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**Mechanism study on stability enhancement of adefovir dipivoxil
by cocrystallization: Degradation kinetics and structure-stability
correlation**

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1 A B S T R A C T

2 The purpose of this study is to determine the mechanism by which cocrystallization can enhance
3 the stability of adefovir dipivoxil (AD), a diester prodrug of adefovir with known chemical
4 stability problem. Three multi-component crystals of AD with biologically safe coformers,
5 including gallic acid cocrystal hydrate (1:1:1), salicylate salt (1:1), and maleate salt (1:1) were
6 prepared and characterized by thermal analysis, infrared spectroscopy, powder and single crystal
7 X-ray diffraction. DVS measurements and stability tests were applied to evaluate the stability. The
8 new crystalline phases exhibit improved stability compared to pure drug in the order AD gallic
9 acid cocrystal > AD maleate > AD salicylate > AD form I. Degradation kinetics and
10 structure-stability correlation studies demonstrate that the stability enhancement mechanism by
11 cocrystallization involves (1) inhibition of hydrolysis of AD by replacement of drug–drug
12 homosynthons by stronger drug–coformer heterosynthons at adenine fragments; (2) suppression of
13 dimerization of AD by separation of adenine fragments by inserting coformers in crystal lattices;
14 (3) further reducing rates of hydrolysis by forming hydrogen bonds with hydrate water at
15 phosphoryl fragments. This study has important implications for use of cocrystallization approach
16 to some easily degradable drugs in pharmaceutical.

17

18 *Keywords:*

19 Adefovir dipivoxil; Stability; Cocrystallization; Cocrystal; Salt; Structure-property correlation

20 *Chemical compounds studied in this article:*

21 Adefovir dipivoxil (PubChem CID: 60871); Gallic acid (PubChem CID: 370); Salicylic acid
22 (PubChem CID: 338); Maleic acid (PubChem CID: 444266)

1. Introduction

In pharmaceutical industry, not more than 1% of active pharmaceutical ingredients (APIs) can come to market due to the poor physicochemical properties [1]. It is a great challenge for pharmaceutical scientists to improve APIs' physicochemical properties and retain their chemical structure and bioactivity at the same time. Recently, crystal engineering of APIs through cocrystallization has gained an increased interest as an emerging approach to optimize physicochemical properties without compromising the structural integrity and bioactivity of APIs [2-10]. APIs cocrystallize with other pharmaceutically accepted molecules (which are usually referred to as coformers) to form neutral cocrystals with hydrogen bonds or ionic salts when a proton transfer occurs, thus new crystalline forms of the API with desirable properties are obtained [11-14]. At present, most researchers about pharmaceutical cocrystallization focus on improving the solubility and dissolution rate of a variety of APIs [2-7], and reports on enhancement of chemical stability are relatively less when we review the literature [9,15]. We now demonstrate a case study of the application of cocrystallization approach to improve a drug's chemical stability.

Adefovir dipivoxil (AD, Fig. 1), with trade names Preveon[®] and Hepsera[®], is an orally-administered acyclic nucleotide analog reverse transcriptase inhibitor used for treatment of chronic hepatitis B [16]. Up to now, five anhydrous polymorphs and four hydrates (including a dihydrate, two polymorphic monohydrates, and a hemihydrate) of AD have been reported [17-23]. Form I is the most stable anhydrous form at room temperature and humidity and used in marketed formulations [17]. However, when

1 leaving form I at 75% RH, it gradually transforms to the dihydrate [23]. AD is the
2 diester prodrug of adefovir (PMEA) and contains phosphoryl moiety and adenine
3 moiety [17]. It is reported that AD is thermodynamically unstable and degrades in two
4 pathways: hydrolysis of the phosphoryl moiety and dimerization of the adenine
5 moiety (Fig. 1) [24]. For this reason, improving the chemical stability of AD is very
6 important for drug product development and quality control. Several techniques, such
7 as addition of insoluble carbonates, formation of liquid capsule and beta-cyclodextrin
8 inclusion, have been reported to enhance the stability of AD [24-26]. Moreover,
9 cocrystallization method has also been used, and the results show that cocrystallizing
10 AD with acidic saccharin exhibited better performance of chemical stability than that
11 with basic nicotinamide [15,27]. However, the lack of single crystal X-ray data makes
12 the detailed structure-property correlation study impossible. The mechanism that
13 leads to stability improvement by cocrystallization remains poorly understood, and
14 cocrystallization protocol, therefore, remains widely empirical.

