potassium bromide (KBr), powdered mass then compressed to form a translucent pellet (Su et al., 2019). The peaks were recorded in the wavelength range of 4000 to 400  $\text{cm}^{-1}$ .

## 2.5. Differential scanning calorimetry (DSC)

Thermal behavior of TDL, GLZ and amorphous solid dispersion (ASD1-ASD3) were analyzed by differential scanning calorimeter (Scinco, DSC N650, Seoul-Korea). The test sample (5 mg) was crimped in aluminum DSC pans and placed in the instrument the sample holder equipped by a cooling system supplied with dry nitrogen at a flow rate of 40 mL/min. The reference (empty pan) and test sample filled pan were heated to the temperature range of 20 to 320 °C at a constant heat rate of 10 °C/min. Analysis of endothermic peaks and overlay processes was carried out by the thermal analysis software of the instrument (Su et al., 2019).

## 2.6. X-ray diffraction (XRD)

Diffraction peaks of TDL, GLZ, and amorphous solid dispersion (ASD1-ASD3) were recorded based on the Bragg's Law by using XRD diffractometer (Ultima IV diffractometer; Rigaku Inc. Tokyo). The operation was performed using Cu anode with Cu K $\alpha$  radiation, voltage/current (40 kV/40 mA). The data were collected in the continuous scan mode at a scan speed of 0.500 deg/min in the range of 3 to 50° $\text{\AA}$  (2 $\theta$ ). The intensity of Bragg peaks will define the crystalline and amorphous or non-crystalline states of solid. The relative degree of crystallinity was calculated by comparing the selected peak intensity of solid dispersion (ASDs) with reference (TDL) (Su et al., 2019).

## 2.7. Scanning electron microscope (SEM)

The surface morphology of amorphous solid dispersion (ASD1-ASD3) were characterized by a focused beam of electrons using (SEM- Ultraplus, Zeiss, Germany). The sample under examination was suspended in an organic solvent (Methanol), spread over glass-slide, and kept for drying in a desiccator. For uniform layering and overloading the sample was coated with gold-palladium for two minutes with 20 mA current using a gold-sputter module under a reduced pressure evaporator. The machine was run at 5 kV with a working distance of 8–11 mm, microphotographs were obtained by scanning and magnification (Al-Shdefat et al., 2016).

## 2.8. Dissolution studies

The dissolution study was carried out by filling the amorphous solid dispersions (ASD1-ASD3) equivalent to ~ 20 mg of TDL in clear vegetarian K-Caps (Capsuline<sup>®</sup>) size 3. The amount of ASDs filled in capsules were 30, 40 and 60 mg for ASD1, ASD2 and ASD3 batches, respectively whereas, 20 mg for TDL. The dissolution test was performed in 0.1 N HCl (900 mL) medium by using USP type-II dissolution apparatus (Dissolution system, Distek Model-2500i, USA) operated at a stirring speed of 50 rpm and, the temperature was maintained at 37 °C. At a predetermined time interval (5 mL) sample withdrawn with the replacement of equal volume fresh medium. The sample was passed through 0.4  $\mu\text{m}$  membrane filter and analyzed for drug dissolved by using UV- spectrophotometer at  $\lambda_{\text{max}}$  284 nm (Jasco V-630 Made in Japan). Percentage drug dissolved in ASDs were compared with TDL. Three sets of the experiment were performed for each batch (Wijiani et al., 2020).

## 2.9. Aphrodisiac activity: In vivo sexual behavior studies

The aphrodisiac activity was carried out in animals procured from animal care house, Department of Pharmacology, Prince Sattam bin Abdulaziz University, Al-Kharj. The permission to carry out animal studies was granted by Bioethical Research Committee (BERC-002-12-19), Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia. Healthy male and female Albino rats were used for the study were held separately in a transparent cage, kept under ventilated-conditions, and fed with solid pellets standard animal feed besides continuous supply of water as required.

Female rats were administered with estrogen benzoate orally (10 mg/kg of body weight) and subcutaneous progesterone (0.5 mg/kg of body weight) two days before the pairing to induce artificial oestrus. Female rat showing maximum receptiveness on exposing them to male rats were selected for the experiment.

The experiment was performed in a calm state under faint red light, Location; Pharmaceutics-testing lab, Department of Pharmaceutics, Prince Sattam bin Abdulaziz University, Al-Kharj. The male rats were trained by exposing them to sexually receptive females before the start of the experiment, at a schedule of once daily for four consecutive days. Sexually experienced male-rats that were showing reactive sexual action (n = 5) were randomly selected and kept in a separate cage. They were grouped into three (n = 5), Group I was the normal control and were administered with vehicle (1% sodium carboxyl methylcellulose). Group II was treated with tadalafil (Herox<sup>®</sup>) at a dose of 10 mg/kg as a positive control. Group III male rats were treated with 10 mg/kg of TDL equivalent amount of optimized formulation ASD3. The normal control vehicle, marketed formulation (Herox<sup>®</sup>), and test formulation (ASD3) were administered orally as a single dose using an oral-gastric tube (Tang et al., 2017).

2.9.1. Sexual behavior-study

After just half an hour, sexually experienced male and sexually active female rats were introduced into the mating cage in a 1:1 ratio under the same lighting condition, state and location. The sexual behaviour of the male animals was immediately begins and continued for the end of the first mating series. Following sexual parameters were analyzed.

Mount latency (ML): The time lap from the entry of a female rat into the cage of the male till the first mount by the male rat with the pelvic thrusting.

Intromission latency (IL): The time period from the introducing of a female rat into the cage until the first intromission by the male (first mount until vaginal penetration).

Ejaculation latency (EL): The time interval between the first intromission of a series until the ejaculation.

Mount frequency (MF): The number of mounts prior to ejaculation.

Intromission frequency (IF): The number of intromissions prior to ejaculation.

