Arabian Journal of Chemistry (2014) 7, 189–195

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Stability and thermophysical properties of azithromycin dihydrate

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Received 1 October 2010; accepted 24 October 2010
Available online 29 October 2010

KEYWORDS

Azithromycin;
Hydrate;
Crystals;
Dehydration;
Thermal stability;
Solubility;
X-ray powder diffractometry;
Differential scanning calorimetry (DSC);
Thermogravimetric analysis

Abstract The aim of this paper was to describe the temperature effect on the stability and the thermophysical properties of azithromycin (AZ). First, the density, the heat capacity and the solubility of original (commercial) AZ were determined. Second, the original samples were heated at 50 °C and 80 °C and their PLM, DSC, TGA and XRD data were compared to those of the original AZ. According to our results, the original AZ was a dihydrate which converted to anhydrate when heated up to 80 °C. The dehydration induced a change of crystal habit while the crystalline lattice remained unchanged.

1. Introduction

Azithromycin (AZ) is a macrolide antibiotic derived from erythromycin. This product has a high bacteriostatic action in front of a wide spectrum of pathogenic bacteria and is used mainly for the treatment of respiratory and dermatological infections. The solid state of azithromycin depends on the solvent used in the crystallisation process (Montejo-Bernardo et al., 2006). When the antibiotic is crystallized in a water acetone mixture, the product obtained (azithromycin) is the dihydrate (Djokic et al., 1988). If the antibiotic is crystallized in a water alcohol mixture the azithromycin form obtained is the monohydrate (Montejo-Bernardo et al., 2006).

The commercial product is formed of dihydrated crystals ($C_{38}H_{72}N_2O_{12}\cdot 2H_2O$). The drying of hydrates is always a difficult step in the industrial process. Indeed, the hydrates can undergo a dehydration which can affect the properties of the pharmaceutical ingredient, such as its stability, dissolution rate and bioavailability. Several mechanisms of dehydration have been reported in the literature (Garnier et al., 2002). They

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.arabjc.2010.10.024

depend on the hydrate crystalline structure and on the dehydration conditions. Harsh dehydration conditions are likely to lead to an amorphous compound, whatever the hydrate type. In some cases, this amorphous compound can transform into a new crystalline form by nucleation and growth. This phase transition can occur simultaneously with the dehydration process. It is also facilitated if the water produced by dehydration is still in liquid state around the particles and favours a solvent mediated phase transition. Obviously, the anhydrate obtained in both cases, i.e. in amorphous state or crystallized in a new polymorphic form will have completely different properties from those of the initial hydrate. On the contrary, gentle dehydration of non stoichiometric hydrates can lead to the formation of isomorphic desolvates (Stephenson et al., 1998). The lattice of these hydrates has the particularity to form tunnels inside of which the water molecules are located. This is often the case with the active principal ingredients because of the big difference of size between the pharmaceutical molecules and the water molecules. The water is then removed without altering the crystal lattice substantially from that of the original solvate form (pseudopolymorphism) (Stephenson et al., 1998). It is rather a continuous transition, although different steps can be observed when the water molecules are involved in different types of bonds in the structure. The isomorphic desolvate is generally unstable (Yu et al., 1998). The lattice of the anhydrous material can undergo a relaxation (i.e. a non-isotropic reduction of the unit cell) in order to improve its stability (Stephenson and Diseroad, 2000). The isomorphic desolvate is also highly hygroscopic since it will always tend to reabsorb water molecules to recover its stability (Liggins et al., 1997). However the dehydration/rehydration loop is not always reversible. The particle size and the chemical stability can be affected. Marcelo et al. (2005) realised an accelerated transformation of three azithromycin pseudo-polymorphs and suggested that the monohydrate and dihydrate crystalline forms of azithromycin are stable whereas the anhydrous form evolves to dihydrate.

In this paper the thermophysical properties of azithromycin and its stability at different temperatures were investigated with the aim to predict its drying behaviour during manufacturing.

2. Materials and methods

2.1. Samples preparation

Three types of samples were used in this study. The first type (AZC) was the commercial (supposed to be dihydrate) azithromycin (maximal total impurities content of 1.43%) provided by the ERCROS Company (Spain). The second (AZ50) was the commercial product heated during 48 h in a batch oven at 50 °C. The third (AZ80) was the commercial product heated during 48 h in a batch oven at 80 °C. In order to prevent the rehydration, the samples were stored and transported from oven to apparatus in hermetically sealed recipients.

2.2. Polarized light microscopy (PLM)

AZC, AZ50 and AZ80 samples were viewed by polarized light microscopy (Leica DML) for a magnification = 100×.