15 This study aims to investigate the mechanism by which cocrystallization can
16 enhance the stability of AD. Based on the hydrogen bonding possibilities of AD
17 molecule (with 13 hydrogen bond acceptors and 2 hydrogen donors), a series of
18 complementary carboxylic acid compounds, which are pharmaceutically acceptable or
19 biologically safe, were selected to react with AD by the solution and solvent assisted
20 grinding methods. Finally, gallic acid, salicylic acid and maleic acid (Fig. 1) can
21 successfully cocrystallize with AD to form new crystalline phases. We employed
22 solid-state material characterization techniques such as DSC, TG, IR and XRD to

characterize these new phases. Their crystal structures were also obtained from single crystal X-ray diffraction data. Further, investigation on hygroscopicity and stability performance were also carried out. In particular, fundamental understanding of mechanism on stability improvement by cocrystallization was explored through degradation kinetics and structure–stability correlation studies. It is expected that such an understanding will enhance the predictability of pharmaceutical cocrystallization strategy for some unstable drugs.

2. Materials and methods

2.1. Materials

AD and PMEA were purchased from Suizhou Hongqi Chemical Co., Ltd. Mono-POM PMEA reference standard was purchased from Shenzhen Feisi Chemical Co. Ltd. Gallic acid, salicylic acid and maleic acid were purchased from Aladdin reagent Inc. All of the other chemicals were commercially available and used as received. All the solvents were pretreated by molecular sieve to remove the residual water.

2.2. Preparation of samples

2.2.1. AD form I

The marketed AD (100 mg) was added to 200 uL acetone, and put to 50 °C water bath for about 30 min. AD was completely dissolved, and the resulting solution was rapidly dried under vacuum to get white solid of form I.

2.2.2. AD gallic acid cocrystal hydrate (1:1:1) (AD–GA)

1 AD–GA cocrystal was prepared by solution and solvent assisted grinding methods:

2 (i) A mixture of AD (501 mg, 1 mmol) and gallic acid (170 mg, 1 mmol) was added to
3 3 mL of ethanol. The resulting solution was stirred at room temperature for 2 h and
4 white solid precipitated. The suspension was filtered and the isolated solid of AD–GA
5 cocrystal was dried under vacuum for one day at ambient temperature. Yield: 489 mg,
6 71%. Single crystals of AD–GA cocrystal were obtained by slowly evaporating the
7 filtrated solution at room temperature. (ii) A 1: 1 mixture of AD (501 mg, 1 mmol)
8 and gallic acid (170 mg, 1 mmol) with 20 uL of ethanol was added to a stainless steel
9 grinding jar. The mixture was ground for 15 min at a frequency of 15 Hz.

10 2.2.3. *AD salicylate (1:1) (AD–SA)*

11 AD–SA salt was obtained via the following two methods: (i) A mixture of AD
12 (501 mg, 1 mmol) and salicylic acid (138 mg, 1 mmol) was added to 2 mL of ethyl
13 acetate. The resulting solution was stirred at 40 °C for 2 h and white solid was
14 precipitated. The suspension was filtered and the isolated solid of AD–SA salt was
15 dried under vacuum for one day at ambient temperature. Yield: 603 mg, 94 %. Single
16 crystals of AD–SA salt were obtained by slowly evaporating the filtrated solution at
17 room temperature. (ii) A 1: 1 mixture of (501 mg, 1 mmol) and salicylic acid (138 mg,
18 1 mmol) with 20 uL of ethyl acetate was added to a stainless steel grinding jar. The
19 mixture was ground for 15 min at a frequency of 15 Hz.

20 2.2.4. *AD maleate (1:1) (AD–MA)*

21 AD–MA salt was obtained via the following two methods: (i) A mixture of AD
22 (501 mg, 1 mmol) and maleic acid (116 mg, 1 mmol) was added to 2 mL of ethyl

1 acetate. The resulting solution was stirred at 40 °C for 2 h and white solid was
2 precipitated. The suspension was filtered and the isolated solid of AD–MA salt was
3 dried under vacuum for one day at ambient temperature. Yield: 555 mg, 90 %. Single
4 crystals of AD–MA salt were obtained by slowly evaporating the filtrated solution at
5 room temperature. (ii) A 1: 1 mixture of (501 mg, 1 mmol) and maleic acid (116 mg,
6 1 mmol) with 20 uL of ethyl acetate was added to a stainless steel grinding jar. The
7 mixture was ground for 15 min at a frequency of 15 Hz.

8 *2.3. Characterization*

9 *2.3.1. Thermal analysis*

10 **Thermogravimetric** (TG) analysis was performed using a Netzsch TG-209
11 instrument. Samples were loaded into aluminum pans and heated under a nitrogen
12 purge at a constant rate of 10 °C·min⁻¹. Scans were carried out within the temperature
13 range of 25 to 500 °C. **Differential Scanning Calorimetry** (DSC) measurement was
14 performed on a Netzsch STA 409 PC Instrument. Samples were placed into closed
15 aluminium pans and heated under a nitrogen purge at a rate of 10 °C·min⁻¹. Scans
16 were carried out within the temperature range of 25 °C to a final temperature where
17 samples began to decompose.