Depending on the beforehand mentioned parameters, the followings sexual behaviour can be calculated by Eqs. (2) and (3).

$$\text{CopulatoryEfficiency(CE)} = \frac{IF}{MF} \times 100 \tag{2}$$

$$\text{IntercopulatoryEfficiency(ICE)} = \frac{IF}{MF + IF} \times 100 \tag{3}$$

2.10. Stability studies

Stability study of prepared optimized formulation was performed to investigate the effects of environmental factors on the drug content and dissolution rate. The optimized amorphous solid dispersion ASD3 was stored in a glass desiccator cabinet at 40 ± 2 °C; saturated NaCl solution was placed at the bottom rack to maintain 75% relative humidity. After 1 month, ASD3 was re-analyzed for drug content estimation as per the aforementioned procedure in the drug content estimation section (Altamimi and Neau, 2017). The degradation of TDL in ASD was calculated as the relative content by the following equation. Eq. (4) (4).

$$\text{RelativeTDLcontent} = \frac{\text{drugcontent}(t = \text{after1month})}{\text{drugcontent}(t = 0)} \tag{4}$$

Evaluation of dissolution profiles of ASD3 before and after 1-month was also done by assessment of the similarity factor (f2) as suggested by Moore and Flanner using Eq. (5).

$$f_2 = 50 \log \left\{ \left[ 1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \right\} \times 100 \tag{5}$$

**Table 1**  
Particle characterizations, process yield and drug content of prepared amorphous solid dispersions (ASD1-ASD3).

Formulation code	TDL / GLZ weight ratio	Particle size (µm) ± SD	PDI ± SD	Process yield (%)	Drug content (%)
ASD1	1:0.5	1.07 ± 0.12	0.49 ± 0.05	58.09	63.67 ± 3.21
ASD2	1:1	1.15 ± 0.15	0.39 ± 0.01	83.10	70.67 ± 2.08
ASD3	1:2	1.92 ± 0.18	0.32 ± 0.00	97.78	85.00 ± 3.10

Data represents average (n = 3) replications ± Standard Deviation. PDI stands for polydispersity index

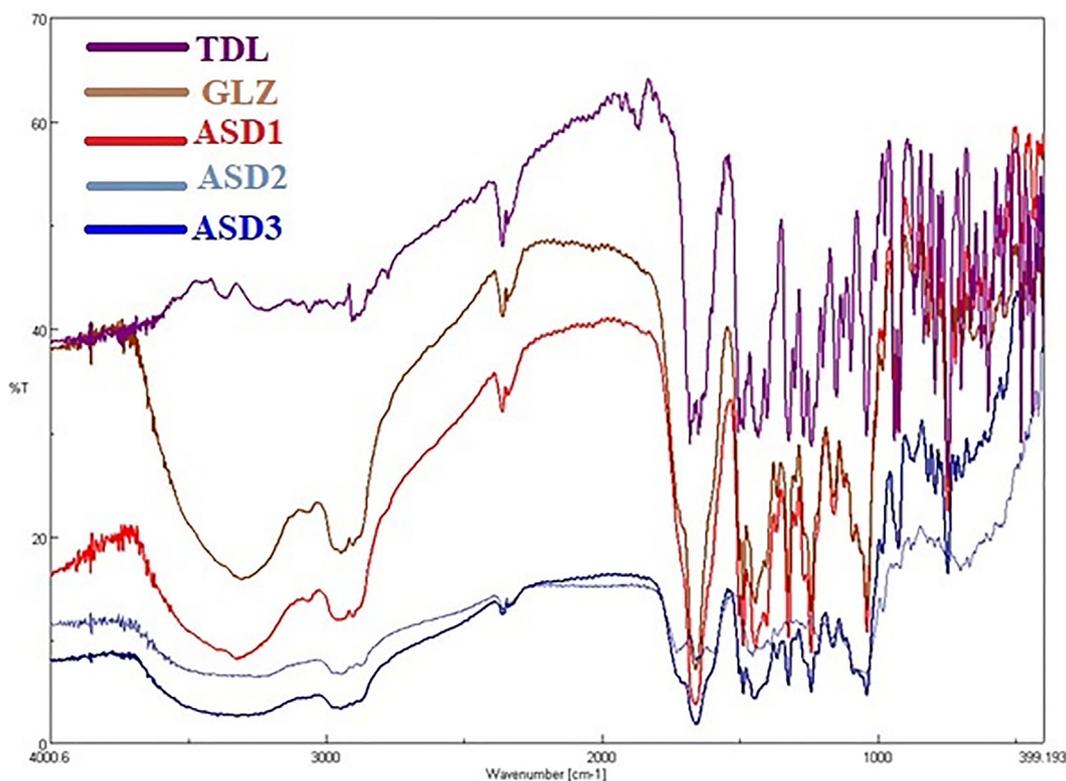


Fig. 1. FTIR spectrums of tadalafil, glycerhizine and its amorphous solid dispersions (ASD1-ASD3).

Where  $n$  is the number of samples,  $R_t$  and  $T_t$  is the percentage of dissolution at the time point  $t$  from the reference sample and test sample respectively.

If the  $f_2$  value found to be  $\geq 50$ , then the dissolution profiles of two samples were considered as equivalent.

### 2.11. Statistical analysis

The Statistical evaluations of the difference between the means/ averages were determined by a one-way analysis of variance (ANOVA) with a post-hoc't' test by using SPSS16 (SPSS Inc., Chi-

