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Article

Spray-dried co-amorphous Tadalafil ternary mixtures: a promising platform towards the enhancement of solubility and bioavailability

Hoda El-Maradny[1], May Saab[2*]

[1]Department of Industrial Pharmacy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt, [2]Department of Pharmaceutical Technology, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon

Tadalafil (Tad) is a poorly water-soluble drug (BCS class II) that is used for the treatment of erectile dysfunction. An enhancement of aqueous solubility is vital to accelerate its onset of action and subsequently enhance its therapeutic effect. Binary and ternary mixtures of Tad with different amino acids (histidine, valine, alanine or arginine) and other excipients (mannitol and SLS) were prepared and then spray dried. The solubilizing efficiency and physicochemical characterization of all spray dried mixtures of Tad were studied. The optimum formulation was investigated in male rats to determine the onset of erection and the pharmacokinetic parameters of Tad. In general terms, the drug solubility of spray-dried formulae was enhanced compared to the crystalline form of the drug as a result of the formation of co-amorphous structures. The final result revealed that the Tad/alanine/mannitol spray-dried mixture (F10) showed the highest solubility and an improvement in its physicochemical characteristics. Moreover, F10 showed a significantly faster erection in rats with an improvement in Tad pharmacokinetic parameters when compared to the crystalline drug. Thus, F10 is selected as a promising formulation that successfully enhanced the bioavailability and the therapeutic efficacy of Tad.

Keywords: Tadalafil. Spray drying. Co-amorphous. Solubility. Bioavailability.

INTRODUCTION

Around 40% of all active pharmaceutical ingredients (APIs) currently available on the market, and 90% of the drug molecules under development display poor aqueous solubility (Babu, Nangia, 2011). The main concern with poorly water-soluble drugs is the low and unpredictable drug absorption that would result in poor bioavailability and inadequate therapeutic efficacy (Babu, Nangia, 2011; Hauss, 2007). Therefore, many competitive pharmaceutical plants have increased their interest in improving the apparent solubility of poorly water-soluble drugs (Jensen *et al.*, 2016).

Several attempts have been made to improve the apparent solubility and, henceforward, the bioavailability of APIs. Among the most common and interesting approaches is the conversion of crystalline APIs into their corresponding amorphous forms (Jermain, Brough, Williams, 2018; Vo, Park, Lee; 2013). However, an enhanced dissolution of pure amorphous drugs cannot be guaranteed due to their high tendency for recrystallization (Hancock, Zografi, 1997). Therefore, the development of polymeric amorphous solid dispersion systems was a solution to such a drawback. However, although amorphous solid dispersions, based on high molecular weight polymers, have improved drug dissolution (Jermain, Brough, Williams, 2018; Vo, Park, Lee; 2013; Zheng *et al.*, 2012), long term stability of such systems is not guaranteed and they face many manufacturing challenges in solid dosage form processing, including flowability and compressibility (Janssens, Van den

***Correspondence:** M. Saab. Department of Pharmaceutical Technology. Faculty of Pharmacy. Beirut Arab University. Beirut 11072809, Lebanon. Phone: +9613761032. E-mail: saabk.may79@bau.edu.lb. ORCID: https://orcid.org/0000-0001-5296-4838

Mooter, 2009; Srinarong *et al.*, 2011). Co-amorphous systems, based on mixing the drug with a low molecular excipient at the molecular level, have been investigated for solubility enhancement of drugs and proved to be a promising alternative to the high molecular weight polymers (Craye *et al.*, 2015; Löbmann *et al.*, 2013). Amino acids are among the low molecular weight co-formers that have been frequently investigated (França *et al.*, 2020; Kasten *et al.*, 2019; Wu *et al.*, 2018). Through their specific and non-specific molecular interactions with drugs, they have shown an improvement in aqueous solubility and the physical stability of the co-amorphous systems (Jensen *et al.*, 2016; Löbmann *et al.*, 2013). Moreover, low molecular weight excipients, such as mannitol and sodium lauryl sulphate, were also studied in co-amorphous systems, and demonstrated high stability and an enhanced dissolution rate of poorly water-soluble drugs (Craye *et al.*, 2015; Yadav *et al.*, 2013).

Tadalafil (Tad) is a crystalline drug that is used for the treatment of erectile dysfunction (Aboul-Enein, Ali, 2005; Hussar, 2003; Reddy, Reddy, 2008). Its poor aqueous solubility is the limiting factor for its absorption, resulting in low bioavailability and reduced therapeutic efficacy (Baek *et al.*, 2015). Various amorphization techniques have been implemented in earlier studies to enhance Tad solubility, including, cryogenic grinding, ball milling, freeze drying, anti-solvent precipitation and spray drying (Rao *et al.*, 2019; Wlodarski *et al.*, 2016, 2015, 2014). Nevertheless, to our knowledge, no study has been conducted on co-amorphous Tad systems using amino acids and other low molecular weight excipients.

In the present study, different amino acids with various aqueous solubilities, namely, histidine, valine, alanine and arginine, are examined for the first time as potential co-formers for amorphous Tad systems. In addition, other low molecular weight excipients, namely, mannitol and sodium lauryl sulphate were investigated as a part of the co-amorphous system towards the further enhancement of drug solubility, and hence, accelerating and optimizing its therapeutic effect. The spray drying technique was adopted to prepare the co-amorphous formulations as a feasible and reproducible method on a large industrial scale (Démuth *et al.*, 2015).