18 *2.3.2. Fourier transform infrared (FTIR) spectroscopy*

19 FTIR (KBr pellet) spectra were recorded on a Bruker Equinox 55 instrument. A
20 total of 64 scans were collected over a range of 4000 to 400 cm⁻¹ with a resolution of
21 0.2 cm⁻¹ for each sample.

22 *2.3.3. Powder X-ray diffraction (PXRD)*

PXRD analysis was performed on a Bruker D2 Advanced diffractometer (Bruker, PHASER) operated with Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) at 30 kV and 10 mA. The data were collected over an angular range of 3–40 ° (2θ) value in continuous scan mode using a step size of 0.014 ° (2θ) and a step time of 0.1 s. Calculated PXRD patterns were generated from the single-crystal structure data using Mercury CSD 3.1 (Cambridge crystallographic data center, UK).

2.3.4. Single crystal X-ray diffraction (SXRD)

SXRD data were collected on an Agilent Technologies Gemini A Ultra system with graphite monochromated Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$). Cell refinement and data reduction were applied using the program of CrysAlis^{PRO} (Agilent Technologies Inc.) [28]. Structures were solved by the direct method and refined by the full-matrix least-squares method on F^2 . All the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in calculated positions with fixed isotropic thermal parameters and included in the structure factor calculated in the final stage of full-matrix least-squares refinement. All the calculations were performed using the SHELX-97 programs [29].

2.3.5. Dynamic vapor sorption (DVS) studies

DVS experiments were carried out on a DVS intrinsic instrument (Surface Measurement Systems, London, UK). Solids of all samples were sieved using standard mesh sieves to provide samples with approximate particle size ranges of 75 – 100 μm and then dried under vacuum for one day at ambient temperature before the experiment. Then the samples were placed onto the DVS sample pan and

1 pre-equilibrated at 0% RH in a continuous flow of dry nitrogen for several hours to
2 establish the equilibrium dry mass. The relative humidity was then increased from 0
3 to 90% in 10% steps. For the desorption process, the RH was decreased in a similar
4 manner. The sorption isotherms were calculated from the equilibrium mass values.

5 *2.4. Chemical stability*

6 Chemical stability studies were performed under two different conditions. Solids
7 of all samples were sieved using standard mesh sieves to provide samples with
8 approximate particle size ranges of 75 – 100 μm . Three physical mixtures (AD/GA,
9 AD/SA and AD/MA) were prepared by 1: 1 equimolar mixture of powder of AD and
10 corresponding carboxyl acid which was added to an EP tube to vortex for
11 well-distributed.

12 *2.4.1. 40 °C/75% RH*

13 Samples were weighed into uncapped glass vials, and then placed in a stability
14 chamber (Mettler Model 500) at 40 °C and 75% relative humidity (RH) for 6
15 months. The residual contents of AD were determined by HPLC at 1, 2, 3, and 6
16 months. The samples for analysis were removed and the left over were discarded at
17 each sampling point. The stability test at 40 °C/75% RH was performed in triplicate to
18 evaluate the standard deviations.

19 *2.4.2. 60 °C/75% RH*

20 Samples were uncapped and stored in a 60 °C/75% RH environment chamber for
21 3 days. The residual contents of AD and the main hydrolysis products (mono-POM
22 PMEA and PMEA) were determined by HPLC at 1, 2, and 3 days. The samples for

1 analysis were removed and the left over were discarded at each sampling point. The
2 stability test at 60 °C/75% RH were performed in triplicate to evaluate the standard
3 deviations.

