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Thermal stability of aripiprazole monohydrate investigated by Raman spectroscopy

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ABSTRACT

Aripiprazole (7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyril) is a well-known antipsychotic oral drug, whose molecular structure makes it suitable for polymorphism. Several crystalline structures were reported in the literature including anhydrate, hydrate and solvate modifications. It was observed that aripiprazole monohydrate exhibits a complex dehydration dynamics which was not completely elucidated. In this work the dehydration process of the aripiprazole hydrate was investigated by using Raman scattering, hot-stage microscopy and differential scanning calorimetry. The temperature evolution of the Raman spectra was analyzed through the multivariate statistical method of principal component analysis. Our results support that the dehydration process of aripiprazole is divided into two steps. First a diffusion-controlled loss of water followed by a fast nucleation and crystallization of the anhydrous form.

1. Introduction

The relevance of controlling polymorphism and solvate formation in crystallization of organic compounds is widely recognized within the industrial and academic communities. Polymorphism is the ability of an active pharmaceutical ingredient (API) to exist in more than one crystalline form. Different polymorphs and solvates of the same drug compound, although having the same active ingredient, exhibit variable physical properties: solubility, dissolution rate, chemical stability, melting points, density, hardness, filterability, color and many other properties. As a result, polymorphism and solvatomorphism can affect the quality, safety and efficacy of a drug product [1,2].

Antipsychotics are characterized by their psychotropic action with psychomotor and sedative effects. Aripiprazole (7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyril, Fig. 1) was the sixth second-generation antipsychotics to be introduced in the market [3–5]. The action mechanism of aripiprazole (APZ) differs from currently marketed typical and atypical antipsychotics [6]. Unlike its predecessors, APZ is considered a partial dopaminergic agonist, acting on both postsynaptic D_2 receptors and presynaptic autoreceptors. It also displays partial agonism at serotonin$_{1A}$ (5-HT$_{1A}$) receptors and antagonism at 5-HT$_{2A}$ receptors [7,8]. APZ was discovered by Otsuka Pharmaceutical Company and approved for the treatment of schizophrenia by the Food and Drug Administration (FDA) in 2002 [9,10]. It is commercialized in the United States and foremost European countries under the name Abilify®.

APZ has a rather simple molecular structure (Fig. 1), the fact of having aliphatic chains and saturated rings makes this molecule suitable for polymorphism. More than 20 patents on the solid forms that this API can assume were applied. APZ was reported to exist in several polymorphs, solvates, salts and co-crystals [11,12]. However, a rigorous investigation of the polymorphism and solvatomorphism of APZ was just recently presented by Braun et al. [12,13] and Tessler and Goldberg [11]. These authors reported the existence of 5 polymorphs and 3 solvates (including one monohydrate). Based on thermal analysis, structural and spectroscopy data, they have established the thermodynamic relationships among the polymorphs and the stability (thermal and moisture) of the solvates.

In the last few years, the investigation of pharmaceutical compounds by means of Raman spectroscopy has attracted much interest and some reports pointed out its pharmaceutical applications [14–16]. The application of this technique to monitor the solid phase during polymorphic and pseudopolymorphic phase transi-

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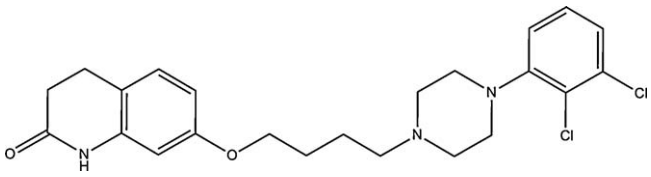

Fig. 1. Aripiprazole scheme.

tions has been described in recent publications [17–19]. The aim of this work is to investigate *in situ* the dehydration process of the aripiprazole hydrate (H_1) by using Raman scattering. The spectroscopic results are correlated with hot-stage microscopy (HTM) and differential scanning calorimetry (DSC) in order to shed some light on the desolvation mechanism of this compound.