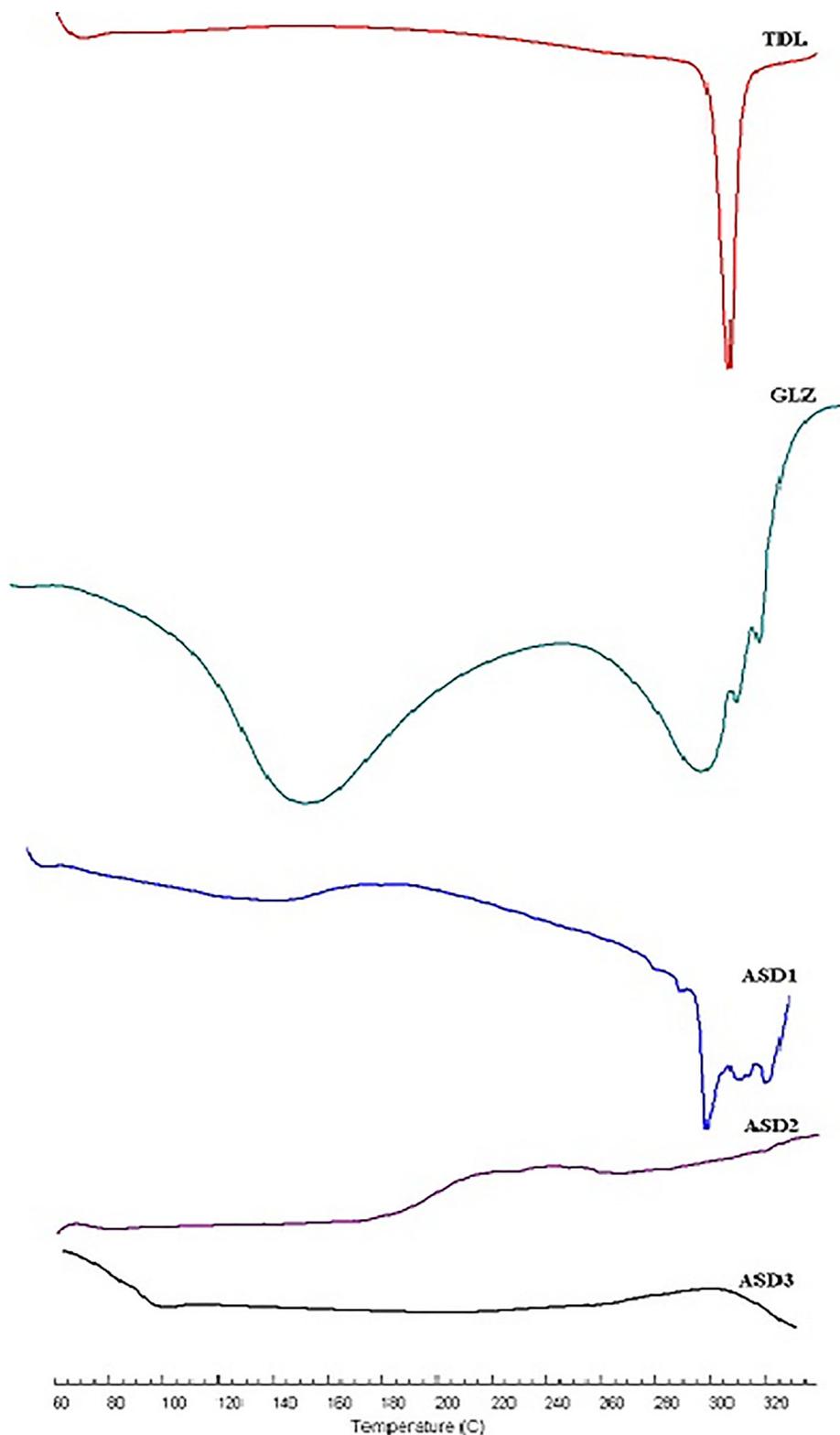


Fig. 2. DSC thermal peaks of tadalafil, glycerhyzine and its amorphous solid dispersions (ASD1-ASD3).

ago, USA). Any differences between the groups were considered significant at  $p \leq 0.05$  level. Animal data are expressed as mean  $\pm$  S.E.M.,  $n = 5$  rats/group.

### 3. Results and discussion

#### 3.1. Particle size and polydispersity index (PDI) measurements

Prepared spray-dried amorphous solid dispersion characterized for size and polydispersity index. ASDs was formed by the transition of drug-carrier liquid droplet to the solid by spray-drying technology. The results of particle size and PDI are presented in Table 1. Particle size was found to be  $1.07 \pm 0.12 \mu\text{m}$ ,  $1.15 \pm 0.15 \mu\text{m}$ ,  $1.92 \pm 0.18 \mu\text{m}$ , whereas PDI was noted to be  $0.49 \pm 0.05$ ,  $0.39 \pm 0.01$ ,  $0.32 \pm 0.00$  for ASD1, ASD2, and ASD3, respectively. Particle size was noted to increase with an increase in the carrier ratio, ASD3 batch showed narrow particle distribution. Batch with PDI value  $\leq 0.3$  considered being monodisperse. Spray-drying technique is a continuous and scalable process that generates particles with a narrow distribution (Szafranec et al., 2019).

#### 3.2. Process yield and drug content estimation

The process parameters and drug-carrier content load will influence the yield (%) of the spray-dried product. The fractions of ASDs to drug-carrier content weight was found to be; 58.09%, 83.10%, 97.78%, whereas estimated drug content was noted as;  $63.67 \pm 3.2\%$ ,  $70.67 \pm 2.08\%$  and  $85.00 \pm 3.10\%$  for ASD1, ASD2, ASD3, respectively. The results of Process yield and drug content are shown in Table 1. ASD1 showed lower yield, which could be due to the stickiness of the product and lowered weight ratio of material used (Smeets et al., 2018). Larger size particle indicated higher drug content, higher drug-carrier proportions yield more ASDs.

#### 3.3. Fourier-transform infrared spectroscopy (FTIR)

Representative peaks of pure TDL drug in the fingerprint region were found at;  $3465 \text{ cm}^{-1}$ ,  $3175 \text{ cm}^{-1}$ ,  $2890 \text{ cm}^{-1}$ ,  $1750 \text{ cm}^{-1}$ ,

$1710 \text{ cm}^{-1}$ ,  $1405 \text{ cm}^{-1}$ ,  $1134 \text{ cm}^{-1}$ ,  $690 \text{ cm}^{-1}$ , corresponding to secondary amine (N-H str), aromatic-stretching (C-H), aliphatic-alkyl stretching, amide (C = O), aromatic (C = C), ketone (C-O-C str symmetry), due to aromatic ring. Prepared ASDs showed identical drug peaks confirmed there was no drug-carrier chemical interaction, whereas, the intensity of these peaks were found to be reduced, which recognized the TDL entrapment in the GLZ. The FTIR spectrums of TDL, GZN and prepared ASDs (ASD1-ASD3) showed in Fig. 1. Some of the identical peaks of the TDL were observed to be shifted, peak smoothening without any additional peaks in the solid dispersions revealed drug-carrier interaction was physical, not chemical (Su et al. 2019; De Mohac et al. 2020)

