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Enhanced dissolution and stability of adefovir dipivoxil by cocrystal formation

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Abstract

Objectives The objectives of this study were to prepare and characterize the novel adefovir dipivoxil–saccharin cocrystal and to demonstrate the enhanced dissolution and stability of adefovir dipivoxil by cocrystal formation.

Methods Adefovir dipivoxil–saccharin cocrystal was prepared using a novel solution crystallization approach and scaled up to 30 g for subsequent studies. DSC, IR and XRPD were used to characterize the novel solid form. The stoichiometry of the cocrystal was analysed by HPLC. Dissolution and chemical stability were assessed and compared with marketed adefovir dipivoxil (form 1) used in marketed Hepsera Tablets.

Key findings A new solid adefovir dipivoxil–saccharin cocrystal with unique melting point, DSC, FTIR and XRPD data was obtained. The molar ratio of adefovir dipivoxil and saccharin in the cocrystal was determined to be 1 : 1. The cocrystal had a pH-independent dissolution profile and showed a two-fold increase in the dissolution efficiency in water and phosphate buffer (pH 6.8) compared with adefovir dipivoxil. The cocrystal was kinetically much more stable than form 1. Form 1 degraded almost completely at 60°C in 18 days, while adefovir dipivoxil–saccharin cocrystal remained unchanged for 47 days at 60°C.

Conclusions This study demonstrated that the dissolution and stability of adefovir dipivoxil could be significantly enhanced by its cocrystal formation with saccharin. The use of cocrystals could be a feasible and valuable approach for improving the physicochemical properties of adefovir dipivoxil.

Keywords adefovir dipivoxil; cocrystal; dissolution; saccharin; stability

Introduction

The solid properties of an active pharmaceutical ingredient may be modulated by some pharmaceutical techniques, such as solid dispersion,[1] spray-drying[2] and hot-melt extrusion,[3] to enhance the physicochemical and pharmacokinetic performance. The solid properties are dominated not only by molecular structures but also by packing motif. Solid-state form selection should be an emphasis in drug development. Pharmaceutical cocrystals, long-known but underutilized, are multiple component crystals made from an active pharmaceutical ingredient and a coformer that are solid at ambient conditions. The molecules are bonded together by interactions other than covalent or ionic bonds.[4] Cocrystals can significantly increase the diversity and applications of the solid-state forms of active pharmaceutical ingredients and are attractive to pharmaceutical scientists because they provide the solid-state form for non-ionizable molecules. The greatest interest in cocrystals lies in the enhancement of physicochemical properties through modifying the original solid forms.[5,6] Carbamazepine had been well explored as a good model molecule for cocrystal formation. Fleischman *et al*.[7] prepared cocrystals of carbamazepine with benzoquinone, terephthalaldehyde, saccharin, nicotinamide, acetic acid, formic acid, butyric acid, trimesic acid, 5-nitroisophthalic acid, formamide and adamantane-1,3,5,7-tetracarboxylic acid via evaporation in appropriate solvent. Hickey *et al*.[8] compared the stability, dissolution and bioavailability of carbamazepine–saccharin cocrystals with the marketed product. The modification of its melting point, stability and dissolution rate was also demonstrated by Rodriguez-Hornedo *et al*.[9]

Adefovir dipivoxil (9-(2-bis(pivaloyloxymethyl)phosphonomethoxyethyl)adenine, bis(POM)-PMEA) (see Figure 1a for DSC thermogram) is a bis(pivaloyloxymethyl) prodrug of the antiviral nucleotide analogue adefovir.[10] It is indicated for the treatment of $HBeAg^+$ and $HBeAg^-$ chronic hepatitis B by inhibiting DNA polymerase.[11] Due to its low

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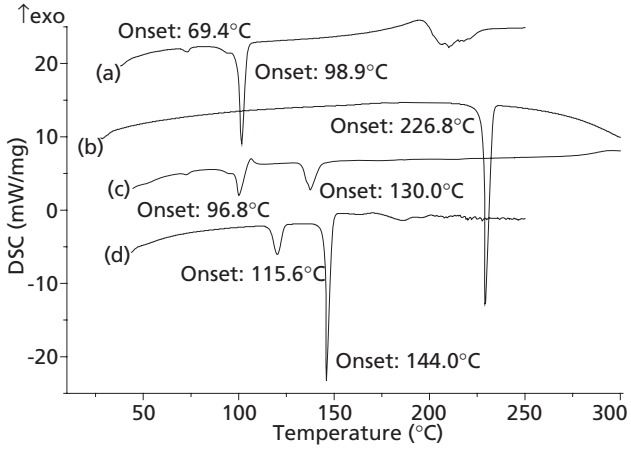

Figure 1 Differential scanning calorimetry thermograms for adefovir dipivoxil (a), saccharin (b), physical mixture of adefovir dipivoxil and saccharin (c) and adefovir dipivoxil–saccharin cocrystals (d).

oral bioavailability, the transdermal delivery of adefovir has been investigated and successful delivery has been demonstrated through porcine skin.[12]