2. Experimental

APZ as a polycrystalline material showing a wide spread of particle size was provided by Maprimed SA (Argentina). Single and well developed crystals of APZ hydrate (H_1) were obtained by dissolving its solid powder in slightly hydrated acetone or ethanol, at 80 °C followed by slow cooling. The clear solution was stored at room temperature and after a low evaporation of the solvent, crystals of APZ, as small colorless plates, were collected from the mother liquor by filtration and dried in air. Amorphous APZ was obtained by melting of the anhydrous and hydrated forms.

Simultaneous thermogravimetric and thermal differential analysis were performed on the single crystals and polycrystalline samples. Thermogravimetric measurements (TGA) had been made under dry nitrogen atmosphere on a Netzsch STA 409 PC LUXX simultaneous thermal analyzer coupled to a Fourier transform infrared spectrophotometer (Bruker Tensor 27) used to analyze the released gasses. The samples were placed in open pans and measured under nitrogen flow.

Optical microscopy studies were performed on a Leitz Ortholux II Pol-BK polarizing Microscope equipped with a Kofler heating stage (Thermovar, Reichert, Vienna, Austria), a full-wave retardation plate and an on-purpose adapted webcam.

Raman spectra were recorded on a Jobin-Yvon T64000 triplemate spectrometer equipped with a liquid N_2-cooled CCD detector. The 514.5 nm line of an Ar+ ion laser (coherent Innova 70) operating at 50 mW was used as excitation.

The principal component analysis of the spectroscopic data was performed using Unscrambler MVDA software (version 9.5, Camo Inc., Woodbridge, New Jersey, U.S.). Data were preprocessed using Multiple Scatter Correction (MSC).

3. Results and discussion

3.1. Hot stage microscopy

Hot stage microscopy was applied to monitor the desolvation process in the APZ hydrate H_1. Transparent tabular single crystals embedded in silicon oil and observed under crossed polarizers were heated using a slow rate (Fig. 2). In this figure, the APZ crystals are observed in yellow, whereas the non-birrefringent regions exhibit an intense red coloration due to the retardation plate. At ca. 80 °C, the loss of some water molecules is evidenced by the observation of few little bubbles, like the one below the APZ crystal at 90 °C in Fig. 2. Since the H_1 has well defined channels that allow the water molecules diffusion, the crystallinity of the sample remains unaltered. On heating, this process continued, but a rather big fraction of the crystal remained unaltered up to complete fusion ca 120 °C, where a simultaneous and clear loss of water is observed. The anhydrous crystals finally melt around 140 °C.

3.2. Thermal analysis

A detailed thermal characterization of polymorphs and solvates of aripiprazole was performed by Braun et al. [12,13]. These authors have determined the thermodynamic relationships among these solid forms. Despite the fact form I (melting point: 148.5 °C) is the thermodynamically most stable modification, all the solvates transform to form III (melting point: 139 °C) after the desolvation process. However, a variable amount of form I can also be observed. In the case of H_1, Braun et al. observed that the dehydration temperature depends on the particle size. It was observed that fine powders start the dehydration first than single crystals. DSC curves provide more detailed information about this process (Fig. 3). In general, the DSC curves can be divided in two regions: the dehydration/melting of H_1 (below ~130 °C) and the melting of anhydrous APZ (above ~130 °C). The melting of the anhydrous form is usually characterized by a peak at 139 °C related to the form III, but in some cases the presence of a small amount of form I (see the curve recorded at 2 °C/min in Fig. 3) is evidenced by a peak at ~148 °C. The recrystallization of amorphous APZ was also investigated by DSC, as it is shown in Fig. 3. One exothermic and two endothermic events are observed in this process. The exothermic event corresponds to the recrystallization of the amorphous form into a polymorph of AZP, which transform into form III around 130 °C. Further investigations must be performed in order to identify the nature of the intermediate form.

