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Co-Amorphization of Acyclovir with Oxalic Acid as an Excipient

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Abstract

The present article provides a novel technique for the co-amorphous formation of acyclovir and oxalic acid as an excipient. Designated as ACV-oxalic acid, methods for the preparation thereof and its use in pharmaceutical applications are described. The co-amorphous ACV-oxalic acid was characterized by powder X-ray diffraction, differential scanning calorimetry and thermogravimetric analysis. Stability with respect to relative humidity (RH) for the co-amorphous ACV-oxalic acid was evaluated and compared with the parent ACV. The aqueous solubility of the co-amorphous ACV-oxalic acid was significantly improved (about 8 times more soluble) at 35°C compared to that of the parent Acyclovir base.

Keywords: Co-amorphous phase, Recrystallization, Solubility, Dissolution rate, Stability, Relative humidity

Introduction

Acyclovir (2-Amino-1, 9-dihydro-9-((2-hydroxyethoxy)methyl)-6H-purin-6-one (abbreviated as ACV, Figure 1) is an antiviral agent used for the treatment of infections viz. herpes simplex (HSV-1 and HSV-2), shingles and varicella i.e., chickenpox Cyclovir, Herpex, Acivir, Acivirax, Zovirax, Zoral, Xovir and Imavir are the brand names of ACV, available in market. The commercial ACV is a 3:2 ACV: Water hydrate [1], whereas the dihydrated form 1:2 ACV: Water has also been reported [2]. One metastable and two stable anhydrous forms were obtained by heating commercial ACV at temperature ranging from 130°C to 180°C [3]. However, the hydrated and anhydrous forms have poor bioavailability [4] because ACV is a class IV drug according to biopharmaceutical system or BCS. Furthermore, hydrated and anhydrous forms of ACV are not stable at all relative humidity conditions [5-7]. It would be desirable to have a solid form of ACV with improved solubility and stability. Therefore, to improve the solubility, dissolution rate and hence bioavailabity of ACV, a novel co-amorphous phase of acyclovir was developed with oxalic acid as an excipient. It was found that the new co-amorphous material is almost stable at all atmospheric relative humidity conditions. Consequently, the developed novel co-amorphous form will be suitable as an anti-viral pharmaceutical drug and has the advantages over the crystalline forms reported earlier.

There is a high demand in the pharmaceutical industry for stable amorphous APIs with high purity. There are several advantages of amorphous forms of a drug substance over crystalline form with respect to solubility and dissolution rate. It has been found that amorphous phases dissolve more rapidly than crystalline forms, and hence, can significantly increase bioavailability of poorly water-soluble drugs. However, the major drawbacks of using the amorphous form is the resulting reduced chemical stability, increased hygroscopicity and physical instability in the final drug product.

The objective of the present research is to develop a technique for the formation of a co-amorphous of ACV with an excipient with favourable properties for the preparation of pharmaceutical formulation to meet the requirements of the pharmaceutical industry. It has been shown that a co-amorphous form of an API, while having the advantage of improved solubility and dissolution rate, it is also physically and chemically more stable than the amorphous phase of the parent API.

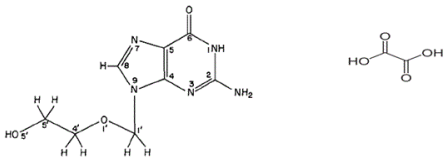

Figure 1: The chemical structures of ACV and Oxalic acid.

The co-amorphous of ACV with oxalic acid has a glass transition temperature at 175°C. This glass transition temperature (Tg) is too high which means that when the co-amorphous ACV-oxalic acid is stored below Tg (for example at 25°C), the kinetics of conversion of the co-amorphous phase to a crystalline material will be slow. Consequently, the co-amorphous product of the present invention should be suitable for formulation and has the advantages over the crystalline and amorphous forms of ACV reported earlier. The co-amorphous ACV-oxalic acid should be suitable for use as an anti-viral therapeutic agent.

Experimental Procedure

Materials

Apotex PharmaChem Inc. (Brantford, Ontario, Canada) donated 3:2 ACV: Water hydrated form. Other chemicals were purchased from Sigma-Aldrich (London, Ontario) and were used as received without

further purification. The anhydrous ACV was prepared by heating the 3:2 ACV: Water hydrated form at 180°C for an hour.

