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Solid-State Characterization of Different Crystalline Forms of Sitagliptin

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Abstract: Sitagliptin is an inhibitor of the enzyme dipeptidyl peptidase-4, used for the treatment of type 2 diabetes mellitus. The crystal structure of active pharmaceutical solids determines their physical and chemical properties. The polymorphism, solvates and hydrates can influence the free energy, thermodynamic parameters, solubility, solid-state stability, processability and dissolution rate, besides directly affecting the bioavailability. Thus, the physicochemical characterization of an active pharmaceutical ingredient is required to guarantee the rational development of new dosage forms. In this context, we describe herein the solid-state characterization of three crystalline forms of sitagliptin: sitagliptin phosphate monohydrate, sitagliptin phosphate anhydrous and sitagliptin base form. The investigation was carried out using differential scanning calorimetry (DSC), thermogravimetry (TG)/derivative thermogravimetry (DTG), spectroscopic techniques, X-ray powder diffraction (XRPD) and morphological analysis by scanning electron microscopy. The thermal analysis revealed that during the dehydration of sitagliptin phosphate monohydrate (Tpeak = 134.43 °C, $\Delta H = -1.15\ J\ g^{-1}$) there is a characteristic crystalline transition event, which alters the physicochemical parameters of the drug, such as the melting point and solubility. The crystalline behavior of sitagliptin base form differs from that of sitagliptin phosphate monohydrate and sitagliptin phosphate anhydrous, mainly with regard to the lower temperature of the fusion event. The melting point (Tpeak) values obtained were 120.29 °C for sitagliptin base form, 206.37 °C for sitagliptin phosphate monohydrate and 214.92 °C for sitagliptin phosphate anhydrous. In relation to the thermal stability, sitagliptin phosphate monohydrate and sitagliptin phosphate anhydrous showed a slight difference; however, both are more thermostable than the base molecule. Therefore, through this study it was possible to establish the most suitable crystalline form of sitagliptin for the development of a safe, effective and appropriate pharmaceutical dosage form.

Keywords: crystallinity; characterization in solid state; physicochemical properties; solubility; bioavailability

1. Introduction

Sitagliptin (Figure 1) is an inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4) and it consequently inhibits the degradation of incretin hormones, like glucagon-like peptide-1 (GLP-1) and

glucose-dependent insulinotropic peptide (GIP), resulting in increased insulin secretion and inhibited glucagon release by the beta and alpha cells of the pancreas, improving glycemic control [1,2].

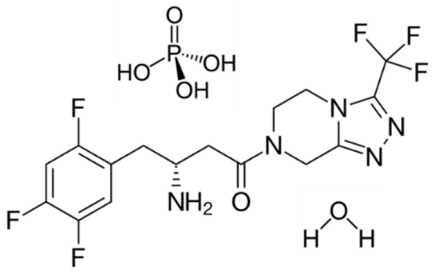

Figure 1. Chemical structure of sitagliptin phosphate monohydrate.

Sitagliptin (7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine) is an effective anti-glycemic drug used for the treatment of type 2 diabetes mellitus (T2DM) [3]. The original product is marketed by Merck & Co., under the trade name Januvia, as sitagliptin phosphate monohydrate, approved in 2006 by the Food and Drug Administration (FDA) [4,5].

Sitagliptin phosphate monohydrate (STG) is white to off-white, crystalline, non-hygroscopic, soluble in water and *N*,*N*-dimethylformamide, slightly soluble in methanol and very slightly soluble in ethanol, acetone and acetonitrile. It has the molecular formula $C_{16}H_{15}F_6N_5O{\cdot}H_3PO_4{\cdot}H_2O$ and the molecular weight is 523.32 g mol^{-1} [6].

The hydrate formation is of great importance in the pharmaceutical industry, considering the ubiquity of water vapor, because hydrates are frequently more stable [5]. Thus, the presence of solvates and hydrates influences the physicochemical properties of the crystals.

A single molecule, such as sitagliptin, may give rise to a variety of crystalline forms with distinct crystal structures and physical properties. The structure of a crystal does not only determine the physicochemical properties of the active pharmaceutical ingredient (API) but also the solubility, stability, processability and bioavailability of the drug [7].

Solubility is one of the most important parameters in relation to achieving the desired concentration of the drug in systemic circulation in order to obtain the required pharmacological response. Poorly water-soluble drugs with a slow absorption, for instance, may show inadequate bioavailability [8,9].