Adefovir dipivoxil is thermally unstable and degrades via two pathways: hydrolysis of the pivaloyloxymethyl moiety and formaldehyde-catalysed dimerization of the adenine ring.[13] It was proposed that some insoluble carbonate salts be added to minimize the dimerization in the solid state while the hydrolysis was not influenced significantly. In addition, polymorphism of adefovir dipivoxil was also exploited to improve its stability.[14,15] Many solid-state forms of adefovir dipivoxil, including crystal form 1, dihydrate and solvates, are protected by patents.[16] However, as the most stable crystal form (anhydrous) used in marketed Hepsera tablets,[17] form 1 still undergoes rapid and extensive degradation in accelerated tests.[18] In our previous cocrystal former screening experiments, it was found that many formers, including some carboxylic acids, may result in the degradation of adefovir dipivoxil during cocrystal preparation. So the neutral saccharin was chosen as the cocrystal former.[19]

In this paper, the cocrystal of adefovir dipivoxil with saccharin was prepared by a solution crystallization approach and characterized by the melting point, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRPD) and stoichiometry. The simultaneous enhancement of dissolution and stability by cocrystal formation was investigated thereafter.

Materials and Methods

Materials

Adefovir dipivoxil (form 1) was recrystallized, as described by Murty,[16] from the marketed adefovir dipivoxil (Bayee Biology, Shanghai, China). Saccharin (99.0% purity) was purchased from Sigma-Aldrich Inc. (Shanghai, China). Mono-POM PMEA reference standard (100577–200401) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of HPLC grade was from E. Merck (Darmstadt, Germany). All other chemical reagents were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Cocrystallization of adefovir dipivoxil–saccharin

Saccharin (1.5 mmol) was dissolved in 10 ml of ethanol. Adefovir dipivoxil (form 1) (1.5 mmol) was added and stirred to obtain a clear solution. The mixture was stirred at 25°C for 10 min and adefovir dipivoxil–saccharin cocrystal precipitated. The product was obtained by suction filtration and dried in a fume hood for 10 h at room temperature. To allow adequate amount of cocrystal for subsequent studies, the adefovir dipivoxil–saccharin cocrystals were then scaled up to 30 g at the same molar ratio, ethanol percentage and temperature mentioned above.

Preliminary characterization

The melting point of samples was determined by RY-1 melting point apparatus (Tianjin, China). Optical micrographs of cocrystals were taken under the Leica DM LM/P polarizing microscope (Wetzlar, Germany).

Differential scanning calorimetry

The thermal properties of samples were characterized by DSC on a Netzsch DSC 204 F1 Phoenix Differential Scanning Calorimeter (Germany), which was calibrated for temperature and cell constants using indium. Samples were placed on non-hermetic aluminium pans. The sample cell was equilibrated at 25°C and then heated at a rate of 10°C/min over the range of 25–250°C. Data analysis was performed using NETZSCH-Proteus software (version 4.2).

Fourier transform infrared spectroscopy

A Thermo Nicolet Impact 410 FTIR Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a spectral resolution of 1 cm^{-1} was used in KBr diffuse reflectance mode for collecting the FTIR spectra of the samples. Sixty-four scans were collected over the range of 4000–400 cm^{-1}. Data were analysed using Nicolet Omnic software (version 8.0).

X-ray powder diffraction

XRPD analysis was performed using a Bruker D8 Advance powder diffractometer (Karlsruhe, Germany) with Cu-Kα radiation (1.5406 Å). The samples were gently consolidated in an aluminium holder and scanned at 50 kV and 50 mA from 7–45°2θ using a scanning speed of 2°/min and a step size of 0.02°. The diffraction patterns were analysed using Materials Studio (version 4.0) and OriginPro 7.0 (OriginLab Corporation, Northampton, MA, USA).

Crystal structure solution, refinement, graphics and generation of publication materials were performed by using Materials Studio, V4.0 software. The diffraction pattern for adefovir dipivoxil–saccharin cocrystals was indexed using X-cell to obtain lattice parameters. The Pawley fit method was performed by the Powder refinement module to fit the peak profiles. The Rietveld refinement was finally performed to demonstrate the cocrystal structure.[20]

HPLC assay

A Shimadzu system consisted of an LC-10AD vp pump and a SPD-10A vp UV detector was applied for stoichiometry

determination of components, dissolution and stability. The analysis was carried out on a Shim-Pack VP-ODS column (4.6 mm × 150 mm, 5 μm) which was kept at 30°C. Isocratic mobile phase was a mixture of methanol and 0.02 mol/l potassium dihydrogen phosphate (pH 6.0) (58 : 42, v/v) at a constant flow rate of 1.0 ml/min. The detector was set at 260 nm and the sample volume solution injected was 20 μl. Quantitation was based on peak area integrated by LC solution (Version 1.24). A mixture of methanol and 0.02 mol/l potassium dihydrogen phosphate (pH 3.0) (58 : 42, v/v) was used to prepare the standard solutions of adefovir dipivoxil (200 μg/ml), mono-POM PMEA (2 μg/ml) and saccharin (36 μg/ml), respectively. A calibration curve containing varied concentrations of adefovir dipivoxil was drawn before the start of the dissolution and stability studies. The total impurities of adefovir dipivoxil were determined by area normalization.

