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Abstract

We demonstrated a facile approach, by adjusting the solvent ratio of water/acetone binary mixture, to alter the intermolecular interactions between Enzalutamide (ENZ) and hydroxypropyl methylcellulose acetate succinate (HPMC-AS) for spray drying process, which can be readily implemented to produce spray-dried dispersions (SDD) with enhanced stability and bioavailability. The prepared SDD of ENZ/HPMC-AS were examined systematically in terms of particle size, morphology, dissolution, solubility, stability, and bioavailability. Our results show that the introduction of water (up to 30% volume fraction) can effectively reduce the hydrodynamic diameter of HPMC-AS from approximately 220 nm to 160 nm (a reduction of c.a. 20%), which increases the miscibility of the drug and polymer, delaying or inhibiting the crystallization of ENZ during the spray drying process, resulting in a homogeneous amorphous phase. The benefits of using acetone/water binary mixture were subsequently evidenced by an increased specific surface area, improved dissolution profile and relative bioavailability, enhanced stability, and elevated drug release rate. This fundamental finding underpins the great potential of using binary mixture for spray drying process to process active pharmaceutical ingredients (APIs) that are otherwise challenging to handle.

***Keywords**:* spray drying, poorly water-soluble drug, particle size, amorphous solid dispersion, drug-excipient interaction

1. Introduction

Spray drying is an essential and widely implemented process for pharmaceutical industry to produce consistently uniform and stable solid dispersions, whereby a solvent containing poorly water-soluble drugs is transformed to solid powder.[1-4] Appropriate solvent system is critical to the success of this process: selection of solvent determines the morphological and physicochemical properties of the solid dispersion powder.[5] An optimal solvent, with suitable volatility, viscosity, and dielectric constant for the spray drying process,[6] will result in a final product of high solubility (>50mg/mL) and excellent bioavailability.

Organic solvents such as acetone, dichloromethane, methanol, and ethanol are commonly used for spray drying processes. To address the solubility limitation imposed by a single solvent, binary solvent mixtures with a controlled volume ratio have been used as feed solution and demonstrated promising effect to enhance miscibility and improve physical stability of active pharmaceutical ingredient (API) that often are hydrophobic.[5,7-9]

To meet processability requirements such as minimal toxicity and residual levels,[10] water was used previously in preparing binary mixtures for spray-drying process.[11-14] It acts as a proton donor[2] to influence the hydrogen bonding between the drug and the polymer, which brings desirable benefits to form drug powders. For example, acetone/water (7:3) binary solvent was used to facilitate a ternary solid dispersion formulation of griseofulvin-PVP-PHPMA whose dissolution characteristics were improved since water is a good solvent for PVP.[15] The same phenomena was reported by Ogawa and co-workers[16] whereby indomethacin-Soluplus particles prepared by ethanol/water (6:4) mixture demonstrated a greater dissolution rate than those prepared by pure

ethanol. It was also suggested that the composition of the binary solvent could affect release profile of the prepared solid dispersion, and the morphological difference is one of the reasons for the improvement with the particles prepared by ethanol/water. According to Zhou et al., when water was added to the solution, porous or honeycomb spray-dried polymethyl methacrylate (PMMA) particles were produced due to the different evaporation rates of solvent and water.[13]

A recent study concerning binary mixture for spray drying[17] argued that the effect of water is not always beneficial: presence of water in alcohol/DCM solvent can cause itraconazole to phase separate from HPMC solution when methanol or ethanol is used, but can enhance the miscibility when *n*-propanol or *n*-butanol is used. This work implies that the subtlety at molecular level, e.g. the interactions between drug, polymer, and solvents, is likely able to control the phase separation,[18] which has a significant impact on spray drying process and the resulting products. It is therefore critical to establish a fundamental understanding of the role of water contained in the binary mixture for spray drying, and its influence on the physicochemical and pharmaceutical properties of the final products, as a new avenue to optimize the current industrial practice.

Enzalutamide (ENZ) is a novel androgen receptor (AR) signaling inhibitor for the treatment of castration-resistant prostate cancer (CRPC),[19] and is also a Biopharmaceutics Classification System class II compound with a low solubility in water (1.5 μg/mL). To produce ENZ containing drugs, hydroxypropyl methylcellulose acetate succinate (HPMC-AS), has been used as polymer matrix for preparing solid dispersion.[7, 20-24] Chemical structures of ENZ and HPMC-AS are shown in **Fig. 1**. Effect of molar mass was examined when HPMC-AS of various grades and ENZ (40:60 w/w) were dissolved in acetone for spray drying.[25] It was reported that the degree of succinoyl substitution has

a notable impact on crystallization inhibition and pH-dependent solubility.[26] In a separate study, a dichloromethane: acetone mixture (75:25 v/v) was used to prepare ENZ/HPMC-AS spray-dried dispersions (SDD) by rotary evaporation.[27] The results confirm that having drug-rich aggregates could enhance the oral absorption of drugs.