2.3. Apparent density

The density of a powder depends on its compaction degree. The apparent density is defined as the average of the aerated and tapped densities. The aerated density was obtained by simply filling a cylinder of 100 ml with the powder. For the tapped density, the cylinder filled with the powder was tapped 10 times on a flat wood plate and the final volume occupied by the powder was measured. Analyses were repeated 10 times.

2.4. Specific heat capacity

Heat capacity measurements were carried out on a DSC apparatus (2920, TA instruments Inc., New Castle). A typical calibration run in modulated mode was performed with 20 mg of sapphire. Ten milligrams AZ samples were placed in sealed aluminium pans. The scan for commercial and heated samples (AZC, AZ50 and AZ80) was run 6 times between 0 °C and 100 °C at a heating rate of 5 °C/min under nitrogen purge (40 ml/min).The modulation amplitude and period were respectively set to ±1 °C and 60 s.

2.5. Solubility

The solubility of AZC in four solvent mixtures with different acetone–water volume fractions (0–100, 25–75, 50–50 and 75–25) was measured as a function of temperature. For each solubility point, around 20 ml of solvent was kept at a given temperature in a 250 ml jacketed vessel equipped with a thermostat and a magnetic mixer. The vessel was also equipped with a condenser to recover the evaporated solvent. When the temperature was stable, AZC powder was added step by step as far as dissolution was observed. The added mass was recorded. Each step could take several hours for the dissolution to be completed. The last step was identified when few particles remained in suspension. The suspension was then maintained under agitation for one day in order to be sure that the particles would no longer dissolve and to detect an eventual phase transition.

2.6. Rehydration curves

Rehydration experiments were carried out in the ambient air (20 °C at 50% of relative humidity). Immediately after being removed from the oven the dehydrated samples (AZ50 and AZ80) were placed in a desiccator until they reached the room temperature. Then they were exposed to the ambient air and their weight was measured at regular time intervals. The relative sample mass M/M_0 (where M and M_0 are the sample's weight at time t and zero, respectively) was plotted as function of time.

2.7. Differential scanning calorimetry – thermogravimetric analysis

The heat flow versus temperature curves were recorded using the DSC apparatus (see Section 2.4). The temperature axis and cell constant of DSC were calibrated with indium. A heating rate of 2 °C/min was employed over a temperature range of 0–250 °C after a 5 min stabilization period under nitrogen

purging. The samples (10–15 mg) were analysed in sealed and open aluminium pans, respectively. An empty pan was used as reference.

The mass versus temperature curves were recorded using the TGA (Mettler, LMI JM Switzerland) apparatus. The samples (1–5 mg) were placed in aluminium pans and heated up to 250 °C at a rate of 1 °C/min under nitrogen purge (40 ml/min).

2.8. X-ray powder diffractometry

Powder X-ray diffractograms of azithromycin samples were acquired at room temperature (20 °C) at a relative humidity of about 50% with an X-ray diffractometer (PANalytical, Xpert pro MPM) using Cu Kα radiation (tube operated at 45 kV, 40 mA). Data were collected over an angular range from 5° 2θ to 50° 2θ.

3. Results and discussion

3.1. Polarized light microscopy

By means of PLM, it was found that the three samples (AZC, AZ50 and AZ80) had different crystal habits (Fig. 1). The AZC and AZ50 samples were quite similar, although more fine particles could be seen in the AZ50 sample. The AZ80 sample was found to contain smaller and more irregular particles. Moreover, the particles of AZ80 had the tendency to stick to each other. It was clear from these observations that the sample dried at 80 °C had undergone a dehydration, which had affected the particle shape and size.

3.2. Density and specific heat capacity

Table 1 gives the results of the density measurement of the commercial sample.

Experimental heat capacities of AZC over the temperature range from 0 °C to 100 °C are plotted in Fig. 2.

It can be seen from Fig. 2 that the heat capacity of the commercial sample increased in a smooth and continuous manner over the whole temperature range. The average heat capacity, between 0 °C and 100 °C was equal to 1611 J kg^{-1} with a standard error of 65 J kg^{-1}.

No thermal anomaly could be observed in this range of temperature. These measurements did not exhibit any phase transition below 100 °C. This should be confirmed by the DSC analysis performed in sealed pan.

3.3. Solubility

The solubility of AZC is plotted versus acetone volume fraction for different temperatures in Fig. 3. The AZ solubility increased with increasing temperature and acetone fraction.

The solubilities given in Fig. 3 were expressed in mg of AZC per ml of initial solvent mixture. In reality the dissolution of one mole of AZC liberates 2 molecules of water, since AZC is dihydrated. The acetone water volume ratio in the solvent mixture is thus modified. However, owing to the high solubility levels and the large difference between the molar masses of AZ and water, this modification of the solvent mixture composition can be neglected.