In the concentration range of 161.92–242.88 μg/ml, the concentration of adefovir dipivoxil (Y_1) correlated well to its peak area (X_1): $Y_1 = 3.6895 + 0.0001385X_1$ ($r = 0.9992$, $n = 5$). In the concentration range of 1.68–2.52 μg/ml, the concentration of mono-POM PMEA (Y_2) correlated well to its peak area (X_2): $Y_2 = -0.02887 + 0.00007038X_2$ ($r = 0.9996$, $n = 5$). After storage for 12 h at ambient temperature, adefovir dipivoxil and PMEA were stable. The values of the limit of detection for adefovir dipivoxil and mono-POM PMEA were 1.62 ng/ml and 2.1 ng/ml, respectively.

Stoichiometry determination of components

The adefovir dipivoxil–saccharin cocrystal stoichiometry was determined. Sample solution was prepared by transferring 13.6 mg adefovir dipivoxil–saccharin cocrystal to a 50 ml volumetric flask and diluting to mark with methanol. Adefovir dipivoxil and saccharin were assayed by HPLC. Samples were analysed in triplicate.

Dissolution

Adefovir dipivoxil, adefovir dipivoxil–saccharin cocrystal and the physical mixture of adefovir dipivoxil and saccharin with a molar ratio of 1 : 1 were sieved using ASTM standard-mesh sieves (mesh size 149 μm) to provide powders with similar particle ranges. The dissolution studies were conducted using a ZRS-8G dissolution tester (TDTF technology Co., Ltd, Tianjin, China). A USP32-NF27 paddle method was used at a rotation speed of 50 rev/min at 37°C. Accurately weighed powders of approximately (or corresponding to, for cocrystal or physical mixture) 10 mg of adefovir dipivoxil were added to dissolution vessels containing 500 ml of medium. Three dissolution media were studied: water, 0.1 mol/l HCl and 0.05 mol/l phosphate buffer (pH 6.8). Sampling was performed at 5, 10, 15, 20, 30 and 45 min and the withdrawn slurry was filtered with 0.22-μm cellulose filter before HPLC analysis for adefovir dipivoxil. Each kind of sample was tested 12 times to calculate the dissolution efficiency, difference factors and similarity factors.

Chemical stability

To evaluate the chemical stability, solid samples were sealed in glass vials and stored in laboratory ovens (MMM Medcenter Einrichtungen GmbH, Planegg, Germany) at 60°C or 40°C. Upon removal at each time point, the contents of adefovir dipivoxil, the main degradation product (mono-POM PMEA) and total impurities in the samples were analysed by HPLC as mentioned above. Samples were assessed in triplicate.

Statistical analysis

In the dissolution test, all data are presented as the mean of 12 individual observations, with standard deviation of mean. Kruskal–Wallis one-way analysis of variance was used to evaluate the effect of dissolution media on pure adefovir dipivoxil, physical mixture and cocrystal. Their dissolution profiles in the same medium were compared using Nemenyi's test. In the chemical stability experiment, properties such as the content of adefovir dipivoxil and the content of impurities at different time points were compared with those at zero time using a one-way analysis of variance. These properties of cocrystal and pure adefovir dipivoxil were statistically compared using a paired-samples *t*-test.

Results

Preliminary characterization

The adefovir dipivoxil–saccharin cocrystals under the optical microscope were observed to be white lamellar-shaped crystals with diffraction quality. A clear melting point around 144°C was observed for adefovir dipivoxil–saccharin cocrystals. This is between the melting points of the individual components of the cocrystal, adefovir dipivoxil (97–99°C) and saccharin (226–228°C). The sharp melting point transition of adefovir dipivoxil–saccharin cocrystals indicates the formation of a new crystalline form.

Differential scanning calorimetry analysis

DSC experiments were conducted to study the thermal behaviour of the adefovir dipivoxil–saccharin cocrystals compared with the individual components.

DSC thermograms for adefovir dipivoxil, saccharin and adefovir dipivoxil–saccharin cocrystals are presented in Figure 1. The onset values for adefovir dipivoxil–saccharin cocrystals and the individual components agreed with the measured melting points. Adefovir dipivoxil showed a single sharp endothermic melting peak with onset at 98.9°C (Figure 1a). This indicates that adefovir dipivoxil was in the form 1.[16] Saccharin demonstrated a steep endothermic melting transition at 226.8°C (Figure 1b), which is in agreement with reported thermal behaviour.[21] The DSC thermogram for adefovir dipivoxil–saccharin cocrystals showed a single endothermic transition attributed to the melting transition (onset = 144°C) (Figure 1d).

The thermal behaviour of the cocrystal was distinct and unique from the individual components; this suggests the formation of a new adefovir dipivoxil–saccharin cocrystal phase. A single endothermic transition for the adefovir dipivoxil–saccharin cocrystals demonstrates the stability of the phase until the melting point and indicates the absence of any unbound or absorbed solvent or water.