#### 3.4. Differential scanning calorimetry (DSC)

DSC spectra of TDL, GLZ, and prepared ASDs (ASD1-ASD2) are shown in Fig. 2. Pure drug TDL showed a sharp endothermic peak at  $302^\circ \text{C}$  corresponding to the melting of the TDL, however, GLZ does not show any sharp peaks, instead indicates by broad peaks. The endothermic peak at  $302^\circ \text{C}$  disappeared completely in ASD2 and ASD3 that reflects amorphousness of powder. In contrary, an endothermic peaks of drug could be seen in ASD1, probably due to low amount of GLZ present in ASD1 (1:0.5, w/w). Drug and carrier form the eutectic mixture lead to a decrease in the melting point, therefore less energy is required for prepared ASDs to get dissolved/dissolutions (Chokshi et al., 2007).

#### 3.5. X-ray diffraction (XRD)

X-ray diffraction patterns are distinct, the superimposition peak intensities of TDL, GLZ, and prepared ASDs were used to determine the crystalline or amorphous state of the prepared (ASD1-ASD3). Bragg peaks and diffraction patterns of pure drug TDL, GLZ, and ASDs are represented in Fig. 3. Diffractogram of pure drug TDL revealed many sharp peaks some of them includes;  $7.30^\circ$ ,  $10.70^\circ$ ,  $12.6^\circ$ ,  $14.60^\circ$ ,  $18.50^\circ$ ,  $21.70^\circ$ ,  $24.30^\circ$  at  $(2\theta)$  diffraction angle. Glycyrrhizin (GLZ) showed the only two peaks of 65 and 235 intensities at  $3.80^\circ$  and  $14.60^\circ$  ( $2\theta$ ). In agreement with the DSC thermal peak of TDL, XRD

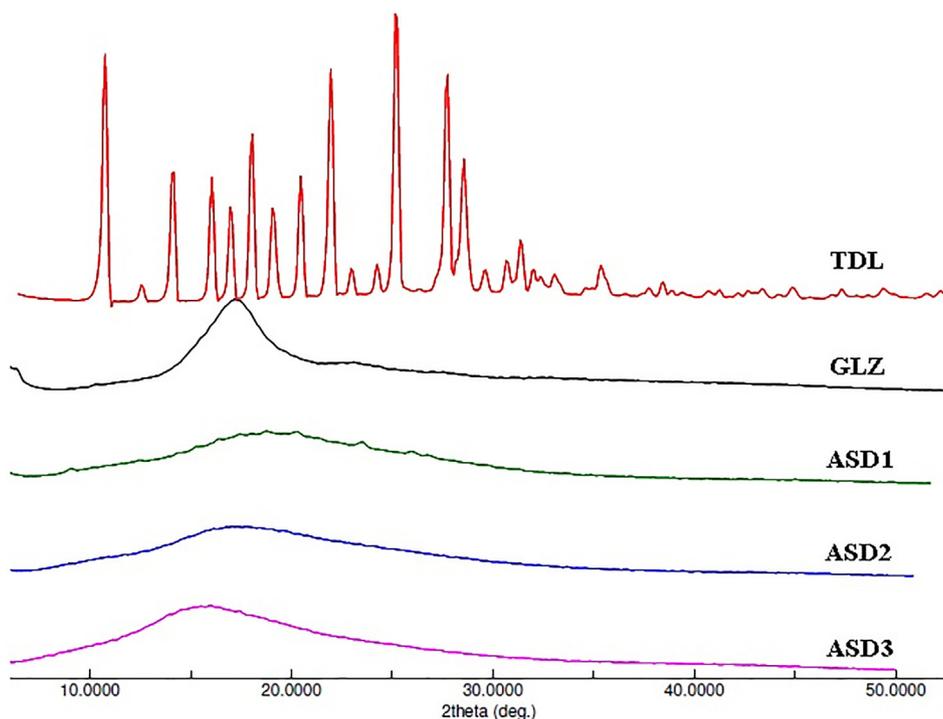


Fig. 3. X-ray powder diffraction (PXRD) of tadalafil, glycyrrhizine and its amorphous solid dispersions (A-ASD1; B-ASD2; C-ASD3).

brags peak of the pure drug confirms TDL was in the crystalline form. In case of XRD patterns of ASDs broad-weak peak(s) denoted as an amorphous state of the prepared solid dispersions. All the three ASDs showed relatively less peak height, confirms the amorphous state of the prepared spray-dried solid dispersion.

### 3.6. Scanning electron microscope (SEM)

The surface electron microscopic study of materials is a qualitative characterization. The SEM images of prepared ASDs (ASD1-ASD3) are represented in Fig. 4. The images of ASD1, ASD2, and ASD3 showed smooth surface and dented-spherical which could be due to the rapid solvent evaporation during the spray drying process. All the three ASDs showed the irregular or quasi-spherical shape, or ruptured spheres. This new solid-state with evident reduced particle size together with amorphousness could enhance the dissolution rate (Kwon et al., 2019).

### 3.7. Dissolution studies

Dissolution means the mass transfer of solid to the solution. XRD results confirms the crystalline nature of the drug (TDL), Fig. 3. Dissolution profiles of amorphous solid dispersions ASD1-ASD3 compared with TDL presented in Fig. 5. ASDs showed fast and complete dissolution within an hour, drug dissolution was found to be  $68.62 \pm 0.8\%$ ,  $88.02 \pm 2.6\%$ ,  $97.78 \pm 0.7\%$  for ASD1, ASD2, ASD3 formulations respectively, in comparison to  $23.99 \pm 1.2\%$  for TDL-crystalline drug (Rahman et al., 2020). The order of dissolution rate was found to be ASD3 > ASD2 > ASD1. Improved dissolution rate could be attributed due to the lack of crystallinity, formation of amorphous, and higher wettability of ASDs by reducing the interfacial tension between TDL and dissolution medium (Sun et al., 2018). Conversion of drug crystal into the amorphous state by formation of solid solution renders an enhanced dissolution rate (Ghanavati et al., 2017). Use of GLZ in the preparation of ASDs was effective in enhancing the dissolution rate an improving the bioavailability. Based on the dissolution result, ASD3 formulation was considered as optimized, exhibits 4.07 folds' increase in dissolution rate compared to pure TDL. Thus ASD3 was further evaluated for therapeutics efficacy in male rats.

