Polymorphism exists extensively in solid drugs during the crystallization process due to changes of recrystallization conditions, and may show different physicochemical properties, such as solubility, dissolution, compressibility and so on. Currently, about 40% of commercial drugs and 75% of drug candidates are poorly soluble, which growing rapidly with oral bioavailability varying greatly, and solubility becomes the limiting factor for the absorption of drugs in vivo. Therefore, it is important to develop effective methods to improve the solubility and dissolution rate of insoluble drugs. The solubility of drugs mainly depends on the interaction of drug molecules as well as the affinity of solute and solvent. There are various pharmaceutical methods to improve the solubility of such drugs including amorphous or metastable crystal forms, solid dispersion systems, cocrystals and so on at modification of raw drug material level. Recently, thermodynamics of drug polymorphism becomes one of the hot spots in the field of physical pharmacy, since it is closely related to the selection and optimization of preparation processes of raw materials during the drug development. The relative stability of polymorphs and amorphous states is a major concern in the pharmaceutical industry, since multiple properties of the active pharmaceutical ingredient (API) will be affected, such as dissolution, bioavailability, machinability, and stability.

Tadalafil (TD, Fig. 1), a phosphodiesterase-5 (PDE-5) inhibitor with poor oral bioavailability. The aim of the study was to prepare and characterize three crystalline polymorphs of TD (II, III, and IV) and the tadalafil amorphous form (TD-AM). TD polymorphs and TD-AM were prepared and characterized by polarized light microscope (PLM), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), X-ray powder diffractometry (XRPD), and Fourier-transform (FT)IR, followed by the dissolution testing, physical stabilities and polymorphic transformation studies. TD-I and TD-II were found to be enantiotropically related, while TD-III was monotropically related to TD-I with heat release. Among all studied polymorphs, TD-AM demonstrated an extremely high intrinsic dissolution rate with most prolonged higher saturated concentration during dissolution, while TD-II, TD-III, and TD-IV converted to TD-I easily by supersaturation-mediated phase transformation. Upon heating under 60°C for 3 h and storing at long-term stability condition for 3 months, no phase transformation was detected for TD-I, TD-III, and TD-AM, while TD-II and TD-IV easily transformed to TD-I and TD-III, respectively. The higher intrinsic dissolution rate, prolonged supersaturated state during dissolution and favorable physical stability of TD-AM made it to be a very promising candidate for further product development.

Key words polymorph; amorphous; transformation; solubility; stability
Experimental

Materials Tadalafil (form I, TD-I, 99.6% purity) was gifted by Zhejiang Yongning Pharmaceutical Co., Ltd. (Zhejiang, China). Acetonitrile and methanol of HPLC grade were purchased from E. Merck (Darmstadt, Germany). All other chemical reagents were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of TD Polymorphs With reference to the information of US 20060111571 A1 patent, three polymorphs of TD crystals were prepared according to following recrystallization procedure. TD-I (2.5 g) was homogeneously dispersed in 1000 mL of dichloromethane at room temperature, and then added 800 mL of petroleum ether slowly with stirring to obtain a precipitate, which was collected by filtration and dried under vacuum at 25°C for 24 h to form II (TD-II).

TD-II (0.5 g) was re-dispersed in 6 mL of methanol or toluene and stirred for 12 h to obtain a slurry. The solvent of slurry was removed by filtration and the resulting solid was dried under vacuum at 25°C for 24 h to obtain form III (TD-III) or form IV (TD-IV).

Amorphous state of TD (TD-AM) was prepared by solvent evaporation method. Briefly, TD-I (0.2 g) was dissolved in a solvent mixture comprising of 20 mL of methanol, 25 mL of acetonitrile and 25 mL of dichloromethane. Then, solvent evaporation method was used to remove the organic solvents by a vacuum evaporator at 40–45°C in a water bath. The residue solid was collected after solvent evaporation. All obtained samples were sieved through 100 mesh (ca. 150 μm) and stored in a desiccator over anhydrous calcium chloride at 25°C for further study.