Co-amorphous acyclovir oxalic acid (1:1 ACV-oxalic acid)

This material was prepared both by solution evaporation and solvent drop liquid-assisted grinding technique. Anhydrous ACV (225 mg, 1 mmol) and oxalic acid (90 mg, 1 mmol) were carefully weighed and dissolved in 10 mL of acetic acid in a sealed tube and heated at 100°C using a hot plate with constant stirring with a magnetic stirrer for 2 h.

The solution was then filtered through 5 µm filter paper (VWR brand; VWR International Ltd., London, Ontario) to remove insolubles, and allowed to evaporate slowly at room temperature under a fume hood. The co-amorphization of acyclovir with oxalic acid was also achieved by grinding 1:1 stoichiometric amounts of anhydrous ACV and oxalic acid in a mortar and pestle for 20 min with gradual addition of 5 drops of methanol from a pipette. The experiment was performed in triplicate to confirm the reproducibility.

Powder X-ray diffraction (PXRD)

The PXRD spectra were collected on a Rigaku-Miniflex benchtop X-ray powder diffractometer (Carlsbad, CA) using CuKα (λ=1.54059 Å) radiation obtained at 30 kV and 15 mA. The scans were run from 5.0° to 35.0° 2θ, increasing at a step size of 0.05° 2θ with a counting time of 2 s for each step. The diffractograms were processed using JADE 7.0 software. Calibration was performed using a silicon standard.

Differential scanning calorimeter (DSC)

Differential scanning calorimetry tests were performed with a Mettler Toledo DSC 822e differential scanning calorimeter (Greifensee, Switzerland). Accurately weighed samples (~ 3 mg) were prepared in a covered aluminium crucible having pierced lids to allow escape of volatiles. The sensors and samples were under nitrogen purge during the experiments. The temperature calibration was carried out using the melting point of highly pure indium in the medium temperature range with a 5 °C/min heating rate.

Thermogravimetric analysis (TGA)

TGA was performed on a Mettler-Toledo TGA/SDTA 851e instrument. Approximately 5 mg sample was heated from 25°C to 250°C at 10°C/min under nitrogen purge.

Relative humidity (RH)

Relative humidity stability tests were conducted at ambient temperature (ca. 20°C) in a sealed glass desiccator containing P_2O_5 for the 0% RH, and at the appropriate saturated aqueous salt solutions for other RH values (K_2CO_3 for 43%; NaCl for 75% and K_2SO_4 for 98%). Co-amorphous ACV-oxalic acid samples were evaluated along with anhydrous ACV and commercial ACV for stability conditions at 0%, 43%, 75% and 98% RH for different time periods (1 day, 3 days, 1 week and 3 weeks).

Powdered ACV-oxalic acid samples with about 40-60 mg were kept in an open glass vials and were stored in the RH chambers at room temperature. The samples were quickly characterized for any form change by PXRD, upon removal from the desiccator.

Solubility and dissolution rate

The solubility of ACV and co-amorphous ACV-oxalic acid samples in water was measured at 35°C. To measure the saturated concentration of each sample, 20 g of the solvent mixture and an excess amount of solids were added to a vial at a given temperature and mixed using a magnetic stirrer plate (AGE Magnetic Stirrer, Newtec Inc., Hull, IA). The suspensions were then placed on a multi-plate mechanical shaker and were left to equilibrate for 72 h in a temperature controlled water bath.

Samples were filtered through 0.45 mm cellulose acetate syringe filters into volumetric flasks. Supernatants were then analyzed by UV spectrophotometric analysis at λ_{max} 252 nm (Cary 100 Bio UV visible Spectrophotometer, Palo Alto, CA).

Subsequently, dissolution rate experiments were conducted on ACV and co-amorphous ACV-oxalic acid samples. The dissolution rate was measured by placing 100 mg of ACV and co-amorphous ACV-oxalic acid in 200 mL of phosphate buffer saline (PBS), as the dissolution medium, for 90 min at 100 rpm in a magnetic stirrer at 35°C. Five mL of aliquots were withdrawn every 10 min. The dissolution medium was replenished after every sampling to maintain the initial sample volume. The concentration of the sample in the solution was measured by UV spectrophotometer (Cary 100 Bio (Palo Alto, CA)).

Results and Discussion

Material characterization

Powder X-ray diffraction: The co-amorphization of ACV with oxalic acid, as a pharmaceutically acceptable drug excipient, was achieved using one unit of ACV with one unit of oxalic acid. The resulting co-amorphous material was a pure compound of ACV and oxalic acid in a 1:1 molar ratio.