### 3.8. Aphrodisiac activity: In vivo sexual behavior studies

Seven sexual behavior parameters were observed and the results are presented in Table 2. The MF and the ML reflect sexual interest or libido, whereas the IF and IL are useful indices of the sexual excitement and the efficiency of erection. Therefore, the reduced duration of ML ( $52.4 \pm 2.50$  sec) and IL ( $78.7 \pm 4.73$  sec) as well as the increased values of MF ( $10.2 \pm 0.37$ ) and IF ( $7.7 \pm 0.19$ ) recorded in Group III ASD3-treated male rats suggests enhanced sexual interest, libido, and erection in comparison with both Group I (normal control) and Group II (positive control).

In addition, ASD3 showed prolonged ejaculation latency ( $439.2 \pm 17.20$  sec) of male rats as compared to the rats of Group I and Group II. The copulatory and intercopulatory efficiencies were found to be 75.49%, 43.02% respectively, uppermost in Group III exposed to ASD3 compared with the Group I and II. All sexual analysis parameters of the optimized ASD3 were remarkably altered compared to control and marketed formulation, these changes are statistically significant. The significant increase in the duration of ejaculation latency confirm that ASD3 has the potential to improve copulatory performance of male rats (Musa et al., 2019). Since the intromission is not possible without adequate erection, therefore increase in IF and prolonged EL by the ASD3 suggests that

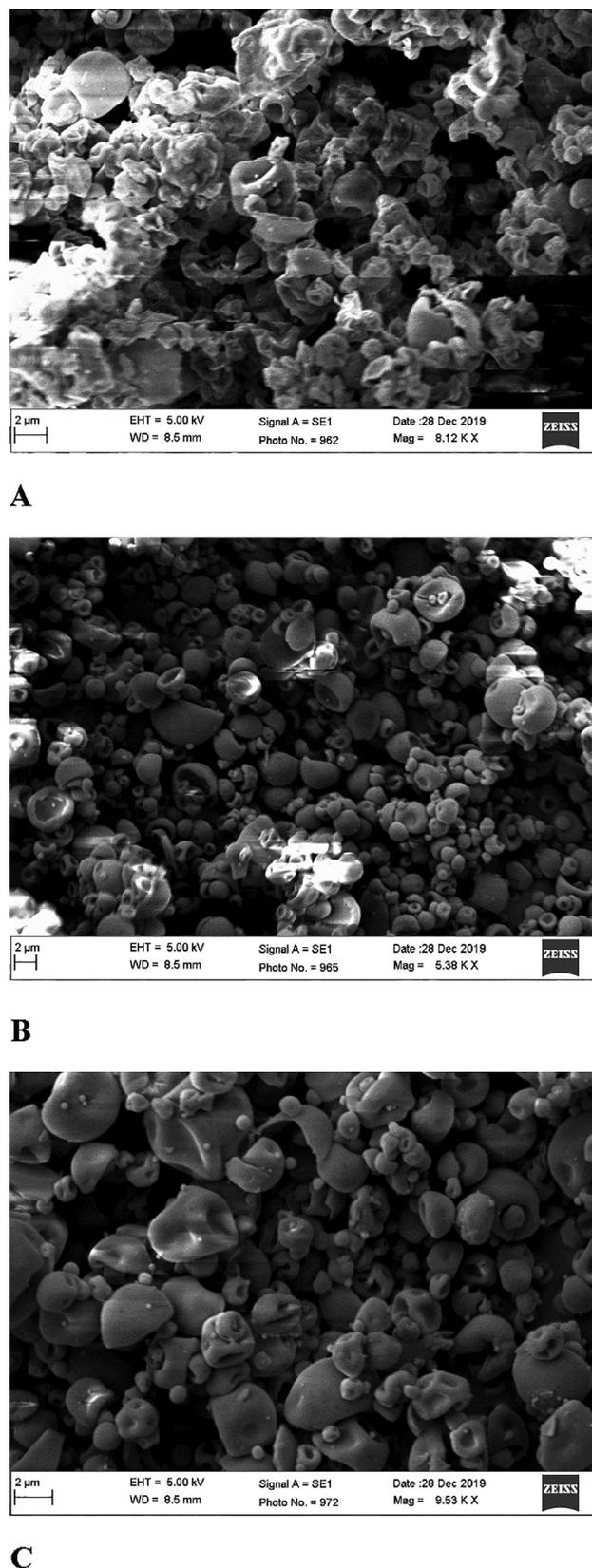


Fig. 4. SEM images of amorphous solid dispersions (ASD1-ASD3).

penile erection was boosted and copulatory performance was enhanced, thereby optimized ASD3 showed the improved aphrodisiac activity with TDL-GLZ amorphous solid dispersion (Chauhan et al., 2014).