MATERIAL AND METHODS

Tadalafil was kindly donated by Benta Pharma Industries, Lebanon.

Mannitol (molecular weight 182.17, aqueous solubility 216 mg/ml) and Sodium lauryl sulphate (molecular weight 288.38, aqueous solubility ~ 100 mg/ml), were purchased from Sigma-Aldrish, USA. L-alanine (molecular weight 89.09, aqueous solubility 164 mg/ml), L-arginine (molecular weight 174.2, aqueous solubility 148.7 mg/ml), L- valine (molecular weight 117.15, aqueous solubility 88.5 mg/ml), L-histidine (molecular weight 155.15, aqueous solubility 41.6 mg/ml), were all obtained from Fluka, Germany. Acetone and all other chemicals of pure grade were also purchased from Fluka, Germany.

Preparation of spray-dried powders

Spray-dried powders were prepared by spray drying Tad alone or with other excipients from acetone/water solutions (Table I). Variation in acetone/water ratios was essential in order to prevent the precipitation of Tad and excipients prior to spray drying.

All mixtures were spray dried separately using a lab plant spray dryer (Lab Plant, SD-O6AG, Fiely, North Yorkshire, England). Solutions were delivered to the fluid nozzle (0.5 mm in diameter) at a flow rate of 5 mL/min by a peristaltic pump, and sprayed with atomizing pressure of 4 bar. Inlet and outlet temperature were maintained at 90ºC and 52ºC, respectively.

Spray-dried powders were gathered from the sample collection unit and stored in a desiccator at 25ºC for further investigations. The percentage yield ranged between 22% and 51%.

TABLE I - Composition of spray dried formulations

Formula		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
% w/v (drug:excipient, molar ratio)	L-histidine	-	0.20 (1:1)	-	-	-	-	-	-	-	-	-
	L-valine	-	-	0.15 (1:1)	-	-	-	-	-	-	-	-
	L-alanine	-	-	-	0.11 (1:1)	-	0.11 (1:1)	0.11 (1:1)	0.11 (1:1)	0.11 (1:1)	0.11 (1:1)	0.11 (1:1)
	L-arginine	-	-	-	-	0.22 (1:1)	-	-	-	-	-	-
	Tad	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Mannitol	-	-	-	-	-	0.25 (1:1)	-	0.5 (1:2)	-	1 (1:4)	-
	SLS	-	-	-	-	-	-	0.37 (1:1)	-	0.75 (1:2)	-	1.5 (1:4)
Acetone:water (v:v ratio)		70:30	60:40	70:30	70:30	75:25	70:30	70:30	70:30	70:30	70:30	70:30
Yield (%)		22	25	26	25	23	43	26	45	24	51	27

Preparation of physical mixtures

Physical mixtures were prepared at the same ratios that were used to prepare spray-dried samples. Homogenous binary or ternary mixtures were obtained by grinding the components for 5 minutes in a mortar.

Solubility study

To study the apparent solubility of Tad from different processed powders, an excess amount equivalent to 10 mg of Tad was weighed and suspended in 10 ml purified water at 37ºC in a 20 ml vial. The vials were shaken (100 cpm) at 37ºC for 24 h in a shaker (WB-MF24, Falc, Italy). The resulting suspensions were filtered through Millipore filters (0.45 μm) and spectrophotometrically assayed at 284 nm using a preconstructed calibration curve ($y=0.0298x+0.0443$, $R^2=0.998$). Each experiment was repeated in triplicate and average results were obtained. Results were expressed as mean ± S.E.D. The level of statistical significance was assessed by ANOVA (one-way analysis of variance) using SPSS 20 (SPSS Inc., Chicago, USA) followed by Tuckey's test. Results with a *p*-value of less than 0.05 were considered statistically significant.

X-ray powder diffraction (XRPD) analysis

XRD analysis of all samples was performed using the Miniflex II powder diffractometer (Rigaku, Japan), equipped with a Cu K-alpha radiation. The X-ray source was operated under 30 kV and 15 mA. The samples were separately placed on a low-background silicon holder and scanned at room temperature over a range of 5–50º at a step size of 0.05°/s.

Fourier Transform Infrared (FT-IR)

FT-IR spectra of individual crystalline and amorphous materials, as well as their physical mixtures and corresponding spray-dried formulations, were investigated using the Bruker Alpha FTIR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) at ambient temperature. Powder samples were compacted by a

spring-loaded mechanical press with constant and reproducible pressure. The spectra were measured with a scanning range of 4000–400 cm^{-1} at a spectral resolution of 2 cm^{-1}.

Scanning Electron Microscope (SEM)

The morphological examination of crystalline, amorphous and co-amorphous Tad was carried out using SEM (ASC-2100, Seron technology, Korea). Powder samples were placed on an aluminium stub and subsequently covered by a carbon strip. The samples were coated with a thin layer of palladium gold alloy using the Cressington sputter coater 10802 at less than 0.1 millibar pressure and 20 mA current. The samples were then scanned in different fields with a specified magnification power (2000x).