Fourier transform infrared spectroscopic analysis

The FTIR spectra for adefovir dipivoxil, saccharin and adefovir dipivoxil–saccharin cocrystals are presented in

Figure 2a–c. The FTIR spectrum of adefovir dipivoxil indicates that adefovir dipivoxil (form 1) was the starting material in the cocrystal formation (Figure 2a).[16] It had peaks assigned to P=O stretching vibration at 1293 cm^{-1}, C=O stretching vibration at 1751 cm^{-1} and N-H stretching vibrations of the amine group at 3275 cm^{-1} and 3124 cm^{-1}. The spectrum of saccharin had peaks corresponding to C=O and N-H stretching vibrations of the secondary amide at 1724 and 3092 cm^{-1}, respectively. In addition, the peaks at 1338 and 1174 cm^{-1} were assigned to the asymmetric and symmetric stretching vibrations of the -SO_2 group (Figure 2b).[21]

The P=O and C=O stretching vibrations of adefovir dipivoxil in adefovir dipivoxil–saccharin cocrystals were observed at 1288 cm^{-1} and 1751 cm^{-1} with N-H stretching vibrations at 3218 and 2971 cm^{-1}. The C=O and N-H stretching vibrations of the cyclic imide group of saccharin were observed at 1697 and 3130 cm^{-1} (Figure 2c). This suggests that both molecules are present in the new phase.

The bathochromic shift in the P=O stretching vibration from 1293 cm^{-1} to 1288 cm^{-1} indicates that a phosphoryl group is likely to participate in a hydrogen bond. The bathochromic shifts in N-H stretching vibrations to 3218 and 2971 cm^{-1} imply another possible hydrogen bond involving the amine group of adefovir dipivoxil. The C=O group underwent no shift in wavenumber.

The hypsochromic shift in the N-H stretching vibration from 3092 cm^{-1} in the imide dimer of saccharin to 3130 cm^{-1} in the adefovir dipivoxil–saccharin cocrystal suggests that the N-H group is participating in the hydrogen bond. A bathochromic shift in the saccharin C=O stretching vibration from 1724 cm^{-1} to 1697 cm^{-1} further explains the formation of adefovir dipivoxil–saccharin cocrystal. The asymmetric and symmetric stretching vibrations of the -SO_2 group of saccharin were also observed in adefovir dipivoxil–saccharin cocrystals at 1333 and 1152 cm^{-1}, respectively.

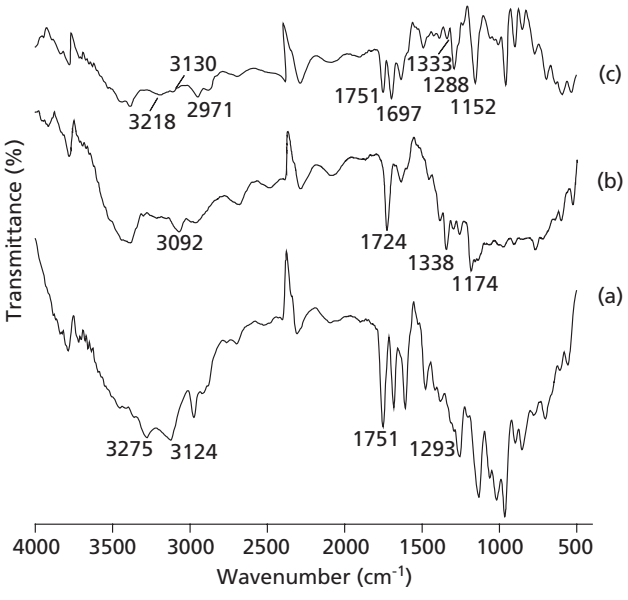

Figure 2 Fourier transform infrared spectra for adefovir dipivoxil (a), saccharin (b) and adefovir dipivoxil–saccharin cocrystal (c).

X-ray powder diffraction analysis and cocrystal structure

The XRPD pattern for adefovir dipivoxil, saccharin and adefovir dipivoxil–saccharin cocrystals are presented in Figure 3. The XRPD pattern for adefovir dipivoxil was similar to that reported for form 1,[16] which had peaks at 12.18°, 12.92°, 15.84°, 17.48°, 20.94°, 21.42°, 22.74° and 23.34°2θ.

Adefovir dipivoxil–saccharin cocrystals exhibited a unique XRPD pattern that allowed them to be distinguished from adefovir dipivoxil and saccharin. It had peaks at the following 2θ angles: 10.66°, 16.46°, 16.90°, 18.34°, 20.36°, 21.66°, 24.10° and 26.88°. This confirms the formation of a new cocrystal phase. The experimental XRPD pattern of adefovir dipivoxil–saccharin cocrystals was X-cell indexed and Pawley refined. The characteristics parameters of the triclinic cell obtained after the Pawley fit are presented in Table 1. A final Rietveld refinement following the Pawley refinement gave a final profile Rwp = 13.58, Rp = 9.49 and Rwp = 39.63. All coordinates of the non-hydrogen atoms were freely refined.