Regarding the dehydration process, DSC curves were recorded in single crystals and powder samples (Fig. 3). In the case of single crystals, the dehydration is dominated by a single event around 123 °C. This event was associated with the inhomogeneous melt-

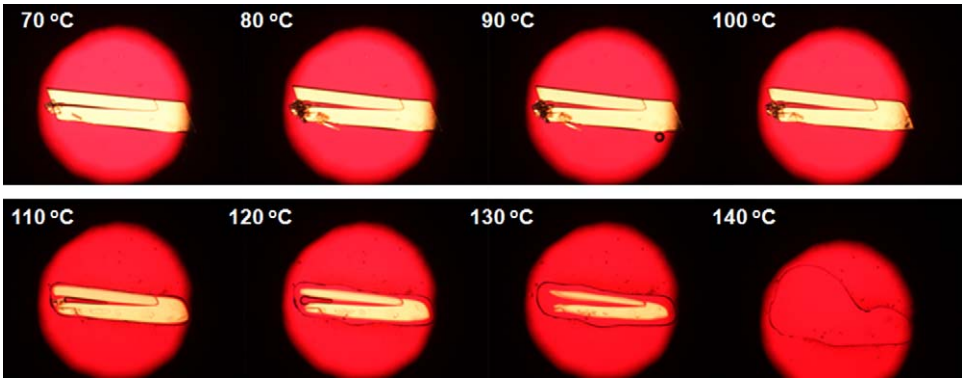

Fig. 2. Hot stage microscopy images of aripiprazole hydrate between 70 and 140 °C.

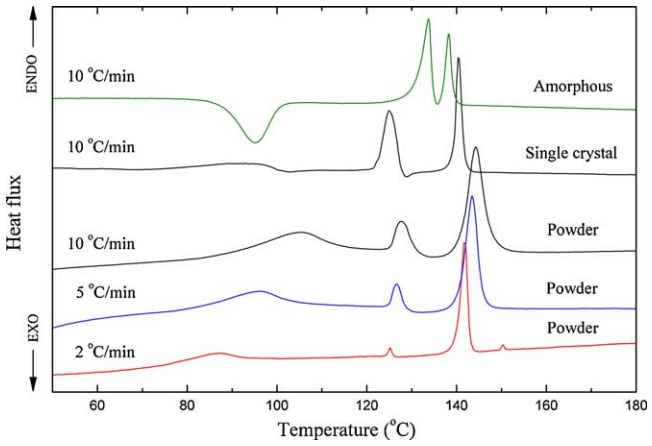

Fig. 3. Differential scanning calorimetry curves of hydrated (single crystal and powder samples) and amorphous aripiprazole recorded at different heating rates.

ing of H_1 consisting of the peritectic melting process (endothermic part) and the crystallization of the anhydrous form (exothermic part). On the other hand, powders exhibit two well defined events: a low temperature broad peak and a sharp peak at 123 °C. The first peak is strongly dependent on the particle size and heating rate, whereas just the intensity of the second one is affected by these parameters. The first desolvation process starts at lower temperatures for slower heating rates, but it involves a higher fraction of the sample. Conversely, a bigger fraction of the sample is converted during the peritectic melting at higher heating rates. Normalizing the transition enthalpies by the one of the melting of the anhydrous form, the fraction of the first process decreases from 76% (2 °C/min) to 58% (10 °C/min), whereas the second process increases from 4% (2 °C/min) to 22% (10 °C/min). In all the investigated samples, the total dehydration enthalpy is around 80% of the melting enthalpy. Based on these results, one may propose that H_1 dehydrates through two processes. The low temperature one could be related to the slow diffusion of the water molecules through the crystalline structure. Considering that the H_1 crystalline structure has channels along the a-axis where water molecules are placed, the diffusion through these channels will allow the low temperature dehydration if the heating rate is slow enough. Furthermore, fine powders exhibit higher superficial area favoring the release of water. On the other hand, the peritectic melting process imposes an upper limit to the stability of the hydrate. Thus, the part of the sample which was not desolvated by diffusion must transform abruptly to the anhydrous form, as observed in the second event. Very slow heating rates or small particles sizes could allow the complete dehydration through the diffusion process [12].

3.3. Raman spectroscopy

Thermal analysis results suggest that the dehydration process of APZ is divided into two steps. First a diffusion-controlled loss of water followed by a fast nucleation and crystallization of the anhydrous form [12,13]. However, DSC and TGA cannot provide direct information about the crystalline structure of the sample. In this kind of problems, Raman spectroscopy is a valuable tool for monitoring *in situ* structural transformations. Since Raman scattering measurement can be easily performed under extreme conditions (temperature, pressure, moisture, etc.), this experimental method is particularly suitable to investigate structural transformations in APIs like polymorphic transitions and solvatation/desolvatation processes [17–19].