In-vitro dissolution study

Purified water was selected as the dissolution medium of the powder since the solubility of Tad is pH-independent (Williams *et al.*, 2013). The dissolution study of powder equivalent to 10 mg Tad was carried out in an USP dissolution apparatus II (type T1500, Erweka, Germany) containing 900 ml purified water kept at 37°C ± 0.5 °C and rotated at 75 rpm (Wlodarski *et al.*, 2015). At predetermined time intervals, aliquots (5 ml) were withdrawn and instantly replaced with a fresh medium. Samples were then analyzed spectrophotometrically at λ max 284 nm. All determinations were carried out in triplicate.

Stability study

To examine the physical stability of the optimum spray-dried mixture, a powder sample of the selected formulation was stored in a desiccator at 40 ºC and 0% relative humidity (Jensen *et al.*, 2016). After 6 months of storage, the powder was analyzed under SEM to detect any recrystallization. Moreover, change in its solubility was also investigated and analyzed statistically using t-test, where the *p* value of less than 0.05 was considered as statistically significant. Additionally, a dissolution study was conducted for the stored sample, and the resulting dissolution profile was compared with that of a freshly prepared sample using the similarity factor (f2). In case of similar dissolution profiles, the value of f2 should range between 50 and 100, indicating that the difference in the percentage of the drug dissolved does not exceed 10 (Soni *et al.*, 2008). The similarity factor is calculated as follows:

$$f2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum(Rt - Tt)^2\right]^{-0.5}\right\} \times 100$$

where n is the number of time intervals, R_t and T_t are the percentage drug release of the reference sample (freshly prepared) and the tested sample (stored), respectively, at time t.

In-vivo study

a. Onset of erection:

Twelve Sprague-Dawley adult male rats (average weight 200 g) were used to investigate the effect of the optimum formulation on the onset of erection. The experimental protocol was ethically approved by the Institutional Review Board (IRB), Beirut Arab University, Beirut, Lebanon. The procedure followed the European directives for animal experiments (2010/63/EU). Rats were maintained at the animal house under a controlled environment (22 ± 2 °C, and 50-60 % relative humidity) for at least 7 days prior to the study. Afterwards, acclimatised rats were separated into 2 equal groups (A and B) and fasted for over 12 hours before starting the experiment. Firstly, the oral dose of the rat was calculated based on a reference guide for converting doses between animals and humans (Nair, Jacob, 2016). From the optimum formulation, an equivalent amount of Tad dose (360 µg/200g) was weighed and suspended in 3 ml of water, and subsequently administered to group A (n = 6) by oral gavage. Similarly, an equal dose of crystalline Tad was prepared and administered to group B (n = 6). Afterwards, the rats were restrained in a supine position in order to detect the time needed to initiate an erection.

b. Bioavailability:

For both groups (A and B), blood samples (approximately 200 µL each) were collected from the lateral tail vein using a 21G-needle cannula at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours following the oral administration. After each blood sampling, the cannula was immediately flushed with heparinized isotonic saline solution (20 units/mL) to prevent blood clotting. Afterwards, blood samples were separated by centrifugation, and plasma aliquots (200-µL) were stored at −80°C until the Tad analysis.

Prior to analysis, samples were freeze-thawed and the Tad was extracted by vortex-mixing a 200 µl plasma with a 500 µl acetonitrile, followed by sonication for 5 minutes and centrifugation at 15,000 rpm for 20 minutes. Then, 100 µl of the supernatant layer was subsequently withdrawn and analyzed using the high performance liquid chromatography method (HPLC) (Mande, Bachhav, Devarajan, 2017). The mobile phase consisted of a mixture of acetonitrile and phosphate buffer pH 3 (1:1 v/v) flowing at a rate of 1.2 ml/minute through the column (C18, waters, Sunfire, 5µm, 4.6*250 mm, Ireland). Samples were detected spectrophotometrically at λ_{max} 285 nm using a UV detector (Waters 2487 Dual λ absorbance, Waters corporation, Milford, MA). Tad plasma concentrations were plotted against time and pharmacokinetic parameters were calculated using pk solver (V 2.0) software. The time to initiate erection and the computed pharmacokinetic parameters in each group were expressed as means ± S.E.D. The level of the significance of difference in the results between the two groups was assessed statistically by ANOVA, where a *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Solubility study

The solubility data of crystalline and spray-dried Tad formulations are illustrated in Table II. Upon investigating the solubility of spray-dried Tad (5.11 µg/ml ± 0.13 µg/ml), a significant increase in drug solubility was observed ($p<0.05$) when compared to its crystalline form (2.49 µg/ml ± 0.04 µg/ml). In an attempt to obtain a higher drug supersaturation, the effect of various amino acids on the solubility of the drug was investigated. Physical mixtures of Tad with different excipients were prepared and solubility studies were conducted. None of the mixtures showed any notable difference in the drug solubility when compared to that of the drug alone (data not shown). On the other hand, when different amino acids were spray-dried with Tad, the effect of the amino acids on increasing the drug solubility was significant ($p<0.05$). Among the binary spray-dried formulations, the highest solubility was recorded for Tad/alanine formulation (F4), recording a supersaturation of 7.29 µg/ml ± 0.04 µg/ml. Regarding the spray-dried ternary formulations F6 and F7 (consisting of equimolar concentrations of Tad, alanine and excipient), no significant increase in drug solubility was observed when compared to F4. Once the concentration of the excipient was doubled (1:2 Tad to excipient molar ratio) in F8 and F9, a significant improvement in drug solubility was observed. Moreover, when the drug: excipient molar ratio increased up to 1:4 (F9 and F10), a remarkable improvement in drug solubility was recorded, where mannitol-based formulation (F10) exhibited the highest apparent solubility (15.12 µg/ml ± 0.13 µg/ml). It should be noted that a further increase in mannitol concentration has shown difficulty in spray drying, hence, a higher concentration was not advocated.