4 2.4.3. *High performance liquid chromatography (HPLC) assay*

5 The residual contents of AD and the main hydrolysis products (mono-POM
6 PMEA and PMEA) were determined by a Shimadzu LC-20A HPLC system, with a
7 C18 column (Inertsil ODS-3, 5 μm $\times$ 4.6 mm $\times$ 150 mm column, GL Sciences Inc.,
8 Japan) and a UV detection wavelength of 260 nm. The mobile phase consisted of a
9 mixture of methanol and 0.1% formic acid solution (pH 2.8). The isocratic elution
10 was used to determine the contents of AD and mono-POM PMEA, with 50% (v/v)
11 methanol and 50% (v/v) formic acid solution for AD, AD–GA cocrystal and AD–SA
12 salt, and 45% (v/v) methanol and 55% (v/v) formic acid solution for AD–MA salt.
13 The flow rate was 1.0 mL $\cdot$ min⁻¹. A gradient profile was used to determine the content
14 of PMEA for all the samples, which was started with 10% (v/v) methanol (8 min),
15 followed by an immediate jump to 90% (v/v) methanol (15 min), and returned
16 immediately to 10% (v/v) methanol (7 min). The eluent flow rate was 0.5 mL $\cdot$ min⁻¹.
17 Linear ranges were determined to be 2–200 $\mu\text{g}\cdot\text{mL}^{-1}$ for AD, 0.5–120 $\mu\text{g}\cdot\text{mL}^{-1}$ for
18 mono-POM PMEA and 0.1–40 $\mu\text{g}\cdot\text{mL}^{-1}$ for PMEA. The relative standard deviation
19 (%RSD) of intra-day and inter-day precision of the assay method was below 2%, and
20 the accuracy was within the range of 98–102%. External standard method was used
21 for the quantitation of AD, mono-POM PMEA, and PMEA. The percentage of
22 hydrolyzed AD was calculated from the contents of the hydrolysis products

(mono-POM PMEA and PMEA). The percentage of dimerized AD was equal to 100% minus the percentage of residual AD and hydrolyzed AD.

3. Results and discussion

3.1. *Characterization of new crystalline forms*

The PXRD patterns for the new crystalline forms are compared with the individual components in Fig. S1. The PXRD patterns of AD–GA cocrystal, AD–SA salt and AD–MA salt are different from those of starting materials, indicating the formation of new crystalline phases. TG and DSC measurements were carried out to study the thermal behavior of the new crystalline forms compared with the individual components (Fig. S2 and S3). The TG curve of AD–GA cocrystal shows a weight loss of 2.5% over the 100–120 °C temperature range, which is consistent with the loss of one water molecule (calcd 2.6%). It indicates that the produced AD–GA cocrystal is a monohydrate. For AD–SA salt and AD–MA salt, no weight loss was detected ahead the melting points, indicating that they are anhydrous forms. The DSC curves show the melting points (onset values of sharp endothermic melting peaks) for AD, AD–GA cocrystal, AD–SA salt and AD–MA salt at 99.1, 140.2, 94.2 and 130.5 °C, respectively. The melting point of AD is identical to that reported for form I [17]. The melting points of AD–GA cocrystal and AD–MA salt are between those of the individual components, while AD–SA salt has a melting point lower than that of either component.

3.2. Crystal structure analysis

The crystal structures of three new crystalline forms were determined by single crystal X-ray diffraction, which can help identify the arrangements and interactions of AD and coformers in the solid states. The crystallographic data and details of refinement are listed in Table 1. Hydrogen bonds play a crucial role during cocrystallization due to their strength and directionality. To compare the hydrogen bonding interactions before and after cocrystallization, the structures of AD are analyzed from a crystal engineering perspective. Form I of AD consists of two point N–H $\cdots$ N intermolecular hydrogens bonds (Fig. S4) between adenine fragments forming a chained structure [23]. When AD cocrystallizes with a guest carboxylic acid, the hydrogen bond donors and acceptors of two components would adjust to achieve a balance. Thus supramolecular homosynthons in pure AD must be interrupted, whereas some new heterosynthons must be generated. Table 2 summarizes the hydrogen bonding distances and angles for the hydrogen bond participating in the synthons.

The crystal structure of AD–GA cocrystal belongs to the monoclinic, $P2_1/n$ space group. The asymmetric unit contains one AD, one gallic acid, and one water molecules (Fig. 2a). The adenine fragments of AD interact with carboxyl groups of gallic acid through N1–H1B $\cdots$ O12 and O13–H13 $\cdots$ N2 hydrogen bonds and hydroxyl groups through O9–H9 $\cdots$ N5 hydrogen bonds forming a chained structure (Fig. 2b). The phosphoryl fragments of AD also interact with water molecules through O1W–H1W $\cdots$ O2 hydrogen bonds (Fig. 2b).

1 The crystal structure of AD–SA salt was solved in a monoclinic, $P2_1/c$ space
2 group. The asymmetric unit contains a 1: 1 stoichiometric ratio of AD cation and
3 salicylate anion, in which proton transfer from carboxyl groups to adenine fragments
4 (Fig. 3a). Protonated AD connect to salicylate anions via $N1-H1A\cdots O10$ and ionic
5 $N2^+-H1\cdots O11^-$ hydrogen bonds forming a dimer structure. Two dimers further link
6 with each other through interdimer $N1-H1B\cdots O9$ hydrogen bonds to generate a
7 tetramer (Fig. 3b).