Physicochemical Characterizations

Polarized Light Microscope (PLM) and Scanning Electron Microscopy (SEM)

Morphological evaluation was performed by PLM (Leica DM LM/P; Pixelink lens, Leica, Germany) and SEM (Hitachi S-3400N; Hitachi, Tokyo, Japan). For PLM, about 2–4 mg of samples was evenly dispersed in liquid paraffin on a glass slide to observe the PLM pattern. For SEM, powder samples were glued and mounted on metal sample plates with gold scraping the tablet. Powders were analyzed by XRPD to examine their crystallinity.

X-Ray Powder Diffractometry (XRPD)

X-ray power diffractometer (Thermo ARL™ XTRA, Thermo Fisher, Waltham, U.S.A.) with a Cu-Kα radiation (λ=1.5406 Å) source was used. The tube voltage and amperage were set at 40 kV and 40 mA, respectively. For each sample, XRPD pattern was collected in the 2θ range of 3–40° with a scanning speed of 4°/min and a step size of 0.02°.

Intrinsic Dissolution Rate (IDR)

Using Wood’s method,20 200 mg of each sample was compressed at a pressure of 110 MPa for 10 s with a hydraulic press machine (Model 4350.L, Carver Inc., Wabash, U.S.A.). The resulting discs with a surface area of 1.3273 cm² were placed in molten beeswax in a way that only the top side of the compact was contacted with the solvent, during the dissolution studies with 1000 mL of water at 37°C by paddle method at 100 rpm. Samples were withdrawn at the predetermined time points (10, 15, 30, 45, and 75 min) and analyzed by the HPLC method.

Dissolution Under Supersaturated Conditions

Supersaturated dissolution test (three replicates) was conducted using a small-volume dissolution apparatus (RC-806 dissolution tester, TDTF Technology Co., Ltd., China) by paddle method with 150 mL of dissolution media (0.01 mol/L HCl, pH 6.8 phosphate buffer and water) and a rotation speed of 100 rpm at 37°C. After excess testing powders (200 mg each) were added to the dissolution media, samples were withdrawn and filtered at predetermined time points (0.25, 0.5, 1, 2, 12, and 24 h) and analyzed by HPLC.

Stability Studies

TD samples were exposed to a 60°C vacuum drying oven up to 36 h or stored in a constant temperature and humidity test chamber with 25°C/60% relative humidity (RH) for 3 months. Pressure stability was also studied. Samples were separately compressed into IDR tablets as described above, then the superficial powder was collected by scraping the tablet. Powders were analyzed by XRPD to examine their crystallinity.
amine the polymorphic transformation.

In addition, about 200 mg of TD-AM was added to a 5 mL test tube with glass stopper sealed. Then, the tube was heated at 80, 105, and 180°C in oil bath for up to 36 h. Moreover, potential solid transformations of TD-I, TD-II, TD-III, and TD-IV were conducted by heating them under 180°C for 30 min. The resultant solids were cooled down to room temperature and analyzed by XRPD.

**Results and Discussion**

**Morphological Analysis** As presented in Fig. 2, TD-I, TD-II occurred as plate and long sharp needle shape, while TD-III and TD-IV presented as short needle shape with particle size decreased significantly, verifying that crystals tend to form needle at higher supersaturation conditions. Because of the rapid crystal growth rate, crystals grow along the direction which is favorable to the heat dissipation. TD-AM was irregular sheet with rough edge, as a result of loss of lattice order upon amorphization. A large number of studies have shown that differences in crystal habits may affect the biological activities significantly and should be controlled strictly.

**XRPD Analysis** As shown in Fig. 3, each polymorphic form shows unique diffraction peaks that can be used for their identification. The 2θ values of TD-I corresponded well with Park’s research. TD-II had strong characteristic diffraction peaks at 7.70° and 22.74° 2θ, while TD-III at 4.62°, 17.78°, 18.20°, 21.12° 2θ, TD-IV at 6.95°, 13.13°, 17.46° 2θ. The difference in diffraction peaks indicated the changes occurred in the crystal structures. The three XRPD figures of crystalline TD (TD-II, TD-III, and TD-IV) were matched with the Figs. 3, 6, 7 in patent US 20060111571 A1, illustrating that the same crystalline structure polymorphs of TD have been prepared. TD-AM had lost its crystalline nature and transferred completely into amorphous form because the characteristic peaks of crystalline TD had completely disappeared.