TABLE II - Solubility data of Tad formulations

Formulation	Solubility (μg/ml)	Formulation	Solubility (μg/ml)
Crystalline Tad	2.49 ± 0.04	F6*	7.42 ± 0.18
F1	5.11 ± 0.13	F7*	7.37 ± 0.08
F2	5.54 ± 0.12	F8**	8.54 ± 0.07
F3	6.02 ± 0.09	F9**	8.22 ± 0.10
F4	7.29 ± 0.04	F10***	15.12 ± 0.13
F5	5.64 ± 0.12	F11***	11.29 ± 0.05

* Ternary system with 1:1:1 Tad/alanine/excipient molar ratio
** Ternary system with 1:1:2 Tad/alanine/excipient molar ratio
** *Ternary system with 1:1:4 Tad/alanine/excipient molar ratio

XRPD

Results of XRPD study conducted for individual materials and binary spray dried mixtures are illustrated in Figure 1. The XRPD diffractogram of original Tad displayed intense peaks at 7.14°, 10.50°, 12.42°, 14.42°, 18.32° and 21.56° with peak intensities of 1849.3, 442, 353.78, 666.97, 553.12 and 456.91, respectively, indicating the crystalline nature of the drug (Nanjwade *et al.*, 2011). The diffraction pattern of spray dried Tad and binary spray dried mixtures showed amorphous halos, thereby, indicating complete amorphization of the drug. The only exception was with Tad/valine spray dried formulation (F3) that revealed a small peak at 7.14° representing crystalline residues of Tad.

Diffraction patterns of ternary spray dried mixtures (F10 and F11) and their pure components are illustrated in Figure 2. All spray dried formulations revealed absence of peaks indicating complete amorphization, except for Tad/alanine/SLS formulation (F11) that showed an emerging small peak corresponding to remaining crystalline domains of SLS and Tad.

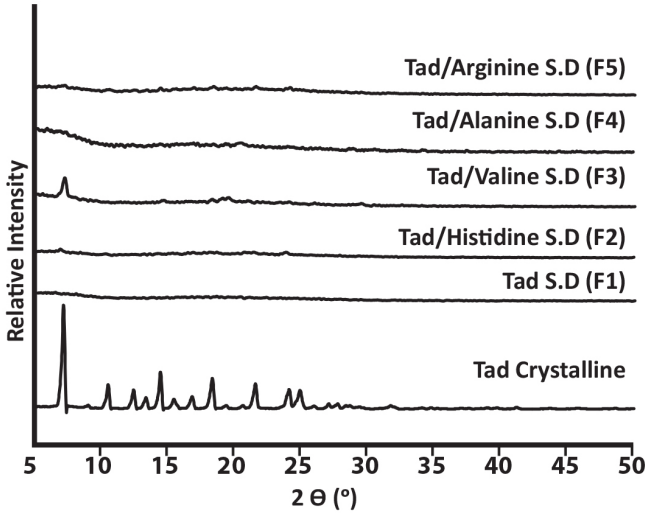

FIGURE 1 - X-ray diffractograms of original Tad, spray dried Tad (F1) and spray dried binary mixtures (F2-F5).

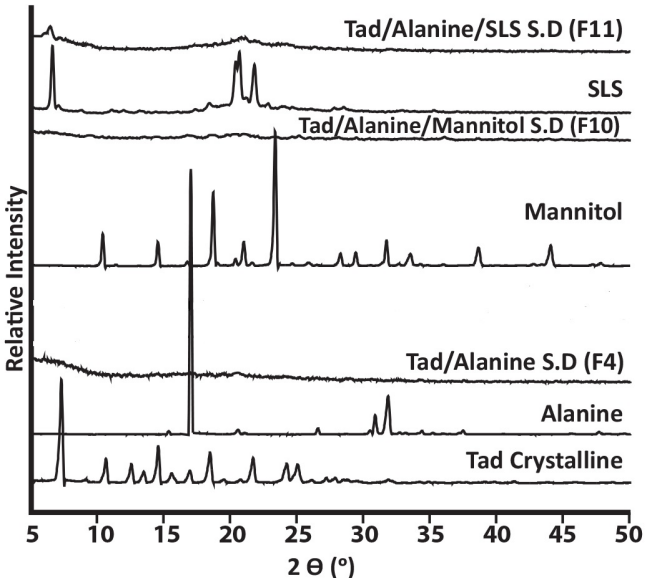

FIGURE 2 - X-ray diffractograms of spray dried binary and ternary mixtures (F4, F10 and F11) and their pure components.