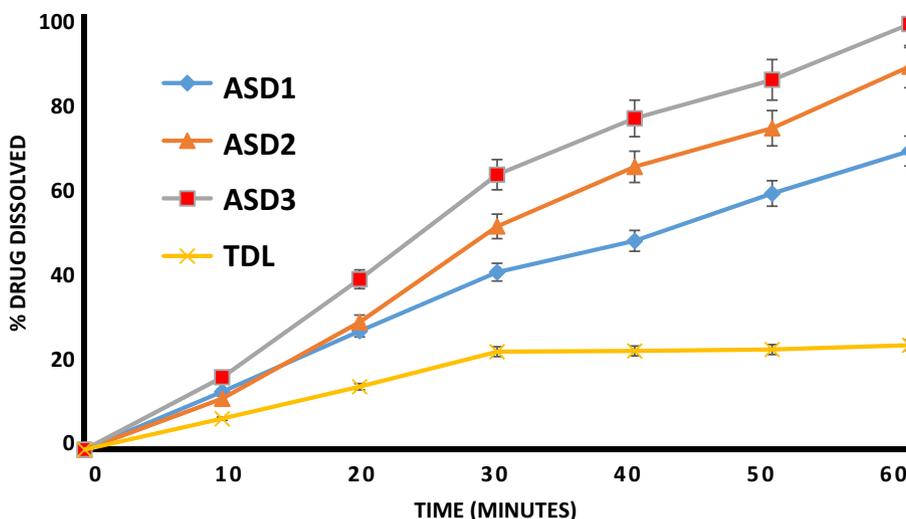


Fig. 5. Dissolution profiles of tadalafil and its amorphous solid dispersions (ASD1-ASD3).

Table 2

Effect of tadalafil loaded amorphous solid dispersion on the mount latency (ML), mount frequency (MF), intromission latency (IL), intromission frequency (IF), ejaculation latency (EL), Copulatory Efficiency (CE) and Intercopulatory Efficiency (IE) of male rats.

Animal Groups	Sexual Behaviour Parameters						
	ML(sec)	MF	IL(sec)	IF	EL(sec)	CE(%)	IE(%)
Group I- normal control	90.1 ± 5.23	5.0 ± 0.32	289.7 ± 2.15	2.9 ± 0.19	275.6 ± 6.12	58.00	36.71
Group II positive control	61.0 ± 0.11 <sup>†</sup>	9.2 ± 0.10 <sup>†</sup>	89.1 ± 1.43 <sup>†</sup>	6.1 ± 0.35 <sup>†</sup>	382.1 ± 11.10 <sup>†</sup>	66.30	39.87
Group III Test-(ASD3)	52.4 ± 2.50 <sup>*</sup>	10.2 ± 0.37 <sup>*</sup>	78.7 ± 4.73 <sup>†*</sup>	7.7 ± 0.19 <sup>†*</sup>	439.2 ± 17.20 <sup>*</sup>	75.49	43.02

Readings are listed as average ± S.E.M., n = 5 rats per group.

<sup>†</sup> Designate significance compared to Group I- normal control at p < 0.05.

<sup>\*</sup> Designate significance compared to Group II positive control at p < 0.05

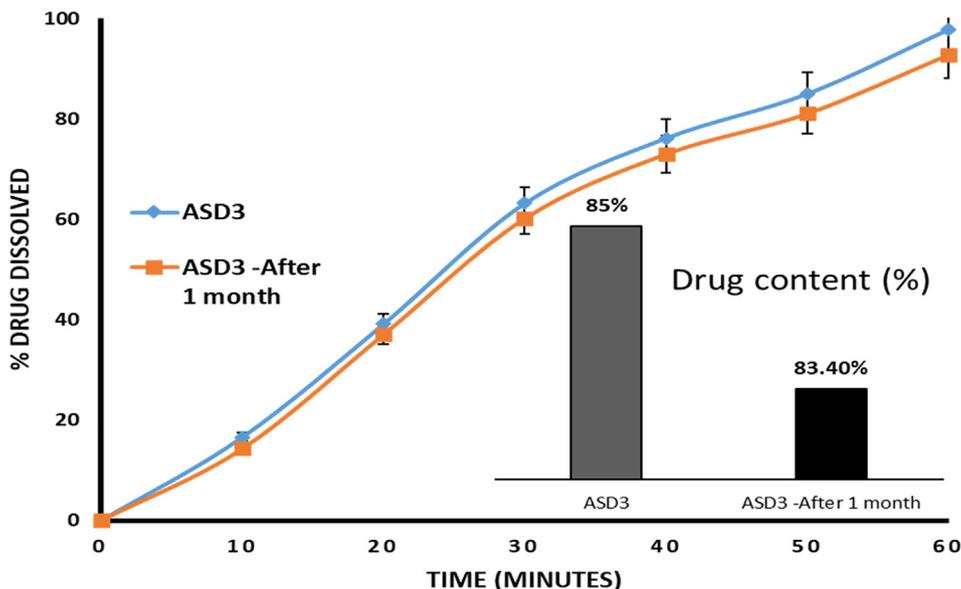


Fig. 6. Effect of stability studies on the dissolution profile and drug content on the optimized ASD3.

### 3.9. Stability studies

During the storage condition, the drug may degrade by light effect, and absorption of moisture may change the solid-state of the drug. As per relative drug content estimation, the amount of drug was found to be 83.40% after 1-month of storage in comparison to 85% to the optimized ASD3 drug content. The percentage of

drug dissolved profiles of ASD3 were also compared after stipulated time and dissolution data of ASD3 before and after 1-month storage was assessed and  $f_2$  value was found to be 51.5, which is within the range of similarity index (Altamimi and Neau, 2017). Stability studies results (Fig. 6) revealed that optimized ASD3 is a stable amorphous solid dispersion.

## 4. Conclusion

Tadalafil is practically insoluble in water, represents lower dissolution and bioavailability. The present study demonstrated the preparation of TDL amorphous solid dispersion with glycyrrhizin using spray-drying technique. FTIR studies revealed the physical state of drug and carrier in the product, DSC clarified the absence of melting endotherm of TDL indicating the absence of crystallinity at higher carrier proportions. Whereas XRD study confirmed the conversion of a crystalline drug into the amorphous state with the use of GLZ, and SEM images showed the formation of dented - spherical drug-carrier amorphous dispersion contrary to the crystalline state of TDL. Dissolution studies revealed the dissolution rate was increased significantly by TDL-GLZ amorphous dispersion compared to crystalline TDL. The higher dissolution rates of ASDs may infer enhance bioavailability due to enlarged wetting and more solubility of TDL in the hydrophilic carrier. Furthermore, In-vivo study shows that the male rats treated with TDL-GLZ amorphous solid dispersion clearly expose significantly improved sexual behavior parameters in the presence of female mice, thus indicating that the optimized ASD3 possesses potent aphrodisiac activity. The stability study revealed optimized ASD3 was stable, reflecting similar dissolution profiles before and after 1-month of storage and assured no recrystallization and degradation of drug during the storage conditions.