**Thermal Analysis** As shown in Fig. 4, TD-I had a sharp melting point (T_m) at 302.5°C as reported, while the endothermic fusion peaks in TD-II, TD-III, and TD-IV agreed with each other, assuming that they converted into TD-I during heating. TD-II underwent an endothermic transition to TD-I during the heating process at 85.2°C. According to the heat of transition rule, TD-II and TD-I were enantiotropically related. TD-III had an exothermic peak at 188.2°C, suggesting that the transition to TD-I occurred when the temperature rose up and being moniotropically related. The small endothermic peak of TD-IV at 175.6°C might be related to the desorption of solvent. TD-AM showed a glass transition temperature (T_g) at 143.8°C, followed by a sharp exothermic peak of crystallization at 188.8°C. The endothermic peak at 303.2°C was attributed to the melting peak of the recrystallized TD.

From TGA curves (Fig. 5), a weight loss of 5.27% from
125 to 200°C of TD-IV was due to the volatilization of toluene, which was consistent with the endothermic event (peak at 175.6°C) on the DSC curve. In addition, the determined percentages of TD and toluene in TD-IV were 94.58±0.67 and 5.49±0.32%, respectively. Such results were very close to their corresponding theoretical percentages (94.42 and 5.58%, respectively) if the stoichiometry of form IV was 4:1 for TD and toluene. Thus, TD-IV was characterized as a toluene solvate (TD·1/4 toluene). On the other hand, little weight loss from 0 to 300°C was detected for TD-II, TD-III, and TD-AM, suggesting that they were not solvates. In addition, amorphization of TD has been also attempted by the quench-cooling method (immediately cooling the melt of TD by liquid nitrogen), but chemical degradation with 3% of impurities was found via HPLC analysis (data not shown).

**IR Analysis** FTIR was carried out to study possible intermolecular interactions of TD in Fig. 6. As previously reported, TD-I showed typical stretching vibrations of the secondary amine group (N–H) at 3326.8 cm⁻¹ and lactam at 1677.3 and 1647.6 cm⁻¹. Comparing with TD-I, TD-II showed bathochromic shifts (3326.8 → 3317.7 cm⁻¹) for secondary amine group. The typical C=O stretching of TD-III demonstrated a significant hypsochromic shift (1647.6 → 1657.9 cm⁻¹) and the peak at 1490 cm⁻¹ split into two peaks as 1501.0 and 1485.6 cm⁻¹. Different to other forms, the N–H absorption peak of TD-IV split into two peaks. While the broadened hydroxyl stretching peak at 3280.4 cm⁻¹ of TD-AM indicated the formation of hydrogen bond between TD molecules with the absence of sharp N–H peak. In addition, only one broadened peak assigned to the C=O stretch was observed for TD-AM, which might be ascribed to the formation of supramolecular chains via N–H…O hydrogen bonding between the indole and lactam carbonyl groups of adjacent TD molecules.

**Solubility and Dissolution Analysis** Crystal forms of TD showed similar solubilities in PBS (Fig. 7) in the range of pH 2–6.8 and exhibited a pH-independent solubility behavior. However, TD-AM showed a 6–9 times higher aqueous solubility.

IDR was expected to correlate more closely with in vivo dissolution dynamics of drug than solubility, which overcomes particle size and crystal habit effects during dissolution at the same time. As shown in Fig. 8, polymorphs showed similar dissolution rate (0.0011–0.0017 mg·min⁻¹·cm⁻²), while TD-AM demonstrated
a 3-fold higher IDR (0.0042 mg·min\(^{-1}\)·cm\(^{-2}\)) than TD-I. Because the relatively large free energy per unit area of the amorphous drug, the easily hydrated surface of the particles during dissolution, and the better deflocculation action of the hydrated film, make the amorphous drug disperse more easily, which results in the increase of the solubility of TD. As a BCS II drug, the oral bioavailability of TD was limited by its slow \textit{in vivo} dissolution, which could be significantly enhanced by amorphization.