FTIR

The IR spectra of the spray dried formulations (F1, F4, F10 and F11) and their pure components are represented in Figure 3. IR spectra of crystalline Tad is characterized by absorption peaks at 3324 cm^{-1} (N-H stretching of secondary amine), 3091 cm^{-1} (C-H stretching of aromatic ring), 2904 cm^{-1}(C-H stretching of aliphatic CH3), 1675 cm^{-1} (C=O stretch of amide), 1645 cm^{-1} (C=C of aromatic ring), 1435 cm^{-1} (C-N stretch), 1041 cm^{-1} (C-O-C stretching) and 744 cm^{-1} (benzene). Physical mixtures revealed all the characteristic bands without any shift in their spectra compared to individual components (data not shown), demonstrating that there were no interactions between the components in the mixture. However, some of the distinctive peaks were difficult to detect due to overlapping of the peaks of each component in the mixture.

Upon spray drying Tad alone, minor variations in its vibrational mode were observed. Such variations were mainly attributed to its molecular rearrangement as a result of its transformation into an amorphous form (Williams *et al.*, 2013). On the other hand, the IR spectrum of Tad/alanine binary mixture (F4) showed a broadening of all bands, a smoothing of the characteristic N-H stretch-peak of Tad and a distortion of the C=O stretch peak of its amide group. Similar findings were also detected in most of FTIR bands of the spray dried ternary mixtures (F10 and F11). However, no additional bands were observed, indicating the absence of any chemical interactions.

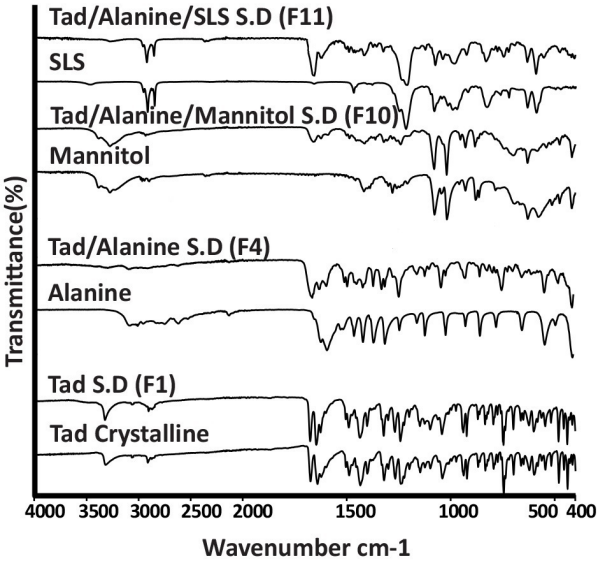

FIGURE 3 - FTIR spectra of spray dried binary and mixtures (F4, F10 and F11) and their pure components.

SEM

SEM images of pure Tad powder, spray dried Tad (F1), Tad/alanine spray dried mixture (F4) and the optimum spray dried ternary mixture (F10) are demonstrated in Figure 4. Pure Tad powder revealed its prismatic crystalline nature (Figure 4a), whereas, spray dried Tad, binary, and ternary mixtures presented an almost spherical microparticles ranging approximately between 2 µm and 10 µm in size, with no irregularities and large surface area (Figures 4b, 4c and 4d).

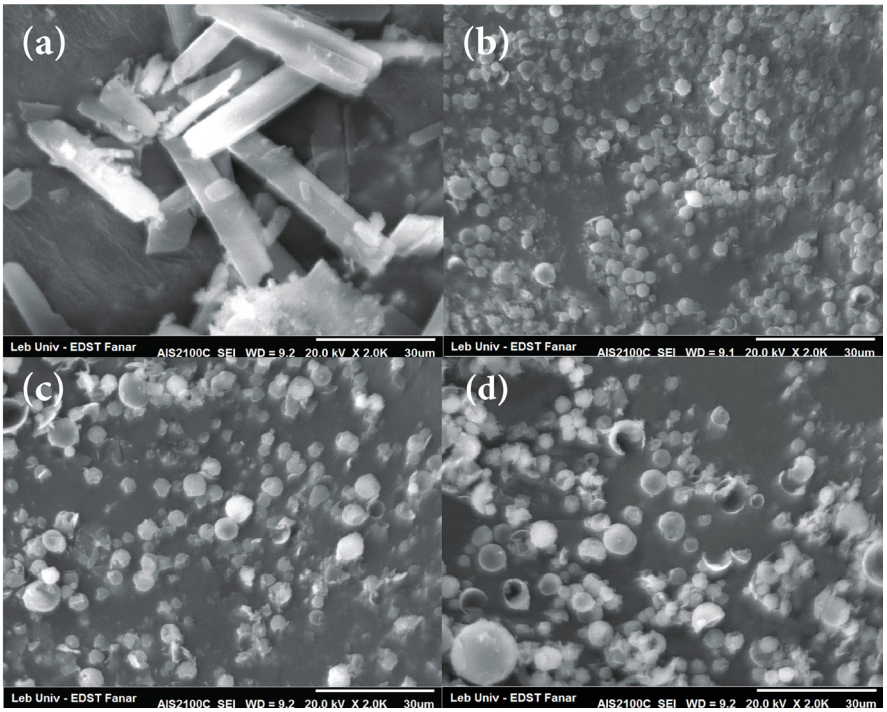

FIGURE 4 - SEM images of Tad powders. (a) Original Tad. (b) Spray dried Tad alone (F1). (c) Tad/alanine binary spray dried mixture (F4). (d) Tad/alanine/mannitol ternary spray dried mixture (F10).