The crystalline structure of the 5 polymorphs and 3 solvates of APZ were reported showing that the conformational flexibility of this molecule plays a very important role in the stability of the solvates [11–13]. In the special case of the dehydration of H_1, the transformation is driven by the flip of the dihydrocarbostyril moiety. Vibrational modes involving torsions of the molecular skeleton are expected in the low wavenumber region, which is hardly accessible by using conventional infrared absorption spectroscopy. In this spectral region, one should also focus one's attention on intermolecular modes i.e., collective translational or rotational motions of the molecules in the unit cell. These modes produce dynamical deformations of the crystal lattice called lattice vibrations or lattice phonons, whose frequencies, involving Raman shifts in the range (10–150 cm^{-1}), probe the intermolecular interactions and are hence very sensitive to different molecular packing [15]. The relationship between each lattice phonon pattern (lattice dynamics) and its corresponding XRD pattern (lattice structure) makes Raman spectroscopy a powerful tool for complementing information on distinct crystal structures.

The temperature dependence of the low wavenumber Raman spectra was measured *in situ* for the anhydrous, hydrated and amorphous forms (Fig. 4). Comparing the room temperature spectra of these solid forms, it is observed that the anhydrous form is characterized by a vibrational mode at 63 cm^{-1}. H_1 exhibit a more complex spectrum whose main features are two modes at 48 and 73 cm^{-1}. Finally, the Raman spectrum of amorphous APZ is dominated by the quasi-elastic scattering characteristic of a glass phase. Thus, the three solid forms observed at room temperature can easily be differentiated using Raman spectroscopy.

Regarding the temperature dependence of the Raman spectra, the three samples were measured as a function of the temperature. The solid–liquid transformation of the anhydrous form is clearly observed in Fig. 4(a) around 130 °C. The Raman spectrum of the melt is also dominated by the quasi-elastic scattering. On heating, the dehydration process of H_1 is evidenced by the continuous transformation between the spectra of the hydrate and anhydrous forms (Fig. 4(b)). Even though DSC and TGA results support the hypothesis of a slow diffusion controlled dehydration, this process could be driven by the continuous transformation from H_1 to form III or through an intermediate phase that could transform into form III at 123 °C. Both thermal and spectroscopic results are ruling out the second possibility because neither a sharp transformation nor a third phase was observed. Fig. 4(c) shows the recrystallization of the amorphous form, which was obtained from the supercooled melt. As stated, at room temperature, the Raman spectra are determined by the quasi-elastic scattering but around 80 °C a shoulder at approximately 60 cm^{-1} was observed. At high temperatures the characteristic spectra of form III were obtained, which transforms into the one of the melt around 140 °C. Since the band determining the observed shoulder is very close to the one that fingerprints form III, it could be related to the nucleation of this polymorph in the amorphous form. However, DSC measurements showed that APZ recrystallizes in an intermediate form which transform into form III before melting.

In order to obtain further information from the measured spectra some data treatment analysis must be applied. The traditional procedure is based on uni- or bivariate approaches considering the bands positions and/or their intensities. However, several non-linear processes could be involved in solid-state transformations that make specific peaks used for identification not well defined. This problem could be overcome using multivariate methods such as principal component analysis (PCA) and partial least-squares (PLS) regression combined with methods of spectral pre-processing. Since, in our case, there is no need for a quantitative analysis, PCA is suitable to monitor the temperature evolution of our sample. PCA is a multivariate projection method which is used to extract and display systematic variation in a data set. The use of PCA allows the number of variables in a multivariate data set