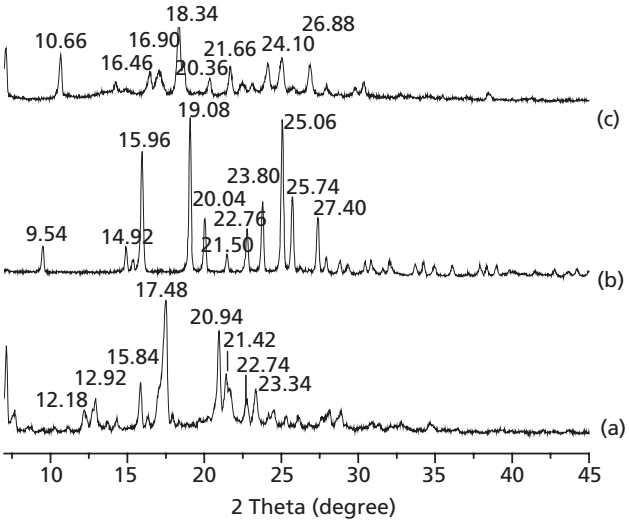

Figure 3 X-ray powder diffraction patterns for adefovir dipivoxil (a), saccharin (b) and adefovir dipivoxil–saccharin cocrystal (c).

Table 1 Crystallographic data and Pawley fit results of adefovir dipivoxil–saccharin cocrystals

Lattice parameters	
System	Triclinic
Space group	P1
a (Å)	8.9589
b (Å)	8.2665
c (Å)	6.4422
α (°)	75.197
β (°)	100.21
γ (°)	111.827
V (Å^3)	426.4
zero point correction	–0.00863
Pawley fit results	
Rwp (%)	8.83
Rwp(w/o bck) (%)	27.61
Rp (%)	5.83

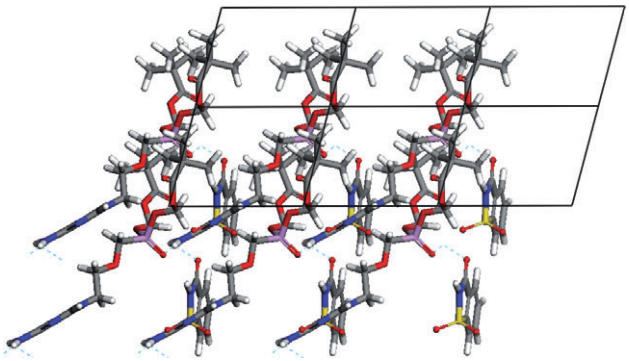

Figure 4 Crystal structure of adefovir dipivoxil–saccharin cocrystal. Note that both the phosphoryl group and imide synthons are interconnected by N–H···O hydrogen bonds.

The final crystal structure of adefovir dipivoxil–saccharin is presented in Figure 4. It revealed the phosphoryl group and imide synthons are interconnected by N–H···O hydrogen bonds.

Cocrystal stoichiometry

Cocrystal stoichiometry was determined by assaying adefovir dipivoxil and saccharin using HPLC. The percentages of adefovir dipivoxil and saccharin were 75.70 ± 0.47 (%) and 26.97 ± 0.55 (%), respectively. The cocrystal stoichiometry was shown to be 1 : 1 since the theoretical percentages of the two components are 73.25% and 26.75%.

Dissolution

A standard in-vitro dissolution powder dissolution test was used to provide a comparison with the dissolution profiles of adefovir dipivoxil and its physical mixture with saccharin. It is generally recognized that buffers with different pH value should be used as dissolution media to evaluate the drug dissolution in different physiological environments. All powder underwent rapid dissolution in 0.1 M hydrochloric acid, with more than 90% of adefovir dipivoxil released after the appropriate 5 min (Figure 5). Cocrystals achieved pH-independent dissolution characteristics and were found to dissolve rapidly in distilled water and phosphate buffer (pH 6.8). After 10 min, the difference in dissolution percentage was not significant in three media at each time point ($P > 0.05$).

However, dissolution media had a significant effect on the dissolution of pure adefovir dipivoxil and the physical mixture, especially their dissolution amount. Significantly less adefovir dipivoxil was dissolved within 45 min in both media compared with the amount dissolved in 0.1 M HCl ($P < 0.01$). The amounts of dissolved adefovir dipivoxil in 45 min were about 42.7% and 62.0%. Compared with them, cocrystals exhibited much faster and complete dissolution even in distilled water and phosphate buffer.

The dissolution profiles of all samples in water and phosphate buffer (pH 6.8) were analysed by two model independent approaches (dissolution efficiency[22] and the similarity factor[23]) and findings are presented in Table 2. Dissolution efficiency (DE) can be calculated by the following equation:

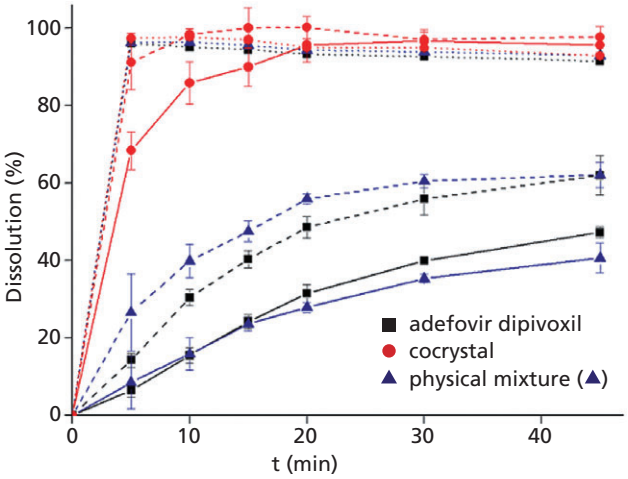

Figure 5 Dissolution profiles for adefovir dipivoxil, cocrystal and physical mixture in 0.1 M HCl (dotted line), water (dashed line) and pH 6.8 phosphate buffer (solid line) ($n = 12$).

$$DE = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100\% \quad (1)$$

where y is the drug percent dissolved at time t.