To further improve the properties of SDD containing ENZ, we implemented water/acetone binary mixture for spray drying process. It was hypothesized that the presence of water could alter the interaction between ENZ and HPMC-AS, resulting in solid dispersions with enhanced physico-chemical properties. The produced SDD was investigated systematically in the present work.

2. Material and Methods

2.1. Materials

Enzalutamide was purchased from Yuancheng Chemical Ltd. (Wuhan, China). Tadalafil (Internal Standard, IS) was purchased from National Institutes for Food and Drug Control (Beijing, China) as standard substance. HPMC-AS 126G was kindly donated by The Dow Chemical Co. (USA). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Fisher Scientific Inc. (San Jose, USA). Ultrapure water was produced by a Milli-Q system with conductivity of 18.8 $m\Omega.cm^{-1}$ (Merck, Darmstadt, Germany). Acetone and all other reagents (analytical grade) were purchased from Damao Co. (Tianjin, China).

2.2 Methods

2.2.1 Spray drying

Solutions of ENZ and HPMC-AS were prepared at a drug loading of 60% (w/w) in four different solvents: acetone and acetone/water mixture at 9:1, 8:2, and 7:3 volume ratio, respectively. The concentration of ENZ in all solutions was kept constant at 10 mg/mL. Spray drying was performed in a Mini Spray Dryer B-290 (Buchi, Switzerland) equipped with Inert Loop B-295 using a closed, inner loop mode with nitrogen. Atomization gas flow was set as 6.83 L/min, whilst the pump speed was 3 mL/min, with inlet and outlet temperatures were 100°C and 70°C, respectively.

2.2.2 Dynamic Light Scattering (DLS)

A Zetasizer Nano ZS90 (Malvern, UK) was used to determine the hydrodynamic diameter (d_{H}) of molecules dissolved in the feed solutions, either pure acetone or acetone/water mixtures. This was calculated based on the Stokes-Einstein equation:

$$d_{\mathrm{H}} = \frac{k_{\mathrm{B}}T}{6\pi\eta D} \qquad (1)$$

where k_{B} is Boltzman's constant (1.38×10^{-23} J/K), T is temperature (K), η is viscosity of the solvent (kg/m.s) and D is the diffusion coefficient (m^2/s). Solutions of pure ENZ, HPMC-AS, and ENZ/HPMC-AS mixture were analyzed by the DLS at 25°C after passing through a 0.22 μm nylon filter then. The averages of three measurements are reported.

2.2.3 Polarized Light Microscopy (PLM)

Spray-dried sample was suspended in olive oil,[4] 1 to 2 drops of which were sandwiched between a glass slide and a cover slip, placed on a hot stage subsequently, and analyzed using an optical microscope (Nikon Eclipse LV100POL) attached by a Q-imaging camera. All pictures were obtained at 20× magnification.

2.2.4 Scanning Electron Microscopy (SEM)

Solid particles were scattered onto a double sided tape, and sputtered with platinum for 200 s at pressure of 0.5 mbar before being analyzed by a SEM (Phenom XL variable pressure, Phenomworld, Eindhoven, Netherlands) at an accelerating voltage of 10 kV.

2.2.5 Specific surface area analysis

The specific surface area of the powder samples was determined by a surface area analyzer (Micromeritics ASAP 2460, USA) with nitrogen absorption at 40°C using the Brunauer-Emmett-Teller (BET) theory.

2.2.6 X-ray Powder Diffraction (XRPD)

XRPD patterns were recorded on an X-ray powder diffraction system (D8 Advance Bruker, Germany) with Cu Kα radiation. Samples were examined over the most informative range of 2θ: 5 – 40°. The generator tension (voltage) and generator current were maintained at 40 kV and 30 mA, respectively.

2.2.7 Modulated differential Scanning Calorimeter (MDSC)

Samples were investigated calorimetrically using a modulated DSC (TAQ2000, TA Co.,USA), where solids powders were heated in an aluminum pan at 3°C/min, with modulations of 0.5°C every 40 s in the temperature range of 50 - 250°C under nitrogen atmosphere.

2.2.8 Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared spectra of samples were collected using a Spectrum 100 FT-IR Spectrometer (PerkinElmer, USA). Approximately 3 mg of the sample and 100 mg of dried KBr was blended

uniformly in an agate mortar and pressed into a translucent disk. Spectra were collected over 4000 – 400 cm^{-1} at a resolution of 4 cm^{-1} at room temperature.