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## References

- Al-Shdefat, R., Ali, B.E., Anwer, M.K., Fayed, M.H., Alalawi, A., Soliman, G.A., 2016. Sildenafil citrate-Glycyrrhizin/Eudragit binary spray dried microparticles: A sexual behavior studies on male rats. *J. Drug Delivery Sci. Technol.* 36, 141–149. <https://doi.org/10.1016/j.jddst.2016.10.004>.
- Altamimi, M.A., Neau, S.H., 2017. Investigation of the in vitro performance difference of drug-Soluplus® and drug-PEG 6000 dispersions when prepared using spray drying or lyophilization. *Saudi Pharmaceutical Journal* 25, 419–439. <https://doi.org/10.1016/j.sjps.2016.09.013>.
- Anwer, M.K., Ahmed, M.M., Alshetaibi, A., Almutairy, B.K., Alalawi, A., Fatima, F., Ansari, M.N., Iqbal, M., 2020. Preparation of spray dried amorphous solid dispersion of diosmin in soluplus with improved hepato-reno-protective activity: In vitro anti-oxidant and in-vivo safety studies. *J. Drug Delivery Sci. Technol.* 60. <https://doi.org/10.1016/j.jddst.2020.102101> 102101.
- Baek, J.S., Cho, C.W., 2016. Transdermal delivery of tadalafil using a novel formulation. *Drug Delivery* 23, 1571–1577. <https://doi.org/10.3109/10717544.2015.1077291>.
- Buvat, J., Hatzichristou, D., Boess, F.G., Büttner, H., Gehchan, N., Henneges, C., Porst, H., 2014. Continuation and effectiveness of tadalafil once daily during a 6-month observational study in erectile dysfunction: The EDAT study. *Int. J. Clin. Pract.* 68, 1087–1099. <https://doi.org/10.1111/ijcp.12449>.
- Chauhan, N.S., Sharma, V., Dixit, V.K., Thakur, M., 2014. A review on plants used for improvement of sexual performance and virility. *BioMed Research International* 2014. <https://doi.org/10.1155/2014/868062>.
- Chokshi, R.J., Zia, H., Sandhu, H.K., Shah, N.H., Malick, W.A., 2007. Improving the dissolution rate of poorly water soluble drug by solid dispersion and solid solution - Pros and cons. *Drug Delivery* 14, 33–45. <https://doi.org/10.1080/10717540600640278>.
- Czyz, S., Wewers, M., Finke, J.H., Kwade, A., van Eerdenbrugh, B., Juhnke, M., Bunjes, H., 2020. Spray drying of API nanosuspensions: Importance of drying temperature, type and content of matrix former and particle size for successful formulation and process development. *European Journal of Pharmaceutics and Biopharmaceutics* 152, 63–71. <https://doi.org/10.1016/j.ejpb.2020.04.021>.
- De Mohac, L.M., Raimi-Abraham, B., Caruana, R., Gaetano, G., Licciardi, M., 2020. Multicomponent solid dispersion a new generation of solid dispersion produced by spray-drying. *J. Drug Delivery Sci. Technol.* 57. <https://doi.org/10.1016/j.jddst.2020.101750> 101750.
- Fahmy, U.A., Aljaeidi, B.M., 2018. Tadalafil transdermal delivery with alpha-lipoic acid self nanoemulsion for treatment of erectile dysfunction by diabetes mellitus. *International Journal of Pharmacology* 14, 945–951. <https://doi.org/10.3923/ijp.2018.945.951>.
- Ghanavati, R., Taheri, A., Homayouni, A., 2017. Anomalous dissolution behavior of celecoxib in PVP/Isomalt solid dispersions prepared using spray drier. *Mater. Sci. Eng., C* 72, 501–511. <https://doi.org/10.1016/j.msec.2016.11.042>.
- Giuliano, F., Varanese, L., 2002. Tadalafil: A novel treatment for erectile dysfunction. *European Heart Journal, Supplement* 4, 24–31. [https://doi.org/10.1016/S1520-765X\(02\)90049-0](https://doi.org/10.1016/S1520-765X(02)90049-0).
- Gupta, S., Kesarla, R., Omri, A., 2013. Formulation Strategies to Improve the Bioavailability of Poorly Absorbed Drugs with Special Emphasis on Self-Emulsifying Systems. *ISRN Pharmaceutics* 2013, 1–16. <https://doi.org/10.1155/2013/848043>.
- Karabakan, M., Keskin, E., Akdemir, S., Bozkurt, A., 2017. Effect of tadalafil 5mg daily treatment on the ejaculatory times, lower urinary tract symptoms and erectile function in patients with erectile dysfunction. *International Braz J Urol* 43, 317–324. <https://doi.org/10.1590/S1677-5538.IBJU.2016.0376>.
- Karen, H.D., Prajapati, P.H., Chaudhary, J.I., 2019. BCS classification and solubility enhancement techniques for bcs class II and BCS class IV drugs. *Eur J Biomed Pharm Sci.* 6, 663–670.
- Khan, Z.G., Patil, A.S., Shirkhedkar, A.A., 2014. Estimation of Tadalafil Using Derivative Spectrophotometry in Bulk Material and in Pharmaceutical Formulation. *International Journal of Spectroscopy* 2014, 1–6. <https://doi.org/10.1155/2014/392421>.
- Kim, J.S., Kim, M.S., Baek, I.H., 2018. Enhanced bioavailability of tadalafil after intranasal administration in beagle dogs. *Pharmaceutics* 10, 7–11. <https://doi.org/10.3390/pharmaceutics10040187>.
- Kwon, J., Giri, B.R., Song, E.S., Bae, J., Lee, J., Kim, D.W., 2019. Spray-dried amorphous solid dispersions of atorvastatin calcium for improved supersaturation and oral bioavailability. *Pharmaceutics* 11. <https://doi.org/10.3390/pharmaceutics11090461>.