The characterization of solid-state properties is a prerequisite in the development of new pharmaceutical solid dosage forms [5]. Thus, the characterization and differentiation of different crystalline forms of sitagliptin is required so that the physical properties of complex solvates and hydrates can be determined. Thermal analysis, for example, is a well-known technique for the characterization of APIs in terms of stability and structural investigations [10,11].

Sitagliptin phosphate monohydrate is relatively characterized and commonly used in the pharmaceutical industry; however, little is known about other crystalline forms of sitagliptin, such as the anhydrous and base form, including the effect of the dehydration process and the production of base form on the physical and chemical stability of this pharmaceutical hydrate.

Few patents such as US patent application 2015/0087834 A1 [12] reported a method for preparation of sitagliptin phosphate and sitagliptin phosphate anhydrous, providing a brief and superficial description of X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC). The US 2009/0221595 A1 [13] reported the processes of preparing polymorphs forms, describing briefly X-ray powder diffraction and differential scanning calorimetry only the base form of sitagliptin. In fact, there is no studies in the literature that reports the detailed characterization these three crystalline forms.

In this context, the novelty of this work aims to provide a detailed solid state characterization of STG, sitagliptin phosphate anhydrous (STGA) and sitagliptin base form (STGB). The physical-chemical characteristics were investigated using DSC, thermogravimetry (TG), non-isothermal kinetics analysis,

spectroscopic techniques (Fourier transform infrared (FTIR) and Raman), XRPD and scanning electron microscopy (SEM).

2. Materials and Methods

2.1. Sitagliptin Phosphate Monohydrate (STG)

The active pharmaceutical ingredient, STG with 99.9% declared content, batch 20170904, was purchased from Baoji Guokang Bio-Technology (Baoji, China).

2.2. Sitagliptin Phosphate Anhydrous (STGA)

The sitagliptin anhydrous form was obtained by dehydration of sitagliptin phosphate monohydrate (STG). The process involved placing 2.5 g of STG in a vacuum oven (model 6030A VACUOTERM, São Paulo, Brazil) for 60 min at 300–400 mmHg and 150 °C.

2.3. Sitagliptin Base Form (STGB)

The base form of sitagliptin was obtained by dissolving 0.008 mol g^{-1} of sitagliptin phosphate monohydrate (STG) in 92.10 mL of purified water and then adding ammonia (10%, v/v) at pH 10.0. The solution obtained was poured into a separatory funnel and washed twice with 83.73 mL of ethyl acetate. The organic phase was dried with anhydrous sodium sulfate and filtered through quantitative filter paper. The filtrate was evaporated in a rotary evaporator and then dried at room temperature in a vacuum desiccator. A white solid crystal powder was obtained with a yield of 92% [12].

2.4. Differential Scanning Calorimetry (DSC)

The DSC analysis was performed in a Shimadzu® DSC-60 calorimeter (Kyoto, Japan). Samples were analyzed in an aluminum crucible containing around 2.0 mg of sample under dynamic synthetic air atmosphere (50 mL min^{-1}) with a heating rate of 10 °C min^{-1} and temperature range of 30 °C to 400 °C. The equipment was calibrated with indium and zinc (reference standards).

The purity was determined using aluminum crucibles with approximately 2.0 mg of sample at a heating rate of 2 °C min^{-1} from 30 °C to 400 °C. The purity of the sample was measured in triplicate using TASYS software (version 1.14, Shimadzu®), based on the Van't Hoff equation:

$$X_2 = \frac{(T_o - T_m)\Delta H_f}{RT_o{}^2} \quad (1)$$

where the purity is determined from the molar percentage of impurities present in the sample, X_2 represents the mole fraction of impurities, T_m is the sample melting temperature, T_o is the melting point of the pure substance (°K), R is a gas constant and ΔH_f is the heat of fusion of the main component (J mol^{-1}) [14,15].

2.5. Thermogravimetry (TG)

The thermogravimetric analysis was carried out on a Shimadzu® DTG-60 thermal analyzer under a dynamic synthetic air atmosphere of 50 mL min^{-1}. Approximately 4.0 mg of sample was placed in a platinum crucible and heated from 30 °C to 400 °C at a heating rate of 10 °C min^{-1}.