Supersaturation dissolution was performed to observe the prolonged period for TD to achieve supersaturated concentration prior to recrystallization (Fig. 9). In all three dissolution media (0.01 M HCl, PBS 6.8, and water), the TD concentration of TD-I increased slowly with time and finally reached a steady state, while other three polymorphs showed different dissolution profiles with peak dissolution amount attained rapidly before 1 h and then declined to approach the dissolution profile of TD-I.

Generally, the lower solubility/dissolution rate of crystals, the more stability of the thermodynamics. It can be inferred from Figs. 8 and 9 that TD-I was the most stable crystal form. The notable dissolution decline was a result of precipitation of a more stable but much less soluble crystalline form, indicating the existence of supersaturation-mediated phase transformation. In order to verify the above speculation, at the end of dissolution tests in water, the rest solids were collected and vacuum-dried for 24 h at room temperature, followed by characterization with DSC and XRPD. It confirmed that TD-II, TD-III, and TD-IV transformed to TD-I during dissolution (Fig. S1), while TD-I remained its original crystalline form after dissolution.

Different to three metastable crystalline forms of TD, TD-AM showed a “spring” effect with an even more rapid dissolution in the first hour of dissolution, followed by a slight decrease in concentration in all three dissolution media and then remained a high supersaturation level till the end of the dissolution. The immediately collected solid residue after dissolution was only found very slight birefringence in the visual field of PLM (Fig. S2), indicating that most of TD-AM remained amorphous state with slight crystallization during dissolution process. XRPD was also carried out for the vacuum-dried dissolution residue of TD-AM, and a halo pattern was observed with detection of a small quantity of crystalline diffraction peaks (Fig. S3), which might be the trace amount of crystal in the residue.

TD-AM exhibited much higher supersaturation level than crystals, indicating almost all of TD-AM still kept its amorphous state during dissolution. In contrast, three TD polymorphs transformed to TD-I. Since amorphous solid lacks periodic arrangement, it could be treated as supercooled liquid thermodynamically which exhibits a miscibility gap with water. When mixing excess amorphous material with water, it will produce supersaturation and exhibit a phenomenon called liquid–liquid phase separation (LLPS), leading to a two-phase system with a lower free energy wherein one phase is a drug-rich phase and the other is a drug-lean continuous aqueous phase. In other word, the highest supersaturation a drug can reach is its amorphous solubility, above which the system will form LLPS. In supersaturation region (upper limit: amorphous solubility; lower limit: solubility of thermodynamically stable crystal), supersaturation provides the driving force for phase separation by crystallization if the barrier for nucleation can be overcome. In the region above amorphous solubility, either LLPS or crystallization could occur since both processes would decrease the free energy of system. LLPS may take place more rapidly than crystallization for complex-structured molecules with high configurational entropy (i.e., unfavorable for organization into a crystal lattice, slow crystallizer). Thereby, such LLPS phenomenon would also contribute to parachute stage during dissolution of amorphous drug if it is a slow crystallizer. In a study of approximately 50 pharmaceutically relevant compounds, around

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Fig. 9. The Concentration–Time Curves of TD-I, TD-II, TD-III, TD-IV, and TD-AM in (A) 0.01 M HCl, (B) pH 6.8 Phosphate Buffer, and (C) Water (\(n=3\))
one third of the compounds evaluated failed to crystallize after 1 h, even though a visible precipitate formed, and were characterized as slow crystallizers.\textsuperscript{34} For TD-AM molecule, only trace amount of birefringence was observed in the view field of PLM after 2 h of the supersaturation dissolution (Fig. S4).