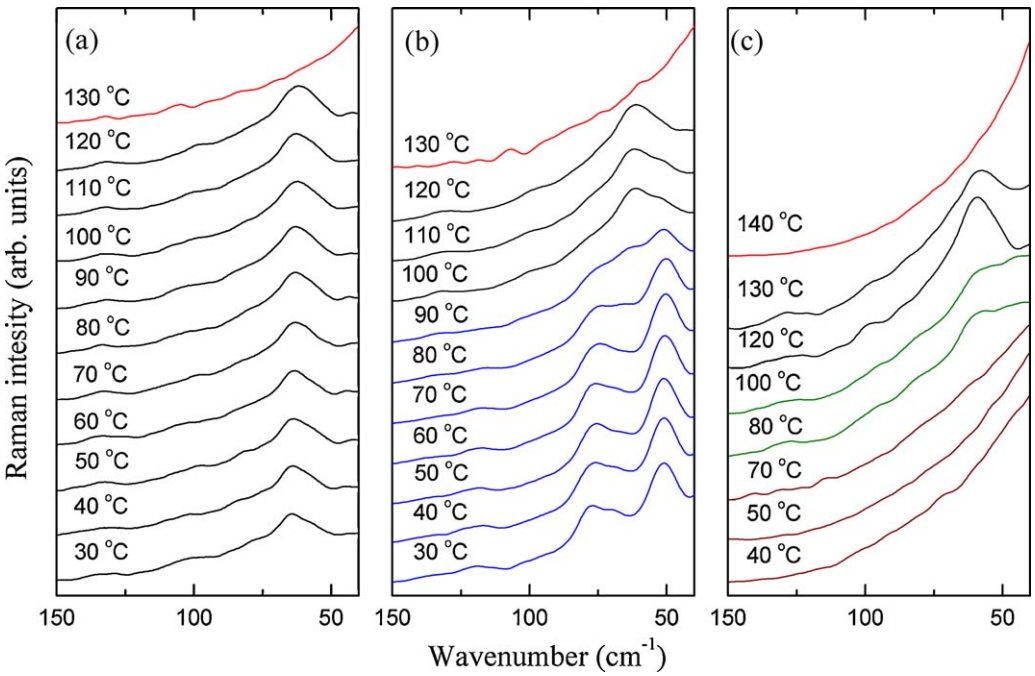

Fig. 4. Temperature dependence of the Raman spectra of (a) anhydrous, (b) hydrated and (c) amorphous aripiprazole.

to be reduced, whilst retaining as much as possible of the variation present in the data set.

In all the samples, just one principal component (PC_1) was necessary to describe the temperature evolution. The spectral region from 30 to 200 was considered in these calculations. The corresponding loadings are plotted in Fig. 5 and the scores as a function of the temperature in Fig. 6. Loadings allow the correlation between the scores and the experimental data. In general, the loadings show that the most relevant bands are those around 60 cm^{-1}. On one hand, the loadings of the anhydrous (Fig. 5(a)) and amorphous (Fig. 5(c)) samples are characterized by one negative peak which is directly related to the main peak of the anhydrous form. On the other hand, the anhydrate–hydrate transformation is depicted by one positive and two negative peaks (Fig. 5(b)) associated with those of the hydrate (48 and 73 cm^{-1}) and anhydrous (63 cm^{-1}) modifications, respectively. From the point of view of the scores, the simplest investigated case is the melting of the anhydrous form (Fig. 6(a)). Since PC_1 describes the amount of variation of the Raman spectra, the result of the calculation is consistent with the expected behavior. PC_1 shows a discontinuity at the melting point separating two regions where it is constant representing the anhydrous (solid)

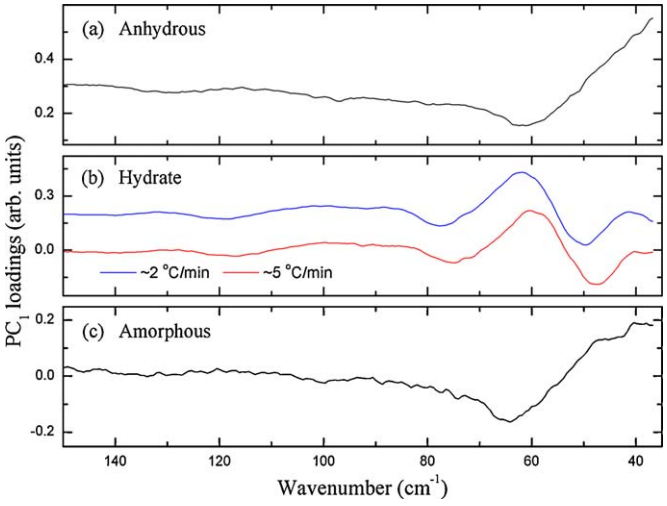

Fig. 5. Loadings of the first principal component (PC_1) of (a) anhydrous, (b) hydrated and (c) amorphous aripiprazole.