For the non-isothermal kinetics studies, the curves were obtained using five different heating rates: 5, 10, 15, 20, 25 °C min^{-1}. The kinetics parameters were determined by the Ozawa method using TASYS software. The equipment was calibrated with calcium oxalate (reference standard).

2.6. Thermogravimetry–Mass Spectrometry (TG-MS)

Thermogravimetry coupled to mass spectrometry was used to study the thermal decomposition of sitagliptin phosphate monohydrate. The analysis was carried out on a TA Instruments®MS

Q600 SDT analyzer (New Castle, DE, USA). Approximately 2.0 mg of each sample was heated to 900 °C under a dynamic nitrogen atmosphere (50 mL min^{-1}) applying a heating rate of 10 °C min^{-1}. The equipment generates separate TG curves and MS spectra, analyzing the mass/charge (m/z) with respect to temperature.

2.7. Melting Point

The melting behavior was determined using a Mettler-Toledo MP70 melting point system (Greifensee, Switzerland). A capillary was used with a closed bottom, applying a heating rate of 10 °C min^{-1} up to a temperature limit of 400 °C.

2.8. Fourier Transform Infrared (FTIR) Spectroscopy

The infrared spectra were recorded on a Bruker Alpha-P FTIR spectrometer (Ettlingen, Germany) using attenuated total reflection (ATR) in the wavelength range of 3500 to 500 cm^{-1}, with a nominal resolution of 4 cm^{-1} and accumulation of 32 scans.

2.9. Raman Spectroscopy

The Raman spectra were obtained with a WITEC-Alpha 300R confocal Raman microscope (Ulm, Germany), using a diode 3 mW source at a diffraction grating of 600 g mm^{-1}, wavelength laser of 532 nm, integration time of 3 s, resolution of 5.0 cm^{-1} and accumulation of 30 scans.

2.10. X-ray Powder Diffraction (XRPD)

The X-ray powder diffraction patterns were obtained on a Shimadzu® XRD-7000 X-ray diffractometer using a sample door of stainless steel of 20 mm with monochromatic radiation CuKα (λ = 1.5406 Å), a voltage of 40.0 kV, current of 20.0 mA, 2θ scanning angle and scan range of 2.0–40.0.

2.11. Scanning Electron Microscopy (SEM)

The morphological evaluation was performed by scanning electron microscopy (SEM) using a JEOL JSM-6360 LV microscope (São Paulo, Brazil). The sample was pre-metallized with gold and analyzed at low vacuum with an acceleration voltage of 15 kV at magnifications of 200×, 600×, 2000× and 5000×.

3. Results and Discussion

3.1. Thermal Characterization

The thermal behavior of STG can be observed based on the DSC and TG/DTG curves in Figure 2. The DSC curve shows four endothermic events and six exothermic events. The first endothermic event corresponds to dehydration of the STG (T_{peak} = 134.43 °C; T_{onset} = 126.76 °C; $\Delta H = -1.15$ J g^{-1}), followed by a well-defined endothermic event (T_{peak} = 142.30 °C; T_{onset} = 140.61 °C; $\Delta H = -42.00$ J g^{-1}), corresponding to the crystalline transition of the sitagliptin phosphate, since no mass loss was indicated by the TG/DTG curve. The third endothermic event (T_{peak} = 206.37 °C; T_{onset} = 203.02 °C; $\Delta H = -104.97$ J g^{-1}) is characteristic of the melting process, and is in agreement with the melting point determined by capillary analysis (205.3 °C) and results reported in the literature [16]. Subsequent thermal events correspond to the decomposition process.

The first mass loss on the TG/DTG curve at between 101 °C and 136 °C (Δm = 2.9%; DTG_{peak} = 117.20 °C) confirms the dehydration event observed on the DSC curve. The second mass loss event indicates that the thermal decomposition occurs in two stages immediately after the fusion event, in the ranges of 192 °C to 243 °C (Δm = 7.9%; DTG_{peak} = 232.81 °C) and 243 °C to 385 °C (Δm = 60.4%; DTG_{peak} = 300.57 °C). Thus, the TG/DTG curve confirms that there is thermal stability up to 192 °C STG.