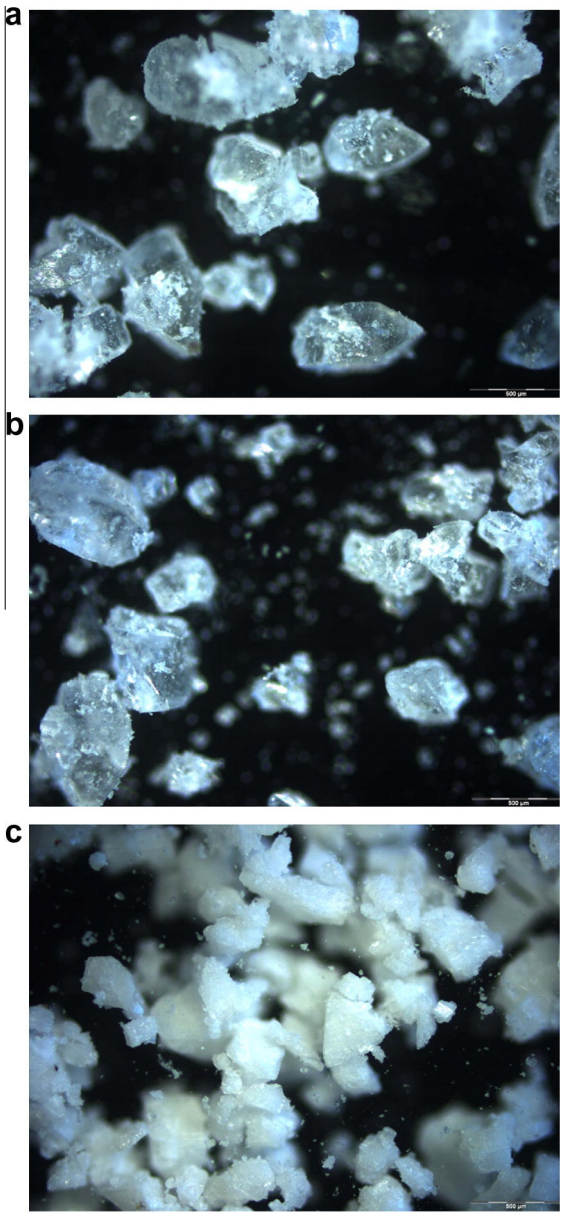

Figure 1 (a) PLM image of AZC crystals; (b) PLM image of AZ50 crystals and (c) PLM image of AZ80 crystals.

Table 1 AZC density.

Aerated density (kg/m^3)	Tapped density (kg/m^3)	Apparent density (kg/m^3)
472.7	575.8	524.2

If the enthalpy of solution, ΔH_{sol}, is considered constant over a given temperature range, a plot of $\ln(S)$ versus $1/T$ is a straight line with a slope equalling, $\frac{\Delta H_{sol}}{R}$, from which ΔH_{sol} can be calculated. The plot of $\ln(S)$ versus $1/T$ curves of AZC in different water acetone mixtures is given in Fig. 4. The straight lines obtained for all studied water/acetone mixtures allowed the estimation of the heats of solution (Table 2).

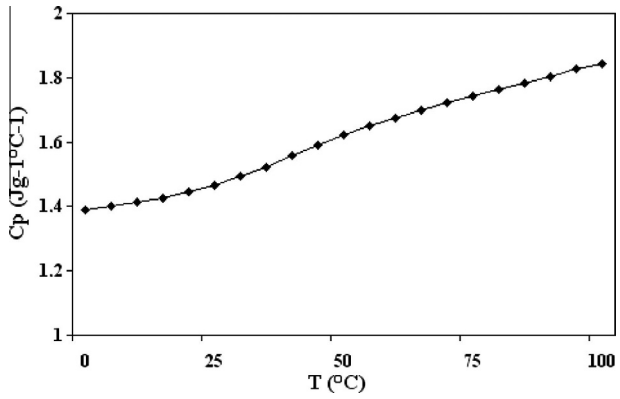

Figure 2 Specific heat capacity of AZC versus temperature.