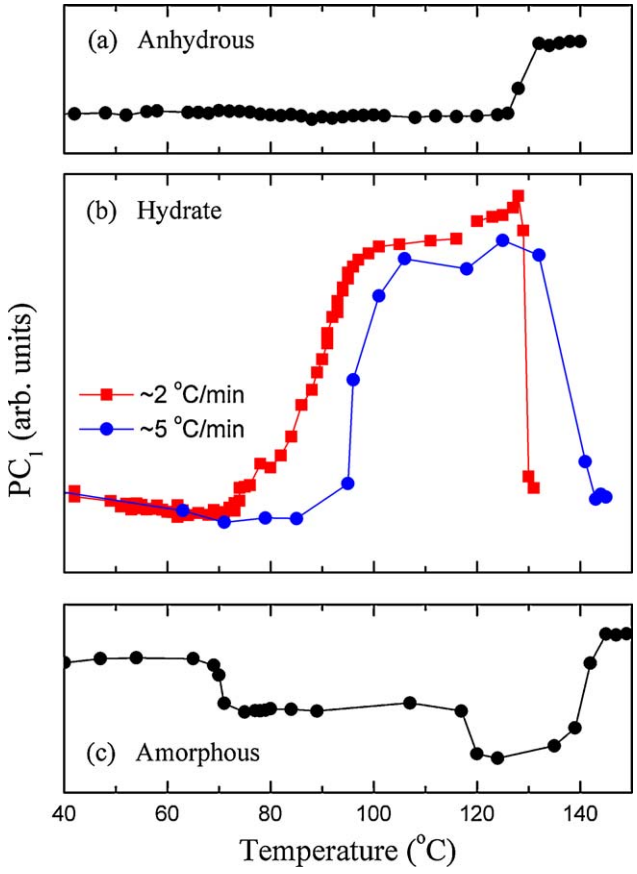

Fig. 6. Temperature dependence of the first principal component (PC_1) of (a) anhydrous, (b) hydrated and (c) amorphous aripiprazole.

and liquid forms. A similar approach was applied to interpret the results of the H_1 sample (Fig. 6(b)). In this case, Raman spectra as a function of the temperature were dynamically recorded using two different heating rates. In both cases, PC_1 changes continuously between two levels, nevertheless, this process depends on the heating rate. Since the two PC_1 levels are related to the hydrate and anhydrous forms, the PCA analysis confirms that H_1 transforms continuously to the anhydrous form. It is also observed that the dehydration start at lower temperatures for slower heating rates, in good agreement with the DSC results. Furthermore, a small discontinuity is present in the curve corresponding to a heating rate of 2 °C/min around 120 °C. This event can be associated to an excess of H_1 which transform abruptly at the temperature of the peritectic melting. Finally, a sharp discontinuity in PC_1 around 130 °C evidenced the melting of the anhydrous form. Fig. 6(c) shows the temperature evolution of PC_1 in the case of the amorphous form. In this case, PC_1 is characterized by three events, which can be directly related to the same number of peaks observed in the DSC curve supporting the existence of an intermediate phase in the temperature evolution of amorphous APZ.

4. Conclusions

Thermal analysis, hot-stage microscopy and Raman scattering were applied to investigate the dehydration process of aripiprazole hydrate. Based on our results, two mechanisms are involved in the releasing of water molecules. At slow heating rates or small particle sizes, the dehydration is driven by a diffusion controlled mechanism. The temperature evolution of the Raman spectra demonstrated that H_1 transforms continuously into form III. Furthermore, the effect of the heating rate was also evidenced by the vibrational spectra, showing that at low heating rates the transformation start at lower temperatures. The second dehydration mechanism is related to the peritectic melting of H_1 followed by the fast growing of the anhydrous form. This process can be observed with a fast heating rate and is dominant in the case of single crystals. A more detailed description of the phase transitions exhibited by amorphous, anhydrous and hydrated APZ was obtained by processing the spectroscopic data using PCA algorithms.

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