The difference factor (f_1) calculates the percent (%) difference between the two curves:

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] / \left[\sum_{t=1}^{n} R_t \right] \right\} \cdot 100 \quad (2)$$

where n is the number of time points, R_t and T_t are the dissolution values of the reference and test batches at time t, respectively.

The similarity factor (f_2) is a measurement of the similarity in the percent (%) dissolution between the two curves:

$$f_2 = 50 \cdot \log \left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \cdot 100 \right\} \quad (3)$$

For curves to be considered similar, f_1 values should be close to 0 (<15), and f_2 values should be close to 100 (>50).

Adefovir dipivoxil–saccharin cocrystals had significantly greater dissolution efficiency than adefovir dipivoxil and the physical mixture in distilled water and phosphate buffer (pH 6.8). They showed a two-fold increase in dissolution efficiency in the two dissolution media. Analysis of the difference/similarity factors suggested that the dissolution profiles of adefovir dipivoxil were similar to those of the physical mixture but significantly different from those of adefovir dipivoxil–saccharin cocrystals.

Chemical stability

A comparison of chemical stability of adefovir dipivoxil–saccharin cocrystals with adefovir dipivoxil was performed to assess whether cocrystallization had the potential to enhance

Table 2 Statistical analysis of dissolution profiles of adefovir dipivoxil, cocrystal and physical mixture in distilled water and pH 6.8 phosphate buffer

Media	Reference	Test	DE (%)	Difference (f_1)	Similarity (f_2)
Distilled water		Adefovir dipivoxil	43.38 ± 2.29		
Distilled water	Adefovir dipivoxil	Cocrystal	85.21 ± 2.95[a]	132.50	12.08
Distilled water	Adefovir dipivoxil	Physical mixture	49.12 ± 2.49[b]	16.34	55.09
pH 6.8 phosphate buffer		Adefovir dipivoxil	29.33 ± 1.11		
pH 6.8 phosphate buffer	Adefovir dipivoxil	Cocrystal	85.85 ± 3.34[a]	223.06	10.51
pH 6.8 phosphate buffer	Adefovir dipivoxil	Physical mixture	26.55 ± 2.51[b]	10.74	70.80

[a]$P < 0.05$, different compared with the values of adefovir dipivoxil. [b]$P > 0.05$, similar compared with the values of adefovir dipivoxil ($n = 12$)

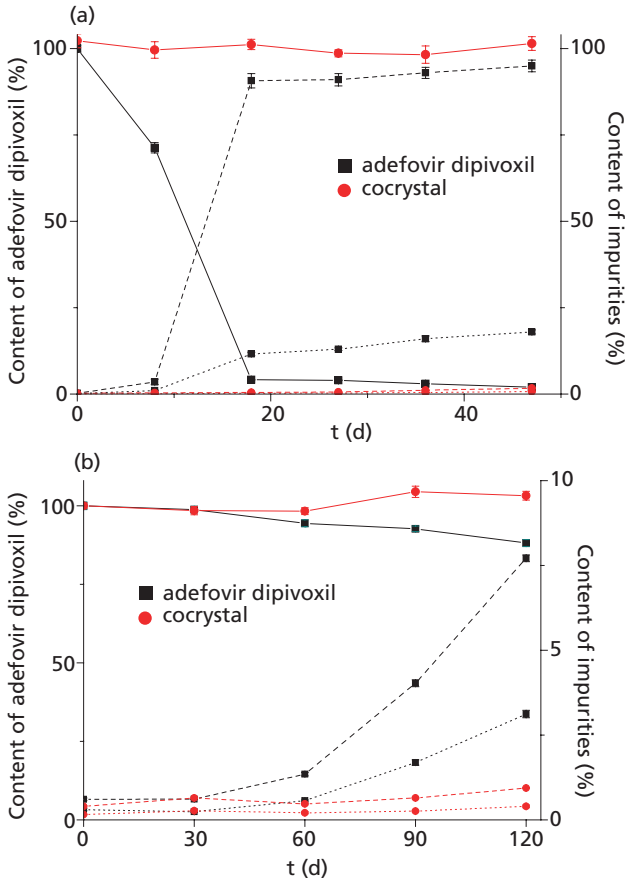

Figure 6 Content of adefovir dipivoxil (solid line), mono-POM PMEA (dashed line) and total impurities (dotted line) assessed for chemical stability of adefovir dipivoxil and cocrystal at 60°C (a) and 40°C (b) ($n = 3$).