8 AD–MA salt crystallizes in the triclinic, $P-1$ space group, with the asymmetric
9 unit containing one protonated AD and one maleate anion (Fig. 4a). The maleate
10 anion adopts a *cis*-conformation, and the neutral carboxyl group is involved in an
11 intramolecular $O11-H11\cdots O10$ hydrogen bond with the adjacent carboxylate group
12 to form a $S_1^1(6)$ ring. Similar to AD–SA salt, one maleate anion and one AD cation
13 form a dimer through $N1-H1B\cdots O10$ and ionic $N2^+-H2A\cdots O9^-$ hydrogen bonds.
14 Then, two dimers link with each other through interdimer $N1-H1A\cdots O12$ hydrogen
15 bonds to generate a tetramer (Fig. 4b).

16 It is generally accepted that a carboxyl anion possesses two close $d(C-O)$ values
17 ($\Delta d(C-O) \leq 0.03$ Å), whereas a neutral carboxyl group possesses two distinctively
18 different $d(C-O)$ values ($\Delta d(C-O) \geq 0.08$ Å) [12]. From bond length analysis (Table 3),
19 the $\Delta d(C-O)$ values are 0.105, 0.029 and 0.024 Å for AD–GA, AD–SA and AD–MA,
20 respectively, confirming that gallic acid forms a cocrystal, while salicylic acid and
21 maleic acid form ionic salts with AD. This result is consistent with the acidity order of
22 maleic acid (pK_a 1.9) > salicylic acid (pK_a 3.0) > gallic acid (pK_a 4.4). Previous

1 reports claimed two cocrystals of AD with saccharin and nicotinamide were prepared
2 [15,27]. According to the strong acidity of saccharin (pK_a 1.6), it is more likely to
3 form salt instead of cocrystal when cocrystallizing with AD.

4 The Earlier reports also showed AD linked saccharin and nicotinamide through
5 intermolecular hydrogen bonding interactions between flexible phosphoryl/carbonyl
6 group of AD and rigid amide group of coformers from imprecise structure data
7 [15,27]. By contrast, no interconnection between phosphoryl/carbonyl group of AD
8 and carboxyl group was observed in our single crystal structures. Our crystal structure
9 analysis shows that the cocrystal has an organization of intermolecular hydrogen
10 bonds to form 1D chains with AD/gallic acid/water trimer units, while both salts form
11 four-component supramolecular structures which consist of two coformers and two
12 API molecules. The $N-H\cdots N$ adenine homosynthons found in form I of AD are
13 replaced by adenine–carboxyl heterosynthons in the new crystal forms, suggesting the
14 degradation of AD might be inhibited after cocrystallization since heterosynthons are
15 usually more stable than homosynthons. In addition, inserting coformers in crystal
16 lattices and subsequently the distance between adenine fragments is increased from
17 4.174 Å for AD to 11.114, 5.864 and 7.253 Å for AD–GA cocrystal, AD–SA salt and
18 AD–MA salt, respectively, which might be help for suppression of dimerization of
19 adenine moiety to some extent (Table 3). For AD–GA cocrystal, hydrate water
20 molecules are linked to the $P=O$ by strong $O-H\cdots O$ hydrogen bonding interactions,
21 which might be help for further reducing rates of hydrolysis of phosphoryl fragments.

22 In addition, the PXRD patterns simulated from SXRD data for three new

1 crystalline forms were compared with the corresponding experimental patterns (Fig.
2 S5). All the peaks displayed in the measured patterns closely match those in the
3 simulated patterns, which indicate that the single crystal structures are representative
4 of the bulk crystalline samples.

5 3.3. DVS study

6 The water sorption kinetics for AD (form I) and three new crystalline forms over
7 the 0-90% RH were studied by DVS experiments to evaluate their
8 moisture-dependent stability. The corresponding isotherm plots are shown in Fig. 5.
9 The phase transitions during the DVS experiment were confirmed by PXRD detection
10 (Fig. S6-9). When exposed to water vapor, AD uptakes significant water (7.2%
11 change in mass) and converts to the dihydrate over 70% to 90% RH range. During the
12 desorption process, the sample maintains the dihydrate stoichiometry until it is dried
13 at 0% RH where it again loses its two hydrate water molecules and returns to form I,
14 which was proven by PXRD measurement (Fig. S6). The above results display a
15 humidity-induced transformation relationship between form I and the dihydrate of AD
16 at room temperature.