In-vitro dissolution study

Dissolution studies were conducted for pure crystalline Tad, spray dried Tad (F1), binary spray dried mixture (F4) and ternary spray dried mixtures with the highest excipient concentration (F10 and F11) (Figure 5).

Spray dried formulations showed a remarkable increase in Tad dissolution rate when compared to that of crystalline Tad; after 1 hour, only 6.2% ± 0.5% of crystalline Tad was dissolved in comparison with 12.9 % ± 0.7 %, 18.4 % ± 1.1 %, 63.8% ± 1.1 % and 41.2% ± 0.8% released from formulations F1, F4, F10 and F11, respectively. Interestingly, Tad/alanine/mannitol ternary mixture (F10) showed the highest release profile of Tad with a complete drug release after 6 hours.

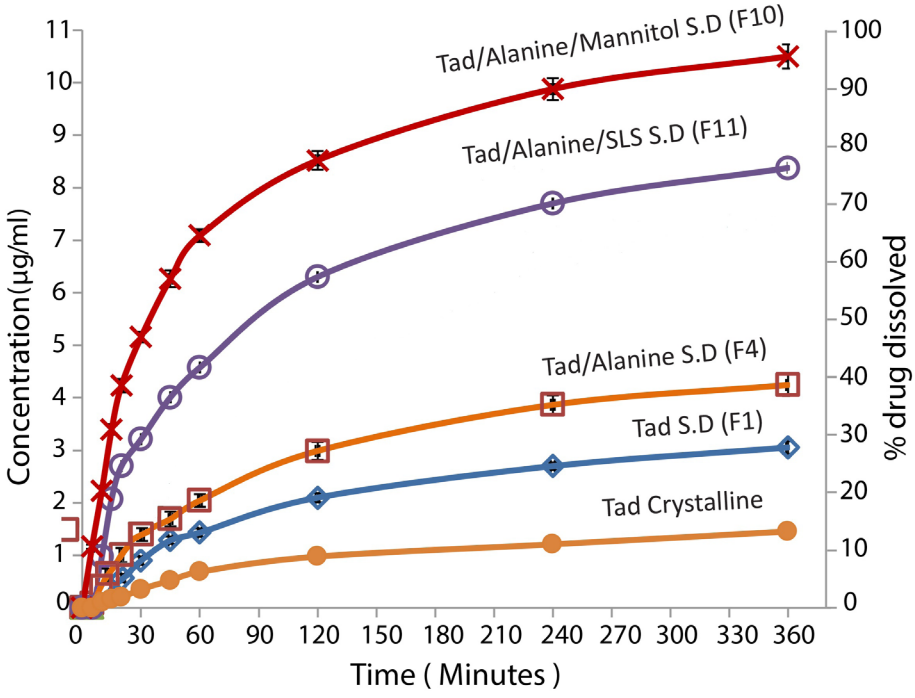

FIGURE 5 - Dissolution Profiles of crystalline Tad and Tad spray dried formulations (F1, F4, F10 and F11) in water at 37 °C

Stability study

The stability study showed that F10 was physically stable after a 6 month storage in the desiccator at 40˚C. This was confirmed by SEM, which revealed that the spherical shape and size of microparticles were still retained (Figure 6). Moreover, an insignificant difference in the solubility of the stored powder was observed (14.47 µg/ml ± 0.22 µg/ml) when compared to the fresh one (15.12 µg/ml ± 0.13 µg/ml), recording a p value of > 0.05. Likewise, the dissolution profiles of the fresh and stored powder were the same, as the value of the calculated similarity factor f2 was higher than 50 (71.7).