2.2.9 Thermal Gravimetric Analyzer (TGA)

A RZY-1 TGA (Jingke Tianmei Scientific Instrument Co., Ltd., China) instrument was used to determine the percentage weight loss (%) of the ENZ solid dispersion during the heating. Approximately 3 mg sample was placed in alumina crucible and then heated from 50°C to 500°C with a heating rate of 10°C/min under 60 mL/min nitrogen gas purging.

2.2.10 In vitro dissolution

According to Dissolution Test with Paddle method,[28] dissolution tests were performed under non-sink condition using a ZRS-8G dissolution tester at a rotation speed of 50 rpm. To eliminate the potential variation due to particle size, raw ENZ was milled and passed through 120 mesh sieves and used as the bench mark. Two mg of ENZ or solid dispersions equivalent to 2 mg ENZ were suspended in 1000 mL 0.1% Tween 80 PBS solution (pH 6.8) at 37°C. One mL aliquots were taken at 5, 10, 20, 30, 45 and 60 min, and immediately filtered through a 0.22 μm filter. The filtered solutions were quantitatively diluted with acetonitrile-water (1:1, v/v) then filtered through a 0.22 μm nylon membrane. Filtrates were analyzed for ENZ using ACCELA UHPLC (Thermo Scientific Inc., San Jose, USA) and Hypersil BDS C18 column (50 × 2.1 mm, 2.4 μm, Thermo Scientific, San Jose, USA), operated in a reverse phase (RP) system with water and acetonitrile mobile phase (52:48, v/v) at a flow rate of 400 μL/min. The detection wavelength was 240 nm, and the injection volume was 5 μL. Calibration curve of ENZ was prepared at 7 concentrations between 0.1 to 10 μg/mL ($A=1225.7+46430.6C$, $R^2 = 0.9999$).

2.2.11 Molecular modeling of HPMC-AS oligomer/ENZ solid dispersions

Molecular environment operating system (MOE, 2009.10) was used to examine the interaction between ENZ and HPMC-AS molecules that were subjected to pronation 3D when water molecules were also introduced. Energy of the system was minimized using MMFF94X force field whilst gradient was set to 0.00001.[29]

The preparation process of solid dispersions was simulated by AMBER18 software. The model of the HPMC-AS oligomer and the Enzalutamide molecule were constructed in a Discovery Studio Visualizer 4.5 software package.[30] HPMC-AS oligomer was constructed according to the commercial product, consisting of 10 monomer units. Based on molar mass of compounds involved and their weight ratio in experiments (40:60), the number of polymer molecules and drug molecule in simulation box was kept at 1:7 . The model of the polymer and drug molecules was constructed using the Packmol program and the LEAP model in AmberTools 14.

2.2.12 In vivo oral bioavailability and statistical analysis

Male Sprague-Dawley rats weighing 180-220 g were supplied by Guangzhou University of Chinese Medicine Experimental Animal Center (Guangzhou, China). The animals were fasted overnight before the study but allowed free access to water. A total of 40 rats were divided randomly into five groups. ENZ/HPMC-AS solid dispersions freshly prepared by acetone, acetone/water (9:1), acetone/water (8:2) and acetone/water (7:3) were given to four groups by oral gavage, at a dosage of 20 mg ENZ/kg and a concentration of 10 mg/mL (suspended in 0.5% methylcellulose vehicle). Raw ENZ of equalivalent dosage was administrated to the fifth group as control.

Blood samples were collected from posterior orbital venous plexus into heparinized tubes at 1, 3, 6, 12, 24, 36, 48, 60, and 72 hours after administration.[25] The blood samples were immediately centrifuged at 12000 rpm for 10 min. Plasma was separated and stored at -20°C for further analysis.

The processing of plasma was: adding 10 μL of plasma samples, 10 μL mobile phase (methanol and ultrapure water contain 0.1% formic acid (50:50 v/v)) and 10 μL 2.5 μg/mL Tadalafil solution (internal standard) into a 2 mL centrifugal tube and vibrated for 2 min. The mixture was vortex-mixed with 200 μL 0.5% sodium bicarbonate aqueous solution and 1000 μL ethyl acetate, followed by centrifugation for 5 min at 12000 rpm. Approximately 900 μL supernatant was recovered and evaporated to dry under nitrogen. Resulting sample was dissolved in 200 μL mobile phase for ACCELA LC-MS (Thermo Scientific Inc., San Jose, USA) analysis. For comparison between each formulation and the raw ENZ, statistical analysis was carried out one-way analysis of variance, followed by the Tukey test using GraphPad Prism 5 (Graphpad Inc., USA).