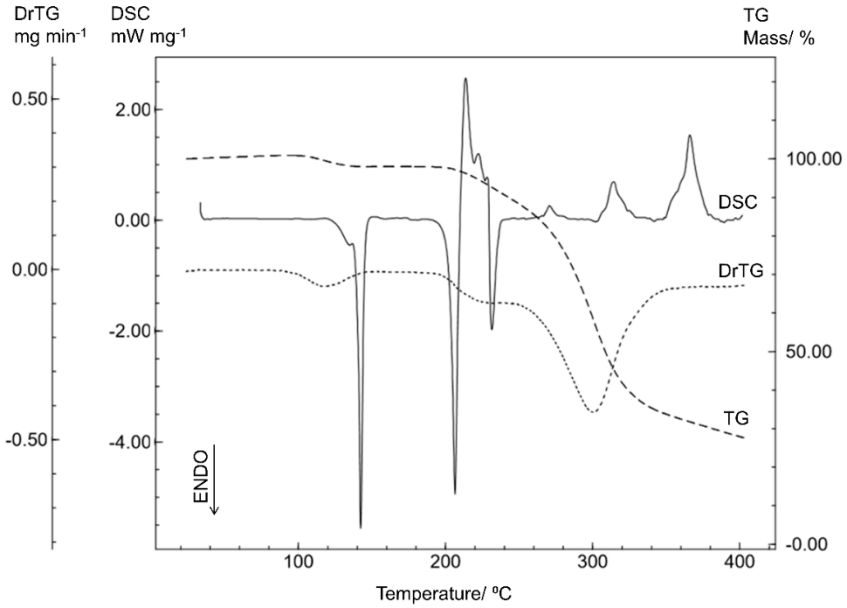

Figure 2. The differential scanning calorimetry (DSC) and thermogravimetry/derivative thermogravimetry (TG/DTG) curves of sitagliptin phosphate monohydrate (STG) obtained with a synthetic air atmosphere (50 mL min^{-1}) and a heating rate of 10 °C min^{-1}.

In order to compare the thermal behavior of the different crystalline forms of sitagliptin, the STGA was analyzed by DSC and TG and the results are shown in Figure 3.

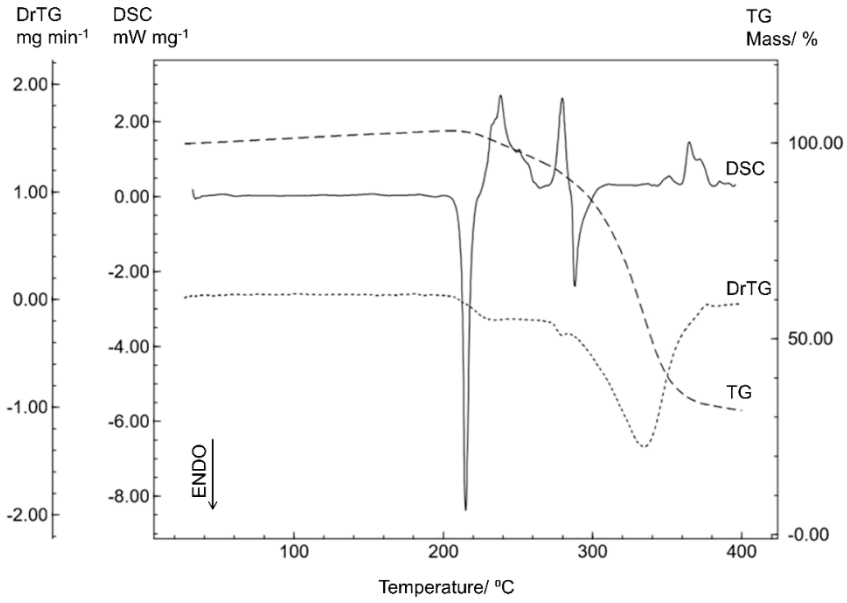

Figure 3. The DSC and TG/DTG curves for sitagliptin phosphate anhydrous (STGA) obtained with a synthetic air atmosphere (50 mL min^{-1}) and a heating rate of 10 °C min^{-1}.

The DSC curve for STGA shows two endothermic events and three exothermic events. The first is related to the melting point (T_{peak} = 214.92 °C; T_{onset} = 212.58 °C; ΔH = −104.84 J g^{-1}), which was confirmed by the capillary method (212.2 °C). The subsequent events correspond to thermal decomposition, confirmed by the TG/DTG curve, indicating that thermal decomposition occurs in three steps starting at 216 °C (DTG_{peak} = 234.24 °C; DTG_{peak} = 278.74 °C; DTG_{peak} = 333.94 °C).

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