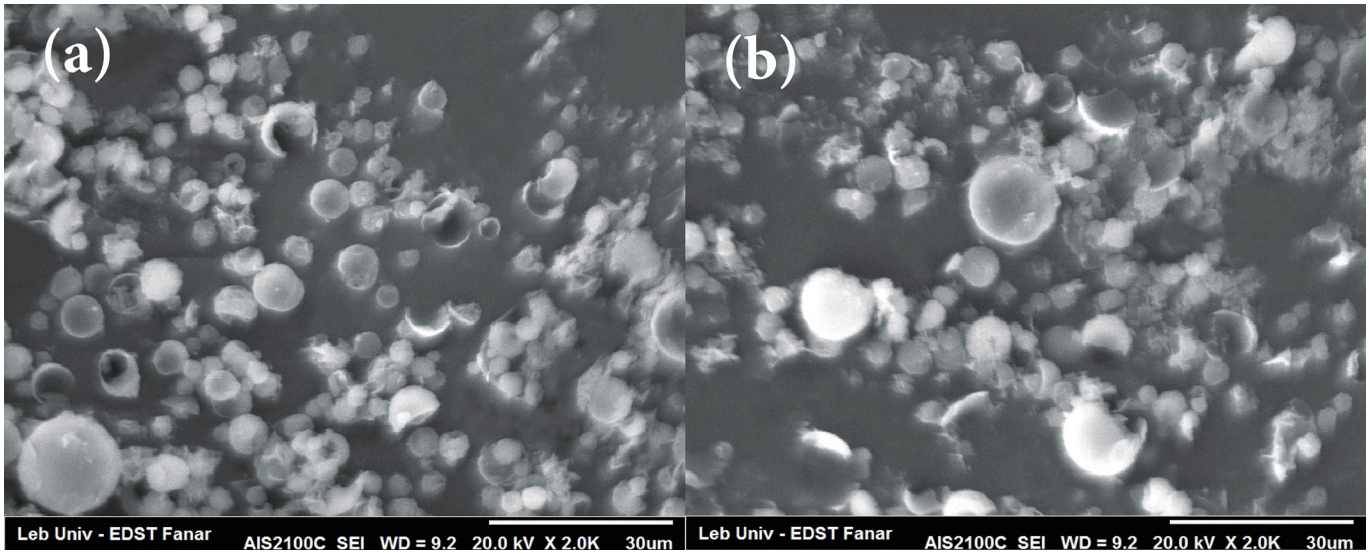

FIGURE 6 - SEM images of spray dried Tad/alanine/mannitol mixture (F10). (a) Freshly prepared powder. (b) Powder stored at 40° C for 6 months.

In-vivo study

Administration of either the optimum Tad formulation (F10) or the crystalline form of the drug to male rats (group A and B, respectively) have resulted in erections, which were observed after a period time. Interestingly, the average onset of erection in group A (15.8 min ± 3.8 min) was faster when compared to that observed in group B (30.8 ± 5.9 min) which was statistically significant with a *p* value of less than 0.05 (0.0004).

Furthermore, to support the aforementioned data, the plasma concentration-time profiles (Figure 7) and the pharmacokinetic parameters (Table III) were investigated; higher drug concentrations were observed in group A when compared to group B, and the pharmacokinetic parameters revealed *circa* a 2.5- and a 2-fold increase in C_{max} and $AUC_{0-\infty}$ values, respectively, and 2.4 times faster T_{max}, for F10 when compared to crystalline Tad.

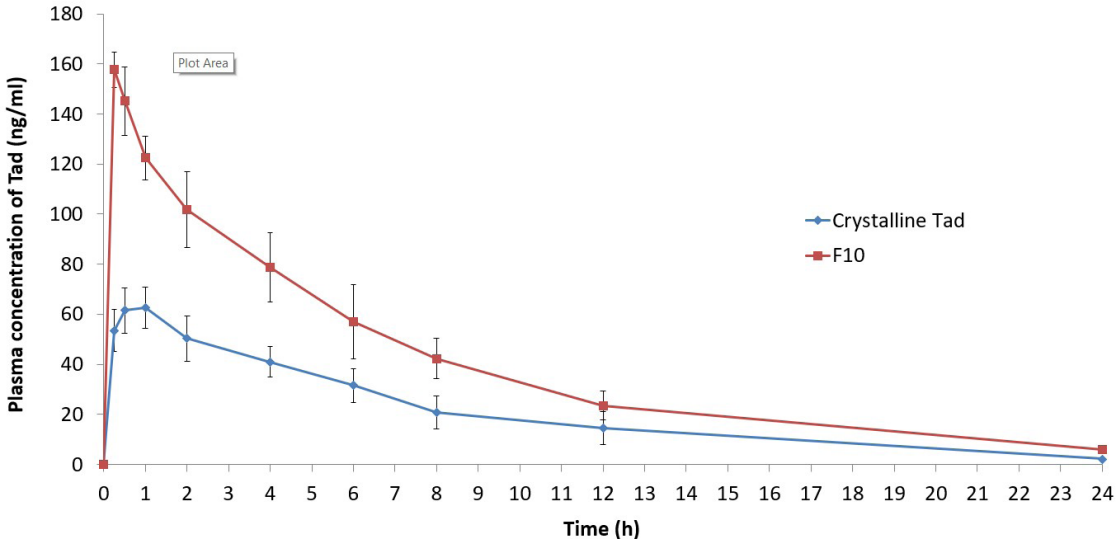

FIGURE 7 - Plasma concentration-time profiles of Crystalline Tad and F10 (n = 6). Values are displayed as means ± S.E.D.

TABLE III - Pharmacokinetic parameters of F10 and Crystalline Tad

Pharmacokinetic parameters	Crystalline Tad	F10
C_{max} (ng/ml)	64.2 ±8.2	159.5 ±7.6**
T_{max} (h)	0.75 ± 0.28	0.31 ± 0.12*
$AUC_{0-\infty}$ (ng/ml.h)	509.3 ± 83.9	1010.2 ± 158.2**
Relative bioavailability	1	1.98

Values are represented as mean ± S.E.D
*p<0.05
** p< 0.01

DISCUSSION

In the current study, a spray drying technique was adopted as a practical method to prepare co-amorphous Tad systems in an attempt to improve drug solubility and accelerate its therapeutic effect. Low molecular weight excipients were investigated for the first time with Tad as potential co-formers to overcome processing challenges that would be encountered with high molecular weight polymers.

Spray-dried Tad (F1) revealed an increase in drug solubility as compared to its crystalline form (Table II). The breakage of the crystal lattice of Tad and the reduction of its particle size to a molecular level by spray drying are assumed to be the cause of the phenomenon of supersaturation (Sun, Lee, 2015; Williams *et al.*, 2013). This was confirmed by the disappearance of XRPD peaks indicating their transformation into an amorphous form (Figure 1).