All animal tests were approved by the University Ethics Committee for the use of experimental animals, in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2.13 Physical stability

ENZ solid dispersions were kept in a controlled environment (40 ± 2°C, 75 ± 5% RH) for 3 and 6 months before XRPD (D8 Advance Bruker, Germany) measurements to evalute their stability.

3. Results and Discussion

3.1 Chemical properties of ENZ/HPMC-AS SDD

Chemical properties of the ENZ/HPMC-AS solid dispersion were characterized by XRPD, DSC, FT-IR, and TGA. The XRPD curve of pure ENZ (**Fig. 2a**) shows several sharp and intense peaks at diffraction angles (2θ) of 9.83°，13.13°，17.45°，19.74°，21.20°, and 26.47°, confirming

that the crystalline nature is consistent with the diffraction pattern of ENZ. However, spectra of the newly prepared SDD show no such distinctive peaks , confirming that they are in an amorphous state. The slight difference between samples of high water content (8:2 and 7:3) and low water content (9:1 and pure acetone) in the region of 10-20 degree is very likely due to the small amount of micro-crystals.

Fig. 2b presents MDSC thermograms of all samples. It is observed that the peaks correspond to the melting point of solid dispersions are close to 195°C, a few degrees less than the melting temperature of pure ENZ (201°C), and their magnitude reduces with an increased water fraction in the binary mixture. Glass transition temperature (T_g), at which point the heat capacity of the solid particles changes, was logged by the MDSC, and found to be approximately 90°C for all samples. Increased fraction of water in the binary mixture is related to samples with high T_g. The increased T_g is likely due to the hydrogen bonds formed between the drug and the excipient.[31] He and colleagues reported that hydrogen bonds in polymer blends could restrict the motion of polymer segments, resulting in an enhanced T_g.[32] This was reported in a separate study whereby introduction of hydrogen bonds can effectively improve the T_g of maleimide isobutene alternating copolymers due to a reduced chain mobility.[33] Furthermore, all samples show a crystallization exothermic peak at about 140°C (**Fig. S3**), confirming the amorphous nature of the solid dispersions.

FT-IR spectrum (**Fig. 2c**) of pure ENZ shows characteristic peaks at 3433 cm^{-1} (N-H stretching), 3091 cm^{-1} (C–H aromatic stretching), 2947 cm^{-1} (C–H aliphatic stretching), 2237 cm^{-1} (C≡N stretching), 1771 cm^{-1}, and 1667 cm^{-1} (C=H aliphatic stretching), as reported in a previous work.[34] Physical mixture of ENZ and HPMC-AS (without spray drying) shows no notable difference from pure ENZ. However, spectra of solid dispersions differ substantially from those of pure ENZ and the physical mixture: it appears that the peak corresponding to secondary amine (3433 cm^{-1}) of the solid dispersions is broad, and the peak for carbonyl stretching at a wavelength of

1771 cm^{-1} is deformed, which suggests the presence of hydrogen bonds in both groups.[35] The shifts and broadening of the stretching vibrant of the amine group and distortion of the carbonyl signal indicate that ENZ interact strongly with HPMC-AS, which is a characteristic of amorphous solid dispersion. There is barely any difference between the spectra of solid dispersions prepared by binary solvent, except that the sample prepared by acetone/water (7:3) shows a weak -OH stretching vibration at 3640 cm^{-1}.

Finally, TGA curves (**Fig. 2d**) of both ENZ and solid dispersions samples show no obvious weight loss before 290°C, suggesting that no degradation occurs during heating process of DSC and solid dispersion preparation. It also shows that the prepared solid dispersions possess no or very little moisture content.

3.2 Physical properties of ENZ/HPMC-AS SDD

3.2.1 Crystallization tendency

To verify the influence of solvent composition on the intermolecular interactions between ENZ and HPMC-AS, PLM and MDSC were used to evaluate the crystallization tendency of the solid dispersions prepared.

From the PLM images (**Fig. 3**), it was found that crystallization disappeared once the supporting substrate (glass slide) was heated to 200°C that is the melting point for ENZ, which confirms there is no interference from impurities. During the quenching process, birefringence detected implies the presence of crystals in the melted blend, including pure ENZ and ENZ/HPMC-AS solid dispersions prepared by either pure acetone and acetone/water binary mixture.

Fig. 3 shows that there is a large quantity of micro-crystals in pure ENZ and ENZ/HPMC-AS SDD prepared using pure acetone, as evidenced by the birefringence observed. However, the

micrographs show a distinctively different feature once acetone/water binary mixture was used: the abundant microcrystals found in SDD prepared by pure acetone nearly diminish upon an increased fraction of water in the binary mixture for the spray drying. These results imply that the ENZ/HPMC-AS are in an amorphous state when acetone/water mixture is used, which suggests that the water molecules included in the feed solution could effectively promote miscibility between ENZ and HPMC-AS, and hence delay or even inhibit the crystallization of ENZ. The benefit of using acetone/water mixture for spray drying is clearly demonstrated between **Fig. 3b**, **3c**, and **3d**: a reduced number of microcrystals as the result of increasing amount of water. It is very likely that the water molecules could facilitate the compatibility between ENZ and HPMC-AS, improving both stability and reducing tendency for crystallization.