the chemical stability of adefovir dipivoxil. The change of the content of adefovir dipivoxil and impurities across six time points at 60°C is presented in Figure 6a. It was found at 60°C that the content of adefovir dipivoxil decreased to 71.30% on the 8th day and abruptly to 4.13% on the 18th day when the degradation product (mono-POM PMEA) was more than 10% and the amount of total impurities was 90.71%. The data for adefovir dipivoxil–saccharin cocrystals were normalized and expressed as the percentage of the adefovir dipivoxil component. Compared with adefovir dipivoxil, adefovir dipivoxil–saccharin cocrystals had a significantly higher content of adefovir dipivoxil and a significantly lower content of impurities at all sampling points ($n = 3$, $P < 0.01$). Over the period of the 47-day study, no significant content change was found in adefovir dipivoxil–saccharin cocrystals ($P > 0.05$). The amount of mono-POM PMEA was less than 1% and the amount of total impurities was less than 2%. Compared with the values at time zero, they were not significantly changed ($n = 3$, $P > 0.05$).

The experimental results at 40°C (Figure 6b) showed a similar variation tendency, further demonstrating that the thermodynamical stability of adefovir dipivoxil was greatly enhanced. The change in appearance of the samples was also visually examined. It was observed that adefovir dipivoxil began to agglomerate to form a cake-like structure at the first sampling time point at 60°C, whereas adefovir dipivoxil–saccharin cocrystals maintained a powder state during the whole course.

Discussion

There are 13 hydrogen bond acceptors and 2 hydrogen donors in adefovir dipivoxil molecule which is potential to form cocrystals based on hydrogen bond. Chang *et al*. revealed that the molecules are linked by N-H···N, O-H···O and O-H···N hydrogen bonding interactions in the crystal structure of adefovir dipivoxil dihydrate.[24] A dimer synthon compromises two adefovir dipivoxil molecules linked by N-H···N hydrogen bonding between adenine rings. Two hetero synthons were formed by O-H···O and O-H···N hydrogen bonding between adefovir dipivoxil and water. Because of the synthons in adefovir dipivoxil molecule, saccharin with a strong hydrogen bond donor and acceptors was selected as the coformer to prepare the cocrystal.

Dissolution enhancement

Adefovir dipivoxil is generally classified as BCS 3 drug and may not exhibit dissolution rate-limited absorption. However, it was found that adefovir dipivoxil is a compound with a pH-dependent dissolution profile. The rate and extent of dissolution of adefovir dipivoxil decreased with an increase of the pH of dissolution media ($P < 0.05$). The dissolution of adefovir dipivoxil was not enhanced by addition of saccharin. It exhibited an incomplete dissolution in neutral and slightly alkaline environments and its complete dissolution could be only obtained in acidic media. It is well known that the pH environment in the stomach can be weakened in some physiological and pathological conditions or by concomitant drug administration, which may diminish the dissolution amount of

adefovir dipivoxil. So the increased amount dissolved in non-acidic condition has the potential for improving the absorption profile.

Adefovir dipivoxil–saccharin cocrystals can provide a pH-independent dissolution profile ($P > 0.05$), exhibiting a complete and rapid dissolution in all experimental aqueous media. This suggests that the cocrystallization of saccharin into the adefovir dipivoxil crystal changed the crystal packing and arrangement and favoured the dissolution of adefovir dipivoxil. By molecular hydrogen bonding, cocrystals provided a new approach for dissolution enhancement, different from solubilization by surfactants, cosolvency by mixed solvents or hydrotropy.

Chemical stability enhancement

The stability of adefovir dipivoxil was significantly enhanced by cocrystallization with saccharin. The cocrystal remained almost intact even at high temperature (60°C). Degradation of adefovir dipivoxil leads to the formation of mono-POM PMEA, pivalic acid and formaldehyde in equal proportion.[13] For phosphonate analogues of nucleotides, water molecules react with the phosphorus atom and carbonyl moiety, resulting in the hydrolysis of adefovir dipivoxil to mono-POM PMEA.[25] Considering the wavenumber shift demonstrated in FTIR analysis and the demonstrated crystal structure, it was inferred that P=O in adefovir dipivoxil was linked up with the NH group in saccharin by hydrogen bonding, which changes the molecular arrangement and crystal packing of adefovir dipivoxil. The hydrogen bond formation through cocrystallization with saccharin may be strong enough to prevent the attack by water molecules and weaken the hydrolysis.

Solid stability has great impact on manufacturing and storage. It was reported in accelerated tests that the most stable crystal form (form 1) still undergoes obvious degradation. Adefovir dipivoxil–saccharin cocrystals with significantly enhanced stability will be beneficial for the stabilization of the solid dosage forms.

The formation of cocrystals brought about enhanced solubility and stability simultaneously. This may be due to the different bonding sites (in adefovir dipivoxil or in cocrystal) with water molecules. In the adefovir dipivoxil–saccharin cocrystal, P=O in adefovir dipivoxil was linked up with the NH group in saccharin by hydrogen bonding and prevented the attack by water molecules at this site in adefovir dipivoxil. On the other hand, it is speculated that the connection of saccharin with strong hydrogen bonding ability to adefovir dipivoxil increases the ability of the resultant whole cocrystal to interact with water molecules through hydrogen bonds, therefore improving the dissolution of the cocrystal.