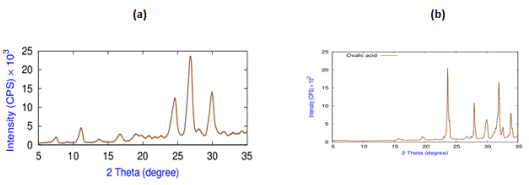

Figure 2: The PXRD patterns of (a) ACV and (b) Oxalic acid.

The PXRD is the main technique to compare different crystalline polymorphic forms of a compound. However, if the sample is in an amorphous state, then no diffraction intensities will be observed and a diffuse broad band will result. Figure 2 shows the PXRD pattern of anhydrous ACV and oxalic acid, and Figure 3 shows the PXRD pattern of the co-amorphous ACV-oxalic acid with a broad diffuse band.

Thermal analysis: The co-amorphous ACV-oxalic acid 1:1 was characterized by DSC (Figure 4) with a glass transition temperature (Tg) of 175°C indicating the sample was amorphous. In addition, an exotherm which is usually accompanied by co-amorphization of an API is also present. From the TGA thermal analysis, it can be concluded that the co-amorphous ACV-oxalic acid is not solvated.

The DSC and TGA thermograms of co-amorphous ACV-oxalic acid are depicted in Figures 4 and 5. The DSC thermogram of the co-

amorphous ACV-oxalic acid was distinguishable from ACV and the corresponding oxalic acid.

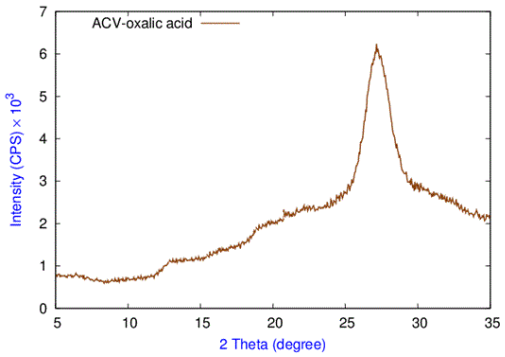

Figure 3: The PXRD patterns of co-amorphous ACV-oxalic acid.

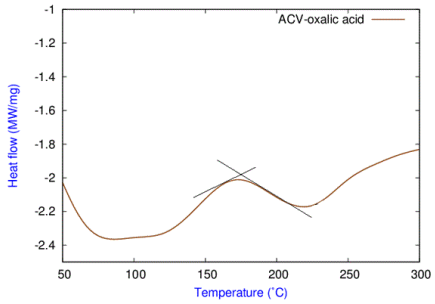

Figure 4: The DSC thermogram of co-amorphous ACV-oxalic acid indicating glass transition temperature and showing an exotherm.

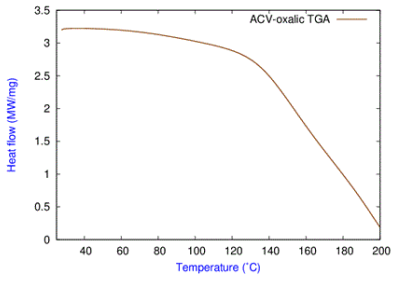

Figure 5: The TGA thermogram of co-amorphous ACV-oxalic acid.

Physicochemical Properties

Stability tests at different relative humidity

The RH stability results of 3:2 ACV: Water (hydrate ACV), anhydrous ACV, and the co-amorphous ACV-oxalic acid are described below and are summarized in Table 1.

1. **The 3:2 ACV: Water hydrate:** The 3:2 ACV: Water hydrate rapidly hydrated at all RH conditions except at 0% RH. The PXRD pattern of hydrated form was evaluated by Sohn and Kim [4]. At 0% RH, the 2:3 hydrated form lost water and as reported earlier it was converted to anhydrous form within 1 day [4].
2. **Anhydrous ACV:** The anhydrous ACV was converted to dihydrated form of ACV within 1 day at all RH conditions, but at 0% RH it remained stable for 3 weeks.
3. **Co-amorphous ACV-oxalic acid:** This material was found to be stable at all relative humidity conditions for 3 weeks, except at 98% RH condition. At 98% RH condition, the material was stable up to 4 days and after that slowly converted to a crystalline material. This study clearly demonstrated enhanced stability of the proposed novel co-amorphous ACV-oxalic acid over hydrated and anhydrous ACV.