The degree of crystallinity can be calculated by separately integrated the exotherm on cold crystallization and endotherm on melting over different temperature regions accordingly. As shown in **Fig. 4**, the sample prepared with pure acetone possesses the highest crystallinity of 16.57%, while the crystallinity of the samples prepared by with the binary solvents presents lower values of 14.23%, 5.68%, and 7.85%, respectively. These data are consistent with the observation made by PLM.

3.2.2 Physical appearance

Due to the nature of spray drying process, evaporation rate of the solvent can have a substantial impact on the processing parameters, as well the final products. Upon the inclusion of water, acetone-water binary mixtures would have a reduced evaporation rate, comparing to the pure acetone, which in principle will affect the resulting solid dispersion, as shown in SEM images (**Fig. 5**).

Pure ENZ, prior to being spray dried, appears as crystals with irregular shape (**Fig. 5a**). Images of low magnification (not show) confirm that the particle size is between 50 and 100 μm. Upon the spray drying process where pure acetone was used, solid particles show a much reduced particle size (3-8 μm). Furthermore, the particles are of regular spherical shape. It is striking to observe that the particles prepared by binary mixture show a very different physical appearance to that prepared by pure acetone: they show a generic concave geometry rather than a spherical shape, with deformed features on the surface. The extent of such 'wrinkles' on the surface is increased with an increasing fraction of water in the binary mixture (from 10% to 30%).

It has been reported previously that the evaporation rate of a solvent has a significant influence on the properties of the final particle, specifically their morphology and size distribution.[6] In the present work, acetone has a faster evaporation rate than water does (less drying time is required), which results in spherical particles with smooth surface, despite that concave features can be seen from some of the particles. With an increased proportion of water in the binary mixture, evaporation rate of the solvent decreases, which prolongs the time takes for the solvent molecules to be removed completely from the ENZ/HPMC-AS blends during spray drying process. The slowed down drying process, determined by the diffusion rate of molecules, is likely the primary reason for the irregular shapes observed in the current work.[36] Similar morphology is reported in other work where binary mixture was used.[37]

During a spray drying process, it was suggested that liquid droplets could shrink in an isotropic manner, whereby both chemical nature and viscosity of the solvent determine the corresponding mass transfer kinetics. Upon the evaporation of the solvent, drug and polymer mixture would

thicken and form a primary shell.[38] It is natural to suggest that the surface morphology of particles is likely determined by the synergistic effect of solvent evaporation and its viscosity. Increasing the fraction of water in the binary mixture have two implications: 1) the viscosity of solvent would increase, resulting in the formation of primary shell at the early stage of the drying;[39] 2) it takes longer for solvent molecules to 'escape' from the polymeric matrix.

As suggested by Taspis and colleagues,[40] the characteristic time for these two process can effectively control the arrangement of components within a mixture during spray drying process. The primary shell formed at the early stage in the present work will inhibit the evaporation of the solvent molecules. Once the pressure within the shell builds up and exceeds a certain threshold, particles would inflate and rupture with 'blow-holes' to release the localized pressure. The rupture characteristics are observed in the SEM images, which is consistent with several studies previously.[40,41] A third possible implication of increasing water fraction is that the formed shell could be thinner than those formed at a late stage. Since the volume of the droplets reduces slowly when having water as solvent, the droplet surface is more readily for buckling than shrinking, which was observed in our study.

3.2.3 Specific surface area

Specific surface area of samples prepared was measured and presented in **Fig. 6**. The specific surface area of the solid dispersion prepared by acetone/water 9:1, 8:2 and 7:3 is 1.3337, 1.9863, and 2.2325 m^2/g, respectively, which is 1.7, 2.5, and 2.8 times greater than that prepared by pure acetone. The results confirm that adding water to acetone as solvent can produce spray dried particles with an increased surface area.

3.2.4 Physical stability

Physical state of the solid dispersions was examined once again using XRPD after being kept at an accelerated condition (40 ± 2°C, 75 ± 5% RH) for 3 and 6 months. The diffraction pattern of solid dispersion prepared by pure acetone (black curve in **Fig. 7**) shows several distinctive peaks at diffraction angles (2θ) of 9.83°, 13.13°, 17.45°, 19.74°, 21.20°, and 26.47°. The magnitude of these peaks reduces as the fraction of water in the binary mixture increases. Taking into account of the MDSC (**Fig. S2**) and PLM data, it is believed that the samples with less micro-crystals are more stable, once water is used in conjunction with acetone as binary mixture.