- Musa, N.H.C., Zain, H.H.M., Ibrahim, H., 2019. Aphrodisiac properties of aquilaria malaccensis leaves aqueous extract in icr mice. *Marmara Pharmaceutical Journal* 23, 130–140. <https://doi.org/10.12991/jrp.2018.117>.
- Noyes, A.A., Whitney, W.R., 1897. The rate of solution of solid substances in their own solutions. *J Amer Chem Soc* 19, 930–934.
- Park, S.I., Heo, S.H., Kim, G., Chang, S., Song, K.H., Kim, M.G., Jin, E.H., Kim, J., Lee, S.H., Hong, J.H., 2018. Comparison of tadalafil pharmacokinetics after administration of a new orodispersible film versus a film-coated tablet. *Drug Design, Development and Therapy* 12, 935–942. <https://doi.org/10.2147/DDDT.S155040>.
- Polyakov, N.E., 2011. Glycyrrhizic Acid as a Novel Drug Delivery Vector: Synergy of Drug Transport and Efficacy. *The Open Conference Proceedings Journal* 2, 64–72. <https://doi.org/10.2174/2210289201102010064>.
- Rahman, M., Ahmad, S., Tarabokija, J., Parker, N., Bilgili, E., 2020. Spray-dried amorphous solid dispersions of griseofulvin in HPC/soluplus/SDS: Elucidating the multifaceted impact of SDS as a minor component. *Pharmaceutics* 12. <https://doi.org/10.3390/pharmaceutics12030197>.
- Selyutina, O.Y., Polyakov, N.E., Korneev, D.V., Zaitsev, B.N., 2016. Influence of glycyrrhizin on permeability and elasticity of cell membrane: Perspectives for drugs delivery. *Drug Delivery* 23, 858–865. <https://doi.org/10.3109/10717544.2014.919544>.
- Smeets, A., Koekoek, R., Clasen, C., Van den Mooter, G., 2018. Amorphous solid dispersions of darunavir: Comparison between spray drying and electrospraying. *European Journal of Pharmaceutics and Biopharmaceutics* 130, 96–107. <https://doi.org/10.1016/j.ejpb.2018.06.021>.
- Sree Harsha, N., Hiremath, J.G., Sarudkar, S., Attimarad, M., Al-Dhubiab, B., Nair, A.B., Venugopala, K.N., Asif, A.H., 2020. Spray dried amorphous form of simvastatin: Preparation and evaluation of the buccal tablet. *Indian Journal of Pharmaceutical Education and Research* 54, 46–54. <https://doi.org/10.5530/ijper.54.1.6>.
- Su, M., Xia, Y., Shen, Y., Heng, W., Wei, Y., Zhang, L., Gao, Y., Zhang, J., Qian, S., 2019. A novel drug-drug coamorphous system without molecular interactions: Improve the physicochemical properties of tadalafil and repaglinide. *RSC Adv.* 10, 565–583. <https://doi.org/10.1039/c9ra07149k>.
- Sun, X., Xu, Y., Zhao, L., Yan, H., Wang, S., Wang, D., 2018. The stability and bioaccessibility of fucoxanthin in spray-dried microcapsules based on various biopolymers. *RSC Adv.* 8, 35139–35149. <https://doi.org/10.1039/c8ra05621h>.
- Szafraniec, J., Antosik, A., Knapik-Kowalczyk, J., Chmiel, K., Kurek, M., Gawlak, K., Odrobińska, J., Paluch, M., Jachowicz, R., 2019. The self-assembly phenomenon of poloxamers and its effect on the dissolution of a poorly soluble drug from solid dispersions obtained by solvent methods. *Pharmaceutics* 11. <https://doi.org/10.3390/pharmaceutics11030130>.
- Tang, X., Olatunji, O.J., Zhou, Y., Hou, X., 2017. In vitro and in vivo aphrodisiac properties of the seed extract from *Allium tuberosum* on corpus cavernosum smooth muscle relaxation and sexual behavior parameters in male Wistar rats. *BMC Complementary and Alternative Medicine* 17, 1–10. <https://doi.org/10.1186/s12906-017-2008-5>.
- Tran, P., Pyo, Y.C., Kim, D.H., Lee, S.E., Kim, J.K., Park, J.S., 2019. Overview of the manufacturing methods of solid dispersion technology for improving the solubility of poorly water-soluble drugs and application to anticancer drugs. *Pharmaceutics* 11, 1–26. <https://doi.org/10.3390/pharmaceutics11030132>.
- Wang, Y., Zhao, B., Wang, S., Liang, Q., Cai, Y., Yang, F., Li, G., 2016. Formulation and evaluation of novel glycyrrhizic acid micelles for transdermal delivery of podophyllotoxin. *Drug Deliv.* 23, 1623–1635. <https://doi.org/10.3109/10717544.2015.1135489>.

- Wijiani, N., Isadiartuti, D., Agus Syamsur Rijal, M., Yusuf, H., 2020. Characterization and dissolution study of micellar curcumin-spray dried powder for oral delivery. *International Journal of Nanomedicine* 15, 1787–1796. Doi: 10.2147/IJN.S245050
- Yang, Z., Wang, L., Tian, L., Zhang, X., Huang, G., 2019. Tadalafil-loaded PLGA microspheres for pulmonary administration: Preparation and evaluation. *Brazilian Journal of Pharmaceutical Sciences* 55. <https://doi.org/10.1590/s2175-97902019000217536>.
- Ziaee, A., Albadarin, A.B., Padrela, L., Faucher, A., O'Reilly, E., Walker, G., 2017. Spray drying ternary amorphous solid dispersions of ibuprofen – An investigation into critical formulation and processing parameters. *European Journal of Pharmaceutics and Biopharmaceutics* 120, 43–51. <https://doi.org/10.1016/j.ejpb.2017.08.005>.