Material	Stability with respect to RH				
	% RH	1 day	3 days	1 week	2 weeks
Anhydrous ACV	0	√	√	√	√
	43	×	×	×	×
	75	×	×	×	×
	98	×	×	×	×
2:3 hydrated ACV	0	×	×	×	×
	43	×	×	×	×
	75	×	×	×	×
	98	×	×	×	×
co-amorphous ACV-oxalic acid 1:1	0	√	√	√	√
	43	√	√	√	√
	75	√	√	√	√
	98	√	√	×	×
The symbol √ and × indicate that the material is stable and unstable respectively at that particular RH condition and time point.					

Table 1: The stability of co-amorphous ACV-oxalic acid with respect to RH.

Solubility and dissolution tests

Solubility of the co-amorphous ACV-oxalic acid material in water at 35°C was compared with the anhydrous ACV and the results are summarized in Table 2. The thermodynamic solubility values revealed that the solubility of the co-amorphous ACV-oxalic acid was about 8 times higher than the solubility of anhydrous ACV.

Material	Solubility in water at 35°C (moles/L)
Anhydrous ACV	1.28×10^{-1}
Co-Amorphous ACV-Oxalic acid 1:1	7.98×10^{-1}

Table 2: The solubility of anhydrous ACV and co-amorphous ACV-oxalic acid materials in water at 35°C.

For Anhydrous ACV

Relative standard deviation RSD=(100 × 0.00158)/1.28 × 10^{-1}=1.23

Our final result can be written as 1.28 × 10^{-1}(moles/L) ± 0.00158

Or 1.28 × 10^{-1} (moles/L) ± 1.23%

For co-amorphous ACV-oxalic acid1:1

Relative standard deviation RSD=(100 × 0.0021)/7.98 × 10^{-1}=0.263

Our final result can be written as 7.98 × 10^{-1}(moles/L) ± 0.0021

Or 7.98 × 10^{-1}(moles/L) ± 0.263%

The highest concentration of anhydrous ACV in the solution (released ACV) was observed approximately after 50 min, whereas the maximum dissolved concentration of the co-amorphous ACV-oxalic acid happened around 20 min after starting the experiment. At the same dissolution time, the concentration of the co-amorphous ACV-oxalic acid in the solution was consistently higher than that of ACV. Moreover, the co-amorphous ACV-oxalic acid had the maximum dissolved concentration at any given time. For example, at 20 min, the concentrations of co-amorphous ACV-oxalic acid and anhydrous ACV were 1.69 mg cm^{-2} and 1.22 mg cm^{-2}, respectively (Tables 3 and 4). Initially co-amorphous ACV-oxalic acid dissolved rapidly and reached equilibrium after 20 min, but dissolution of ACV was slow from the beginning and reached the equilibrium point after 50 min (Figure 6). Solubility was performed in triplicate.

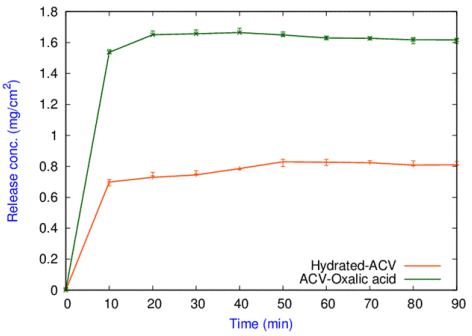

Figure 6: The release profile of co-amorphous ACV-oxalic acid salts compared with hydrated ACV.

Time point	10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min	90 min
RSD	1.8	1.2	1.4	1.8	0.8	0.6	0.4	0.8	1.8

Table 3: RSD values for ACV-Oxalic acid salts.

Time point	10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min	90 min
RSD	2.1	2.4	1.8	0.2	2.2	2.1	0.4	1.2	1.8

Table 4: RSD values for hydrated ACV.

Conclusions

Co-amorphization of acyclovir with oxalic acid in 1:1 stoichiometric ratio has been described. The stability of the co-amorphous ACV-oxalic acid with respect to RH has been improved compared to the hydrated and anhydrous ACV. In terms of physicochemical properties, such as solubility and dissolution rate, the co-amorphous ACV-oxalic acid shows remarkably higher solubility and faster dissolution rate compared to the hydrated ACV. Taking into account, the improved physicochemical properties and stability of the proposed co-amorphous form of acyclovir with oxalic acid reported herein, novel formulations of ACV using the suggested co-amorphous ACV-oxalic acid can be developed as a medicament.

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