3.3 Intermolecular interactions between ENZ and HPMC-AS

3.3.1 Dynamic light scattering

Light scattering was used to measure the size of polymeric globules dispersed in a continuous phase,[42] establishing the molecular interactions between ENZ and HPMC-AS in the presence of solvent (acetone and water). HPMC-AS was introduced to two series of acetone/water mixtures (ratio: 10:0; 9:1; 8:2; and 7:3), with or without the presence of ENZ, whose size were measured and presented in both **Table 1** and **Fig. 8**. It can be seen that the hydrodynamic diameter of HPMC-AS measured in pure acetone is approximately 220 nm, with or without the presence of ENZ. This size is consistent with a hydrodynamic radius (R_h) of 100 nm reported in previous studies where HPMC-AS molecules were dispersed in PBS buffer[39] or aqueous solution with controlled pH.[43] Such value is however far greater than what is expected for an individual molecule, suggesting that the HPMC-AS molecules interact with each other and self-assemble to form large aggregates in acetone due to its amphiphilic nature. Its ability to interact with the drug molecules, ENZ in the

present work, implies that there are ENZ molecules being either contained or attached to the HPMC-AS matrix,[20,44] which might be correlated with its supersaturation performance.[45]

It is worth noting that no signal was detected when ENZ was dissolved in acetone, as the molecule is too small for detection, suggesting that ENZ is fully solvated. However, once HPMC-AS was added to the acetone containing ENZ, a bright beam pathway can be seen visibly, which is a direct evidence of large aggregates formation.

Hydrodynamic diameter of HPMC-AS was subsequently measured in acetone/water mixture as a function of water content. It was observed that, upon the addition of 10% water, the size of HPMC-AS reduces nearly 18% to 183 nm, and carries on decreasing with additional water in the solvent. Considering the amphiphilic nature of HPMC-AS, the reduced molecular size is likely the result of losing the competition for acetone molecules available against water: the HPMC-AS matrix is less solvated as opposed to that in pure acetone. Such phenomena has been reported previously where the micellar morphology of a synthetic amphiphilic polymer, polystyrene-*b*-poly (acrylic acid-*co*-methyl acrylate), was controlled by the fraction of acetone content in acetone/water mixture.[46] Similar principle was applied in a selection of works where the self-assembly of amphiphilic polymers was controlled.[47,48]

Another series of data present in **Fig. 8** is the hydrodynamic diameter of HPMC-AS in acetone/water mixture, with the presence of ENZ. The size of ENZ/HPMC-AS matrix in pure acetone is very similar to that of pure HPMC-AS . This suggests that HPMC-AS molecules remain solvated by acetones, and the interaction between ENZ and HPMC-AS is too weak to make any impact. The size of ENZ/HPMC-AS is notably smaller (approximately 14 nm) than that of pure

HPMC-AS in the corresponding binary mixture, which is already reduced in comparison to that in acetone. It is very likely that the presence of water molecules facilitates molecular interaction between ENZ and HPMC-AS. The synergistic effect of reduced molecular size of HPMC-AS and the enhanced ENZ/HPMC-AS interaction provides an opportunity to tightly constraint a concentrated amount of ENZ in HPMC-AS matrix, which results in an improved stability and compatibility, with less tendency for crystallization. This is supported by the FT-IR results showing that the NH function group of ENZ form hydrogen bond with HPMC-AS.

We speculate that there are two possible interactions present between HPMC-AS and ENZ: enhanced hydrophobic interaction and ionic interaction, upon addition of water to the solvent. As illustrated in **Fig. 9**, both or either interactions would force the HPMC-AS matrix that accommodates ENZ to either shrink or to dissociate, evidenced by the reduced hydrodynamic diameter. The proposed mechanism was verified using two other API, tadalafil and celecoxib, of which results are summarized in **Table S1** and **Fig. S1** in the Supplementary materials.

Interaction between polymer and drug has a profound impact on the bioavailability of the spray-dried solid dispersions. This concept was demonstrated in several previous work[49,50] where chemistry of HPMC-AS was controlled in terms of amphiphilic balance, hydrogen bonding capability, glass transition temperature etc. It was shown that the presence of an amphiphilic, ionizing polymer could enhance the stability and bioavailability of the SDD via intermolecular forces of different nature, e.g. ionic repulsion, hydrogen bonding, or hydrophobic interaction, depending on the chemical nature of the drug used. Such principle is likely applicable in the present work whereby the interaction between HPMC-AS and ENZ is affected by the presence of water

molecules in the solvent mixture. It is worth noting that the interaction between ENZ and HPMC-AS in acetone/water mixture at 7:3 ratio is not as notable as that in the other two binary mixtures examined, and results in an opposite effect on the size distribution of the ENZ/HPMC-AS aggregates. We speculate that the increased fraction of water changes the configuration of the ENZ/HPMC-AS, of which the specific nature warrants some future work using advanced technique such as neutron scattering.