In addition to possibility of LLPS formation for TD-AM during supersaturation dissolution, the difficulty of homogeneous nucleation could not be neglected. It is well known that the work done in homogeneous nucleation is much higher than heterogeneous nucleation under the same pressure, since the former one forms a spheroid and the latter one forms a spherical sector with lower volume. The relationship between the Gibbs free energy required to form a critical spherical nucleus of new phase during homogeneous nucleation ($\Delta G^s_{\text{hom}}$) and heterogeneous nucleation ($\Delta G^s_{\text{het}}$) could be expressed as follow:

$$
\Delta G^s_{\text{het}} = \Delta G^s_{\text{hom}} \cdot \phi(\theta)
$$

where

$$
\phi(\theta) = \frac{1}{4}(1 - \cos \theta)^2(2 + \cos \theta)
$$

is a function of wetting angle and accounts for the catalytic potency of the substrate with respect to the nucleus formation. $\theta$ is the wetting angle between the substrate plan and the tangent to the droplet surface, and is determined through the Young equation from the specific surface energies of interfaces between the nucleus droplet and the solid substrate, the liquid phase and solid substrate, and between the nucleus droplet and the liquid phase, denoted by $\sigma_s$, $\sigma_l$, and $\sigma_{sl}$, respectively, in the situation of nucleus formation on a solid substrate in a liquid phase.\textsuperscript{35} The relationship between $\sigma_s$, $\sigma_l$, $\sigma_{sl}$, and $\theta$ could be expressed as

$$
\sigma_s = \sigma_t + \sigma \cos \theta
$$

In Eq. 2, $\phi(\theta)$ varies from 0 to 1 when $\theta$ varies from 0 (complete wetting) to $\pi$ (complete no wetting). In the axis, $\phi(\theta)$ is a monotonically increasing S-shaped curve between 0 to 1 in the wetting angle $\theta$ range of 0 to $\pi$. Since $\sigma_t$ and $\sigma_l$ are constant, if $\sigma_s > \sigma_t$, then $\cos \theta_s < \cos \theta_t$, and $\theta_s > \theta_t$, which makes $\phi(\theta_s) > \phi(\theta_t)$. This means the higher surface energies between the nucleus droplet and the solid substrate, the higher $\phi(\theta)$ value, leading to the higher barrier of heterogeneous nucleation. Therefore, the surface tension between TD metastable crystals (i.e., TD-II, TD-III, and TD-IV) (as solid substrate) and hydrophobic TD-I nucleus should be higher than that between TD-AM and TD-I nucleus, since the surface of amorphous solid is easier to be hydrated by surrounding water than crystalline solid. This might be another reason for TD-AM keeping higher supersaturation level with small amount of crystallization during its supersaturated dissolution.

Raina \textit{et al.} investigated the impact of amorphous solubility and LLPS formation on passive membrane permeability using a side-by-side diffusion cell. The flux, measured as a drug diffusion rate, is correlated to the solute thermodynamic activity or free drug concentration.\textsuperscript{33} When LLPS occurred, the numerous nanosized drug droplets are in equilibrium with the aqueous phase. Once the free drug molecules initially dissolved in aqueous phase are absorbed, the nanosized drug-rich phase rapidly releases more drug to replenish the lost drug molecules in solution, thus maintaining the highest solute thermodynamic activity for absorption.\textsuperscript{36} Therefore, the accelerated IDR and prolonged supersaturation of TD-AM in aqueous media would benefit the \textit{in vivo} absorption of TD.

**Physical Stability**

\textbf{Influence of Temperature on Crystalline Forms}

After heating under 60°C for 3 h, XRPD pattern of TD-I did not show significant changes, demonstrating its good physical stability. The intensity of the typical XRPD peaks (\textit{i.e.}, 7.70° and 22.74° 2$\theta$) of TD-II decreased with the appearance of typical peaks of TD-I (\textit{i.e.}, 7.36° and 21.75° 2$\theta$) (Fig. 10A-b) and the diffraction peak of TD-IV (Fig. 10A-d) was consistent with TD-I, which both illustrated the transformation to TD-I. After heating TD-II and TD-IV at 180°C for 30 min, only characteristic diffraction peaks of TD-I could be observed, indicating they completely transformed to TD-I. For TD-III, although it did not show any change in XRPD at 60°C for 3 h (Fig. 10A-c), it also changed to TD-I after heating for 30 min at 180°C (Fig. 10B-c). These results were in agreed with DSC results (Fig. 4). Crystalline solid–solid transitions are usually observed in single component systems. Different from crystallization–melting–recrystallization process, crystalline solid–solid transitions take place solely in the solid state. By changing temperature (or/and pressure), a crystalline solid can be transformed into another crystalline solid without entering an isotropic liquid phase. These transitions result in material polymorphs. In most cases, crystalline solid–solid transitions are first-order transitions which undergo discontinuous changes in volume, enthalpy, and entropy due to crystal packing changes by positional changes of the molecules from one crystal structure to the next.\textsuperscript{37}