17 In contrast, three new crystalline forms of AD exhibit simple sorption/desorption
18 behavior (Fig. 5). The hydrate water molecules of AD–GA cocrystal are not lost even
19 at 0% RH (Fig. S7). As the humidity is increased up to 90% RH, these three
20 crystalline forms uptake negligible water (< 0.5%) on the surface and no phase
21 transitions are observed (Fig. S7-9). During the desorption process, they reversibly
22 lose the absorbed water, and no hysteresis gap is observed in these three instances,

1 demonstrating that the nonhygroscopic property of AD is significantly improved after
2 cocrystallization.

3 *3.4. Stability study*

4 Cocrystallization may modulate the stability of an API since it leads to changes in
5 molecular arrangements and interactions of the relevant chemical entity. In the present
6 study, a systematic stability study of all new crystalline forms of AD, with form I as
7 the control, was performed to assess how cocrystallization would modulate the
8 physical and chemical stability of AD and further explore the degradation kinetics to
9 get in depth understanding on the mechanism of chemical stability enhancement that
10 may occur by cocrystallization.

11 Accelerated stability test at 40 °C/75% RH is commonly investigated to evaluate
12 the stability in the development of pharmaceutical materials. To compare the phase
13 transformation tendency before and after cocrystallization, the PXRD measurements
14 for samples at 40 °C/75% RH were performed. Form I converted to the dihydrate in
15 one month, while three new crystalline forms of AD retained their initial crystal forms
16 after 6 months at 40 °C/75% RH (Fig. S10-13). This result is consistent with the
17 observations made in DVS experiments. Thus these three crystalline forms are
18 physically stable over a period of at least 6 months under direct (unpackaged)
19 exposure to ICH accelerated conditions. The chemical stability of samples was further
20 evaluated by HPLC assay. The residual contents of AD after stored at 40 °C/75% RH
21 for 6 months are presented in Fig. 6. The data for all new crystalline forms of AD
22 were normalized and expressed as the percentage of AD component. For form I, it

1 was found that the residual content of AD decreased to 93.4% after 6 months. In
2 contrast, three new crystalline forms showed no significant decrease ($< 1.3\%$) of
3 residual AD contents during storage, representing a superior chemical stability
4 compared to form I.

5 To understand the difference in stability behavior of the new crystalline phases of
6 AD and the physical mixtures of AD and coformers, the stability experiments were
7 also conducted on the respective physical mixtures. Fig. 7 depicts the residual
8 contents of AD for three physical mixtures and the control sample. After 6 months of
9 exposure to 40 °C/75% RH, the percent AD remaining in AD/GA (92.1%), AD/SA
10 (90.4%) and AD/MA (91.6%) physical mixtures was less than the control (93.4%),
11 suggesting that the addition of carboxylic acids can accelerate rather than reduce the
12 degradation rate of AD. It may be attributed to the carboxylic acids in physical
13 mixtures which can acidify the surroundings of AD and subsequently catalyze the
14 hydrolysis and dimerization reaction [24]. This conclusion is opposite to the opinion
15 of Zhang, et al, who claimed that the weakly acidic micro-environment provided by
16 saccharin might inhibit rather than catalyze the degradation of AD [27]. According to
17 our study, if carboxylic acids are used in AD formulations, they should be
18 incorporated in the crystal lattice, and thus improve the stability of AD by increasing
19 the physicochemical interactions with AD molecules.

20 Degradation kinetics of AD is governed by two temperature-dependent
21 degradation pathways: hydrolysis of the phosphoryl fragment and dimerization of the
22 adenine fragment. Thus exposure of samples to a relative violent condition

(60 °C/75% RH) allows exploring how cocrystallization effects on the kinetics of hydrolysis and dimerization pathways, respectively, on a shorter time scale. Fig. 8 depicts the contents of residual AD, hydrolyzed AD and dimerized AD for samples stored at 60 °C/70% RH. For form I, it was found that the residual content of AD decreased to 62.6% on the 2nd day and abruptly to 21.9% on the 3rd day; meanwhile, the percentage of hydrolyzed AD and dimerized AD respectively increased to 26.9% and 51.2% on the 3rd day. In contrast, three new crystalline forms of AD had significantly higher residual contents of AD at all sampling points. Over the period of the three-day study, the residual contents of AD were 93.3%, 86.5% and 88.7% for AD–GA cocrystal, AD–SA salt, and AD–MA salt, respectively. Chromatographic analysis of the degradation product distribution revealed that cocrystallization of AD with carboxylic acids significantly reduced the rates of both hydrolysis and dimerization as compared to the control sample (Fig. 8).

3.5. *Structure–stability correlation*

It can be seen that incorporation of carboxylic acids to the crystal lattice can significantly improve the stability of AD, while the addition of carboxylic acids as physical mixtures can further accelerate the rate of degradation. Thus a key factor for stability enhancement of AD is the formation of new crystal structures with stronger physicochemical interactions between AD and coformers. Herein we attempt to correlate the stability properties with hydrogen bonding interactions of crystal structures, which may help improve the predictability of pharmaceutical cocrystallization strategy for the aim of stability enhancement.