Conclusions

In this study, a cocrystal form between adefovir dipivoxil and saccharin was created and prepared in gram quantities using solution crystallization approach. The novel AD-SAC cocrystal was a new solid phase with unique DSC, FTIR and XRPD and a stoichiometry of 1 : 1 between adefovir dipivoxil and saccharin. Through the hydrogen bond formation through cocrystallization between the two compounds, the molecular arrangement and crystal packing of adefovir dipivoxil was accomplished to improve its important characteristics. Overall, the study manifested that the use of cocrystals is a feasible and valuable approach for improving the physicochemical properties of a compound.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Dhumal RS *et al*. Cefuroxime axetil solid dispersion with polyglycolized glycerides for improved stability and bioavailability. *J Pharm Pharmacol* 2009; 61: 743–751.
2. Bothiraja C *et al*. Evaluation of molecular pharmaceutical and in-vivo properties of spray-dried isolated andrographolide-PVP. *J Pharm Pharmacol* 2009; 61: 1465–1472.
3. Andrews GP *et al*. Physicochemical characterization and drug-release properties of celecoxib hot-melt extruded glass solutions. *J Pharm Pharmacol* 2010; 62: 1580–1590.
4. Zhang GZ, Zhou D. *In Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice*. Burlington: Academic Press, 2009.
5. Shan N, Zaworotko MJ. The role of cocrystals in pharmaceutical science. *Drug Discov Today* 2008; 13: 440–446.
6. Gao Y *et al*. [Pharmaceutical cocrystals.]. *Chem Prog* 2010; 22: 829–836 [in Chinese].
7. Fleischman SG *et al*. Crystal engineering of the composition of pharmaceutical phases: multiple-component crystalline solids involving carbamazepine. *Cryst Growth Des* 2003; 3: 909–920.
8. Hickey MB *et al*. Performance comparison of a co-crystal of carbamazepine with marketed product. *Eur J Pharm Biopharm* 2007; 67: 112–119.
9. Rodriguez-Hornedo N *et al*. Cocrystals: design, properties, and formation mechanisms. In: Swarbrick J, ed. *Encyclopedia of Pharmaceutical Technology*. New York: Informa Healthcare USA, Inc, 2007: 615–635.
10. Starrett JE *et al*. Synthesis, Oral bioavailability determination, and in vitro evaluation of prodrugs of the antiviral agent 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA). *J Med Chem* 1994; 37: 1857–1864.
11. Patrick M *et al*. Adefovir Dipivoxil for the treatment of hepatitis b e antigen–positive chronic hepatitis B. *N Engl J Med* 2003; 348: 808–816.
12. Vávrová K *et al*. Transdermal and dermal delivery of adefovir: effects of pH and permeation enhancers. *Eur J Pharm Biopharm* 2008; 69: 597–604.
13. Yuan L *et al*. Effect of carbonate salts on the kinetics of acid-catalyzed dimerization of adefovir dipivoxil. *Pharm Res* 2000; 17: 1098–1103.

14. Zhang D *et al*. [New crystalline form and preparation of adefovir dipivoxil]. CN Patent 1303089C, 2007 [in Chinese].
15. Zhang X *et al*. [Crystalline form of adefovir dipivoxil]. CN Patent 1211391C, 2005 [in Chinese].
16. Murty NA *et al*. Nucleotide analog compositions. US Patent 006451340B1, 2002.
17. The Committee for Medicinal Products for Human Use. *European Medicines Agency, Scientific Discussion* [online]. 2009. Available at: http://www.emea.europa.eu/humandocs/Humans/EPAR/hepsera/hepsera.htm (accessed 27 January 2011).
18. Sun WJ *et al*. Accelerated test's stability comparison of three adefovir dipivoxil products. *Chin J New Drug Clin Remedy* 2010; 29; 923–927 [in Chinese].
19. Basavoju S *et al*. Indomethacin–saccharin cocrystal: design, synthesis and preliminary pharmaceutical characterization. *Pharm Res* 2008; 25: 530–541.
20. Guguta C *et al*. Crystal structure of aspartame anhydrate from powder diffraction data. structural aspects of the dehydration process of aspartame. *Cryst Growth Des* 2006; 6: 2686–2692.
21. Matos MAR *et al*. Saccharin: a combined experimental and computational thermochemical investigation of a sweetener and sulfonamide. *Mol Phys* 2005; 103: 221–228.
22. Menegola J *et al*. Dissolution test for citalopram in tablets and comparison of in vitro dissolution profiles. *Eur J Pharm Biopharm* 2007; 67: 524–530.
23. US Food and Drug Administration. *Guidance for Industry: Dissolution Testing of Immediate Release Solid oral Dosage Forms*. Rockville: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 1997.
24. Chang Y *et al*. 9-{2-[Bis(pivaloyloxymethoxy)phosphinylmethoxy]ethyl}adenine dihydrate. *Acta Crystallogr Section E* 2007; 63: o1014–o1015.
25. Yuan L *et al*. Degradation kinetics of oxycarbonyloxymethyl prodrugs of phosphonates in solution. *Pharm Res* 2001; 18: 234–237.