3.3.2 Molecular modeling

To further elucidate the effect of water on the interaction between ENZ and HPMC-AS, molecular modelling was carried out using MOE software package. The molecules were built within Amber12 force field with Solvation R Field, followed by application of partial charges and assignment of ionization states and position hydrogens. Energy was subsequently minimized to find atomic coordinates that are local minima of a potential energy function. The resulting conformation was subject to molecular dynamic simulation, in which the Nose-Poincare-Anderson equations of motion was used to generate ensemble trajectory during a heating from 0 K to 300 K in 60 ps, running time of 1 ns, and a cool-down to 0 K in 60 ps.

The final model (**Fig. 10**) presents the occupational molecules around the ENZ molecule (magenta) are water molecule (oxygen atom in red) and HPMC-AS molecule (grey). The result confirms that it is plausible for ENZ and HPMC-AS to form more hydrogen bonds when water molecules are present. In our study, the compatibility between ENZ and HPMC-AS was changed with the participation of water. That could be attributed to one oxygen atom of water acted as a hydrogen bond acceptor and two hydrogen atoms as donors, enhancing the interactions between

drug and polymer by forming H-bonds. The red frame highlights the forming of hydrogen bonding reduces the distance between HPMC-AS and ENZ molecules.

ENZ molecule has an NH moiety that is capable of forming hydrogen bonds. Previous studies show that the position of the NH peak is sensitive to the strength of the hydrogen bond formed.[51] FT-IR studies show that hydrogen bonding occurs between the NH group and the carbonyl function in amorphous ENZ, and that the average hydrogen bonding is stronger than in the crystalline state. It is possible that ENZ processes a hydrogen bond donor >N-H and accept ester groups that are capable of forming various hydrogen bonds with HPMC-AS. **Fig. 11** shows that the number of hydrogen bond changed with the time in the preparation process: nearly 20 hydrogen bonds are formed between 21 drug molecules and 3 HPMC-AS oligomers (10 monomers for each oligomer). During the formation of an amorphous solid dispersion, ENZ and HPMC-AS molecules extend and form hydrogen bonds. These results may partly attribute to a certain content of water that combined ENZ molecules with HPMC-AS molecules. Both experimental and computational results show that suitable fraction of water in the binary mixture could help to increase the drug-polymer interaction and decrease the size of polymer, forming a drug-rich polymeric matrix with reduced size, which is beneficial to produce SDD with enhanced performance. Such mechanism, applied on three different APIs, can potentially be applicable to a broad range of SDD by manipulating molecular size and drug-polymer interaction.

3.4 In vitro dissolution

Dissolution profiles (%) of the SDD prepared, with pure ENZ as benchmark, are presented in **Fig. 12**. It shows that the dissolution of pure ENZ is just 20% up to 60 min. As a contrast, SDD produced using HPMC-AS in pure acetone could reach 42.3% in 10 min, which is a substantial improvement, demonstrating the technical advantage of spray drying process and the excellent

capability of excipient polymer. Within the same timeframe, the SDD samples prepared by binary solvents (acetone: water ratio 9:1, 8:2, and 7:3) show an even better performance, reaching 52.2, 59.1, and 62.3% respectively. Such improved dissolution characteristics (ca. 47%) is likely attributed to a synergistic effect of an increased specific surface area, as confirmed by both SEM and BET results, and the amorphous nature of the solid particles. This shows, once again, the benefit introduced by the revised spray-drying process where water is used in the binary solvent.

3.5 In vivo oral bioavailability

Plasma concentration-time profile and pharmacokinetic parameters for ENZ and SDD samples prepared by acetone and acetone/water mixture are presented in **Fig. 13** and **Table 2.** The SDD samples clearly show a greater T_{max}, C_{max}, and AUC than those of pure ENZ. The T_{max} for SDD samples varied from an average of 2.7-5.6 h, which is about the transit time in the stomach and small intestine of rats (approximately 4 h). More importantly, our results confirm that the solid dispersion prepared by using the higher water content solvent can maintain a high blood concentration in the rat for a longer time than using the lower water content solvent.