From DVS data we can find that the nonhygroscopic property of AD is improved after formation of the cocrystal and salts with replacement of drug–drug homosynthons by drug–coformer heterosynthons, which can not only resist the formation of AD dihydrate but also inhibit the hydrolysis of AD at high RH. Chromatographic analysis of the degradation product distribution at 60 °C/75% RH demonstrates that besides reducing the rates of hydrolysis, the formation of cocrystal and salts also diminishes the extent of dimerization, which might be attributed to the increased distance between adenine fragments by inserting coformers in crystal lattices. From chemical stability data, we can find that the rank order of the stability is AD form I < AD–SA salt < AD–MA salt < AD–GA cocrystal. AD–GA cocrystal possesses a highest stability. If we carefully examine its crystal structure, then we can see that not only the hydrogen bonding interactions between adenine fragments and carboxyl groups as other two salts, but also combined those between phosphoryl fragments and hydrate water molecules, may play a role in further improving the stability of the drug. Although AD–SA salt and AD–MA salt have similar hydrogen bonding patterns in their crystal structures, the stability of AD–SA salt is relatively lower than that of AD–MA salt. It might be due to the weaker interdimer hydrogen bonds with smaller angle of 138 ° for AD–SA salt (vs 157 ° for AD–MA), which may also be responsible for its lower melting point of 94.2 °C. (vs 130.5 °C for AD–MA).

4. Conclusion

Adefovir dipivoxil is a diester prodrug of the antiviral agent, adefovir, with known

degradation problem. The present study demonstrates that cocrystallization of adefovir dipivoxil with carboxylic acids can effectively improve the physical and chemical stability. Three new crystalline forms, including one cocrystal hydrate with gallic acid and two salts with salicylic acid and maleic acid, were prepared and fully characterized. The crystal structure analysis reveals that AD–GA cocrystal generates chained structure with AD/coformer/water trimer units, while AD–SA salt and AD–MA salt form four-component supramolecular structures which consist of two coformers and two API molecules. DVS study and stability test indicate that these new crystalline forms of AD exhibit superior solid-state stability. Degradation kinetics and structure-stability correlation studies reveal that the mechanism responsible for stability enhancement involves (1) inhibition of hydrolysis of AD by replacement of drug–drug homosynthons by stronger drug–coformer heterosynthons at adenine fragments; (2) suppression of dimerization of AD by separation of adenine fragments by inserting coformers in crystal lattices; (3) further reducing rates of hydrolysis by forming hydrogen bonding interactions between phosphoryl fragments and hydrate water molecules. Our findings have important implications for use of cocrystallization approach to some unstable drugs in pharmaceutical.

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Appendix A. Supplementary material

Please refer to CCDC: 1402509-1402511 for cif files. Other supplementary data associated with this article can be found, in the online version, at <http://www.sciencedirect.com>.

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1 **Figure and table captions**

2 **Fig. 1.** Degradation of adefovir dipivoxil (AD) and molecular structures of coformers in this study.

3 **Fig. 2.** The asymmetric unit (a) and chained structure (b) in AD–GA cocrystal.

4 **Fig. 3.** The asymmetric unit (a) and the tetramer structure (b) in AD–SA salt.

5 **Fig. 4.** The asymmetric unit (a) and the tetramer structure (b) in AD–MA salt.

6 **Fig. 5.** DVS isotherm plots for AD (form I) and three new crystalline forms of AD.

7 **Fig. 6.** Content of residual AD for pure AD (form I) and three new crystalline forms of AD at

8 40 °C /75%.

9 **Fig. 7.** Content of residual AD for pure AD (form I) and three physical mixtures at 40 °C /75%.

10 **Fig. 8.** Contents of residual AD, hydrolyzed AD and dimerized AD for pure AD (form I) and three

11 new crystalline forms of AD at 60 °C /75%.

12 **Table 1** Crystallographic data and refinement parameters for AD–GA cocrystal, AD–SA salt and

13 AD– MA salt.

14 **Table 2** Hydrogen bonding distances and angles of AD–GA cocrystal, AD–SA salt and AD–MA

15 salt.

16 **Table 3** Bond length of carboxyl groups and distances between amine N of adjacent adenine

17 fragments for AD–GA cocrystal, AD–SA salt and AD– MA salt.

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Fig. 1. Degradation of adefovir dipivoxil (AD) and molecular structures of coformers in this study.

(a)

(b)

Fig. 2. The asymmetric unit (a) and chained structure (b) in AD-GA cocrystal.