On the other hand, TD-AM kept in amorphous state at 60°C even for 36 h (Fig. 10C-a). In order to explore whether TD-AM would be changed to crystalline state, it was placed under further higher temperatures (80, 105, and 180°C). Under 80°C for 36 h, TD-AM was still in amorphous state (Fig. 10C-b), while it showed weak diffraction peaks of TD-I (\textit{i.e.}, 7.35°, 10.71°, and 21.76° 2$\theta$) after heating under 105°C for 36 h, suggesting the partially crystallization into TD-I. Moreover, TD-AM completely transformed to TD-I with its typical diffraction peaks (\textit{i.e.}, 7.36°, 10.70°, 12.63°, 14.63°, and 21.75° 2$\theta$) after heating under 180°C for 30 min.

The physical stability was investigated by long-term stability testing for three months. It was found that TD-I was stable, while TD-II completely transformed to TD-I during storage (Fig. 11). For TD-III, the diffraction peaks of the XRPD did not change, while TD-IV transformed to TD-III under room temperature. However, TD-AM was quite stable without any phase transformation, which might be due to its relatively high $T_g$. The ease of glass forming tendency could be defined by the ratio of $T_g/T_m$ and for an excellent glass former this ratio is greater than 0.7.\textsuperscript{38} TD-AM exhibited a $T_g/T_m$ of 0.72, which expected to be a good glass former. Since the $T_g$ of amorphous form is relatively high (143.8°C) with a low molecular mobility, the conversion of the glass state to crystalline is inhibited under long-term condition. When increasing temperature, the molecular mobility accelerates the molecular collision, which facilitates the nucleation and crystallization.\textsuperscript{39}

For TD-AM,
the higher temperature accelerates its recrystallization to TD-I (105 vs. 180°C). In summary, TD-III and TD-AM are less susceptible to crystal transition under room temperature with the stable kinetic properties, while TD-II and TD-IV are easily transformed to TD-I and TD-III, respectively.

Influence of Pressure on Crystalline Forms

Pressure may lead to solid–solid transformation of polymorphic drugs 40) and would affect the crystal interface structure, resulting in crystal lattice distortion or disorder. As shown in Fig. 12, TD-I and TD-III had no change in the position and relative intensity of the diffraction peaks. However, TD-II and TD-IV exhibited the XRPD signals of TD-I and TD-III, respectively, indicating that they partially transformed to TD-I and TD-III during compressing process. The good physical stability of TD-AM was also proved by no crystalline diffraction peak detected after compressing. In addition, moisture absorption measurement and powder compaction analysis of TD-AM have also been conducted and found that TD-AM shared comparable hygroscopic properties and tabletability with the commercial form TD-I (see sections S5 and S6 in supplementary materials). However, it took advantages of significantly higher solubility and dissolution in comparison to the studied four crystalline polymorphs.

Conclusion

Three crystalline polymorphs and an amorphous form of TD were prepared and physicochemically characterized.
TD-II was found to be enantiotropically related to TD-I, while TD-III was monotropically related to TD-I. TD-IV was a solvate of four TD molecules with one toluene molecule. While the amorphous form TD-AM with a high $T_g$ of 143.8°C showed a significantly higher dissolution rate and prolonged supersaturated concentration level during dissolution. In addition, it demonstrated relatively higher physical stability during dissolution, heating and compressing. Thus, TD-AM could be a very promising candidate for the further dosage form development.

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Conflict of Interest The authors declare no conflict of interest.

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