Binary spray-dried mixtures (F2-F5) revealed higher drug solubility when compared to F1. Mixtures were completely amorphous, as indicated by the disappearance of their characteristic peaks in XRPD diffractograms, with the exception of the Tad/valine mixture (F3) that displayed an emerging small peak of Tad, thereby, signifying its incomplete transformation to an amorphous state (Figure 1). The highest solubility was recorded for the alanine-based binary spray-dried mixture (F4), with an around 3-fold increase in Tad solubility when compared to the crystalline drug form (Table II), and hence alanine was selected as the optimum amino acid for further investigations. The superiority of alanine in increasing the drug solubility may be attributed to the complete co-amorphization of the corresponding spray-dried mixture and its higher hydrophilicity when compared to histidine, valine and arginine. Similarly, in a previous study, an enhanced dissolution of naproxen was mainly attributed to the hydrophilicity of the amino acid (proline) and the production of a co-amorphous mixture (Jensen *et al.*, 2014). A further increase in the molar concentration of alanine in the binary system did not reveal any additional improvement in drug solubility. Accordingly, ternary mixtures were investigated.

Spray-dried ternary mixtures with a molar ratio of 1:1:1 Tad/alanine/excipient (F5 and F6) did not show any significant enhancement in drug solubility. On the other hand, spray-dried ternary mixtures with a higher molar ratio of mannitol or SLS demonstrated a remarkable increase in drug solubility (F8-F11) when compared to the binary system F4 (Table II). Such findings may suggest that the enhancement of Tad solubility in a ternary system was mainly dominated by the molecular dispersion of the system's components within the excipient (namely, mannitol or SLS), rather than their individual interactions at the molecular level with the latter. Although the hydrophilic surfactant SLS contributed in enhancing the drug solubility which would be attributed to micellar solubilization, the solubilizing effect of the highly hydrophilic mannitol was superior. F10 recorded around two-fold higher drug solubility compared to F4, and the total increase in drug solubility with respect to the original crystalline Tad was around 6 folds (Table II).

The physico-chemical characterization of binary and ternary spray-dried formulations (F4 and F10) depicted no distinct XRPD peaks, indicating the complete amorphization of the system (Figure 2). On the other hand, crystalline residues, manifested as small visible peaks corresponding to Tad and SLS, were demonstrated in the diffractogram of the Tad/alanine/SLS spray-dried mixture (F11) (Figure 2). This is in accordance with a previous study conducted by Craye *et al.* (2015), where a spray-dried co-amorphous system of simvastatin,

lysine and SLS revealed amorphous halos with residual crystallinity of its components. The remaining crystalline domains in F11 would explain the superiority of the F10 formulation in attaining a higher Tad solubility. The FTIR spectrum of spray-dried mixtures revealed a smoothing of the characteristic N-H stretch peak of Tad and a distortion of the C=O stretch peak of the amide group of the latter, which could be attributed to a hydrogen bonding with other excipients (Wlodarski *et al.*, 2016, 2015). Furthermore, a broadening of the bands in spray-dried samples could also be an indication of the physical interactions of the various components in the mixture (Wlodarski *et al.*, 2016). The interactions of these hydrophilic excipients with Tad in an amorphous state may have contributed to the improvement in its solubility, as a result of an increase in the surface contact of Tad particles with the aqueous medium (Wlodarski *et al.*, 2015). On the other hand, some of the characteristic bands of Tad in F11 were not remarkably broadened when compared to that of F10, which indicated an incomplete amorphization, confirming the results obtained by XRPD, and signifying the advantage of mannitol over SLS in enhancing Tad solubility.

In conclusion, the enhancement of Tad solubility and its improved dissolution rate in F10 was mainly attributed to an increase in the surface contact of Tad particles with the aqueous medium that would be explained by the complete co-amorphization of the drug with hydrophilic excipients. This was also elucidated by the visualization of spherical particles under SEM (Figure 4d).

Finally, the in-vivo investigation of the selected formulation (F10) in rats (group A) demonstrated an approximately 50% reduction in the time needed to initiate erection ($p<0.05$) when compared to the crystalline Tad (group B). Such findings were further confirmed by the remarkable improvement in the plasma concentration-time profile (Figure 7), with a significant acceleration in T_{max}, a higher C_{max} and an enhanced oral bioavailability as compared to the crystalline untreated form (Table III). This implies that alanine and mannitol were successful co-formers in preparing amorphous Tad formulation; such excipients were able to remarkably improve the bioavailability and therapeutic effect of Tad.

CONCLUSION

Based on the above findings, the spray-dried ternary mixture F10 was selected as the optimum powder formulation. It exhibited the highest solubility (15.12 ± 0.13 µg/ml) and dissolution rate (complete dissolution at 6 hours) as a result of the co-amorphization of Tad with alanine and mannitol. The F10 formulation has also demonstrated a physical stability for more than 6 months at 40˚C and 0 % relative humidity. Moreover, the in-vivo study in rats revealed an acceleration and enhancement in the therapeutic effect of the drug that was manifested by a significantly faster erection, higher plasma concentrations and improved bioavailability. Thereby, F10 showed to be a promising formulation that increased the Tad dissolution and accelerated its onset of action and subsequently optimized its therapeutic effect.

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