(a)

(b)

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Fig. 5. DVS isotherm plots for AD (form I) and three new crystalline forms of AD.

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4 40 °C /75%.

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Fig. 8. Contents of residual AD, hydrolyzed AD and dimerized AD for pure AD (form I) and three new crystalline forms of AD at 60 °C /75%.

Table 1

Crystallographic data and refinement parameters for AD–GA cocrystal, AD–SA salt and AD– MA salt.

	AD–GA	AD–SA	AD–MA
formula	C ₂₇ H ₄₀ N ₅ O ₁₄ P	C ₂₇ H ₃₈ N ₅ O ₁₁ P	C ₂₄ H ₃₆ N ₅ O ₁₂ P
formula weight	689.61	639.59	617.55
crystal system	Monoclinic	Monoclinic	Triclinic
space group	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> -1
<i>a</i> (Å)	17.2208(2)	12.7057(3)	9.5949(6)
<i>b</i> (Å)	11.11360(10)	6.8697(2)	10.1010(6)
<i>c</i> (Å)	17.5357(2)	35.3932(12)	17.5958(11)
<i>α</i> (deg)	90	90	77.536(5)
<i>β</i> (deg)	99.8420(10)	95.414(3)	84.298(5)
<i>γ</i> (deg)	90	90	63.601(6)
<i>V</i> (Å ³)	3306.68(6)	3075.49(16)	1491.50(16)
<i>Z</i>	4	4	2
<i>D</i> _c (g·cm ⁻³)	1.385	1.381	1.375
<i>R</i> _{all} , <i>R</i> _{obs} ^a	0.0613, 0.0464	0.0555, 0.0395	0.1072, 0.0875
<i>wR</i> _{2,all} , <i>wR</i> _{2,obs} ^b	0.1252, 0.1146	0.0987, 0.0897	0.2397, 0.2213
GOF	1.035	1.018	1.042

^a $R_I = \sum ||F_o| - |F_c|| / \sum |F_o|$. $wR_2 = [\sum [w(F_o^2 - F_c^2)^2] / \sum w(F_o^2)^2]^{1/2}$, $w = 1/[\sigma^2(F_o)^2 + (aP)^2 +$

$bP]$, where $P = [(F_o^2) + 2F_c^2]/3$

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3 **Table 2**

4 Hydrogen bonding distances and angles of AD–GA cocrystal, AD–SA salt and AD–MA salt.

	hydrogen bond	D···A (Å)	∠D-H···A (deg)		hydrogen bond	D···A (Å)	∠D-H···A (deg)
AD–GA	N1-H1B···O12	2.882(3)	169(3)	AD–SA	N1-H1A···O10	2.684(2)	173.3
	O13-H13···N2	2.654(3)	167.9		N2-H1 ⁺ ···O11 ⁻	2.734(2)	176(3)
	N1-H1A···O9	3.093(3)	157(3)		N1-H1B···O9	2.845(2)	138.0
	O9-H9···N5	2.646(3)	155.6		O9-H9···O10	2.477(2)	147.2
	O1W-H2W···O13	2.967(3)	157(3)	AD–MA	N1-H1B···O10	2.769(5)	178(6)
	O1W-H1W···O2	2.732(3)	168(4)		N2-H2A ⁺ ···O9 ⁻	2.779(5)	172.5
					N1-H1A···O12#1	2.808(5)	157(5)
					O11-H11···O10	2.434(4)	174(7)

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7 **Table 3**

8 Bond length of carboxyl groups and distances between amine N of adjacent adenine fragments for
9 AD–GA cocrystal, AD–SA salt and AD–MA salt.

	d(C-O) (Å)	Δd(C-O) (Å)	d(N···O) (Å)
AD–GA	1.327(3), 1.222(3)	0.105	11.114
AD–SA	1.278(2), 1.249(2)	0.029	5.864
AD–MA	1.261(6), 1.237(6)	0.024	7.253

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1 Graphical Abstract for

2 **Mechanism study on stability enhancement of adefovir dipivoxil by**

3 **cocrystallization: Degradation kinetics and structure-stability**

4 **correlation**

5 **Rui-Zhen Lin, Peng-Jie Sun, Qian Tao, Jia Yao, Jia-Mei Chen, and Tong-Bu Lu**

6

7 Adefovir dipivoxil (AD) was successfully cocrystallized with gallic acid, salicylic

8 acid, and maleic acid and exhibits improved chemical stability in the order AD gallic

9 acid cocrystal > AD maleate > AD salicylate > AD form I. Degradation kinetics and

10 structure-stability correlation studies reveal the stability enhancement mechanism.