The relative bioavailability of solid dispersions prepared by using acetone and acetone/water (9:1, 8:2 and 7:3) as solvent are 2.655, 3.014, 3.336 and 3.639 times greater than the AUC of ENZ, and the relative bioavailability of solid dispersions prepared by using acetone/water (8:2 and 7:3) are 1.256 and 1.371 times greater than the AUC of ENZ by using acetone as solvent. This confirms that the bioavailability of the solid dispersion increases with an increased water content in the binary solvent for spray-drying process. It is likely that the difference in the environmental conditions of in-vitro and in-vivo experiments is responsible for the contrast between fast dissolution and slow absorption rate reported in **Fig. 12** and **Fig. 13**. It is well documented in the literature that HPMC-AS is not readily dissolved in gastic acidic condition,[7,52,53] which resulted in a

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slow absorption rate for the first 3 hours. However, once the ENZ/HPMC-AS matrix are exposed to the mild condition in small intestine, HPMC-AS molecules would swell and release ENZ, which explains the prolonged release profile between 6 and 36 hours for samples prepared by high water content. The in-vitro dissolution experiments were carried out in media of pH 6.8 (close to that in small intestine), which supports our rational above.

4. Conclusions

In this work, we systematically investigated the effect of using acetone/water mixture on the spray-dried dispersion of ENZ/HPMC-AS, the underpinning molecular mechanism, and the resulting benefits in stability and bioavailability. Both experimental (light scattering and FT-IR) and computational simulation reveal that the molecular interaction between drug and polymer is controlled by the presence of water molecules, which not only has a substantial impact on the properties of SDD, but underpins a facile approach to improve spray drying process. Polymeric matrix with reduced molecular size could effectively constrain drug molecules when being exposed to acetone/water mixture, which inhibits the crystallization tendency of ENZ and facilitates the formation of a homogeneous amorous phase. The synergistic effect of decreased size for polymeric matrix and increased drug-polymer interaction is both fundamental and instrumental for producing SDD with enhanced properties. The benefits of using acetone/water binary mixture, rather than pure acetone, for ENZ/HPMC-AS spray drying process include increased specific surface area, improved dissolution profile and relative bioavailability, enhanced stability, and elevated drug release rate. Such understanding, evidenced by a comprehensive suite of characterization, could be adapted by a range of spray drying processes that are widely used by the pharmaceutical industry.

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Figure captions

Fig. 1. Chemical structures of (a) Enzalutamide and (b) HPMC-AS.

Fig. 2. Characterization of ENZ and solid dispersions, (a) XPRD patterns of ENZ and solid dispersions; (b) Reverse heat flow curves of ENZ and solid dispersions; (c) FT-IR spectra of pure ENZ, physical mixtures, amorphous mixture, and solid dispersions; (d) TG curves of ENZ and solid dispersions.

Fig. 3. PLM images of (a) pure ENZ, and solid dispersion of ENZ/HPMC-AS prepared with different solvent: (b) acetone, (c) acetone/water 9:1, (d) acetone/water 8:2, and (e) acetone/water 7:3.

Fig. 4. The degree of crystallinity of solid dispersions prepared by pure acetone and acetone/water binary mixtures.

Fig. 5. SEM images of ENZ (1500×) and solid dispersions prepared with different solvent (10000×): (a) ENZ; (b) acetone; (c) acetone/water 9:1; (d) acetone/water 8:2; (e) acetone/water 7:3.

Fig. 6. Specific surface area results of solid dispersions prepared by pure acetone and acetone/water binary mixtures.

Fig. 7. XRPD patterns of the solid dispersions after storage at 40°C and 75% RH for 3 months and 6 months.

Fig. 8. (a) Averaged hydrodynamic diameter of HPMC-AS, with and without the presence of ENZ, measured as a function of water content in acetone/water mixture; (b) size distribution of HPMC-AS and ENZ complex.

Fig. 9. Schematic diagram illustrating the possible molecular configuration of HPMC-AS and ENZ aggregate in acetone and acetone/water mixture.

Fig. 10. Potential interactions among ENZ (magenta), HPMC-AS (gray) and water (red). Hydrogen-bonds are depicted in dashed lines.

Fig. 11. The number of H-bond change with the time in the preparation process.

Fig. 12. Dissolution profiles of raw ENZ and ENZ/HPMC-AS solid dispersions at 60% drug loading (n=3, mean ± SD).

Fig. 13. Plasma concentration-time profiles of ENZ in rats after oral administration. All formulations were administered in aqueous suspensions (n=8, mean ± SD).

Supplementary materials

Fig. S1. Averaged hydrodynamic diameter of HPMC-AS and two HPMC-AS mixture with different API measured in pure acetone and acetone/water mixture of different water content.

Fig. S2. The degree of crystallinity of solid dispersions prepared by pure acetone and acetone/water binary mixtures at 0 month and 1 month.

Fig. S3. Heat flow curves of ENZ and solid dispersions.