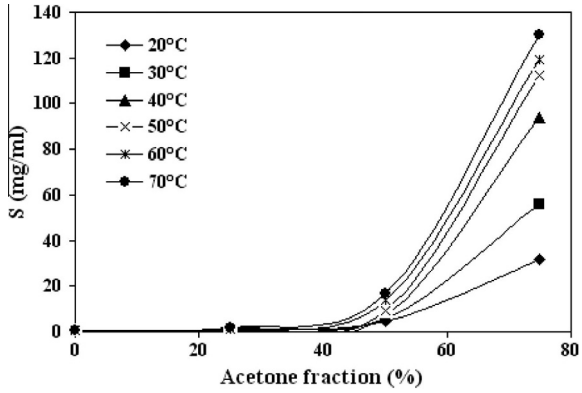

Figure 3 Solubility of AZC as function of water/acetone mixture composition at different temperatures.

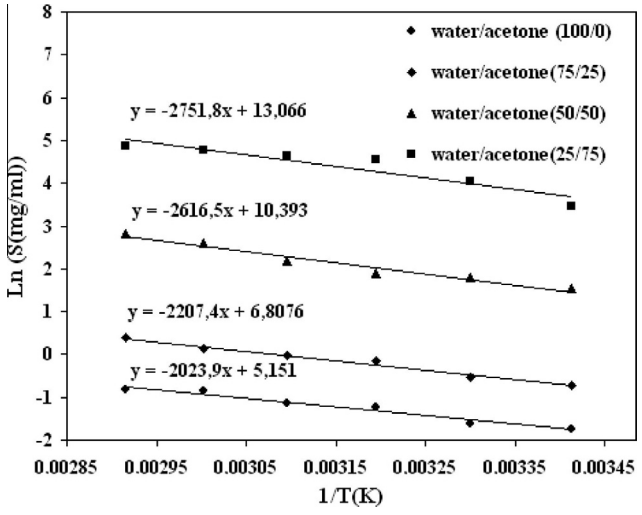

Figure 4 Solubility of AZC as function of temperature for different water/acetone mixtures.

ΔH_{sol} increased with the acetone content. ΔH_{sol} is the sum of the enthalpy of fusion, ΔH_f (endothermic) and of the enthalpy of mixing, ΔH_{mix} (exothermic). Using absolute values, this gives:

$$|\Delta H_{sol}| = |\Delta H_f| - |\Delta H_{mix}|$$

Table 2 Enthalpy of solution of AZC in different water/acetone mixtures.

Mixture	ΔH_{sol} (J/g)	$\Delta(\Delta H_{sol})$
Water/acetone (100/0)	21.43	0.64
Water/acetone (75/25)	23.38	0.54
Water/acetone (50/50)	27.71	0.17
Water/acetone (25/75)	29.14	0.59

The different solubility curves obtained between 20 °C and 70 °C were probably relative to the same crystalline phase, i.e. the dihydrate. This imply that the value of ΔH_f is the same, whatever the solvent mixture composition is. The values of ΔH_{sol} given in Table 2 seemed then to indicate that ΔH_{mix} decreased when the acetone content increases.

For each solubility point and especially at high temperature and high solubility, the suspension of few crystals obtained after the last powder addition was maintained under agitation for one day in order to detect any phase transition towards a less hydrated or an anhydrous crystalline more stable phase. Indeed, it is rather common that a dihydrate is no more thermodynamically stable at high temperature. If a more stable crystalline phase exists, a phase transition may occur by nucleation and growth of this more stable phase. Such transitions are facilitated in solution and are more likely to occur at high solubility. However, no phase transition had been observed. The amount and the habits of the crystals in suspension remained unchanged. This indicated that the dihydrate was still the most stable phase at 70 °C. But it was not an absolute proof, since the phase transition towards a more stable form did not necessarily occur if the supersaturation relative to this more stable phase remained too low.

3.4. Thermogravimetric analysis (TGA) and rehydration experiments

These experiments allowed the determination of the hydration degree of the tested samples. The TGA thermogram obtained with the AZC sample is given in Fig. 5. This thermogram exhibited the transition from the dihydrate to the anhydrous form of azithromycin. The observed weight loss of 4.38% corresponded to the stoichiometric weight loss of two water molecules. This result correlated with those obtained by Ghandi et al. (2002). The dehydration started as soon as the sample was placed under the nitrogen flow at 25 °C, proceeded

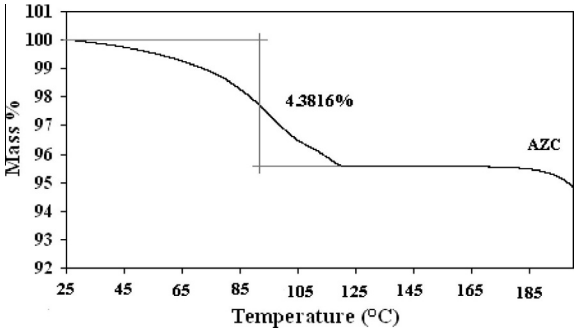

Figure 5 TGA thermogram of AZC.

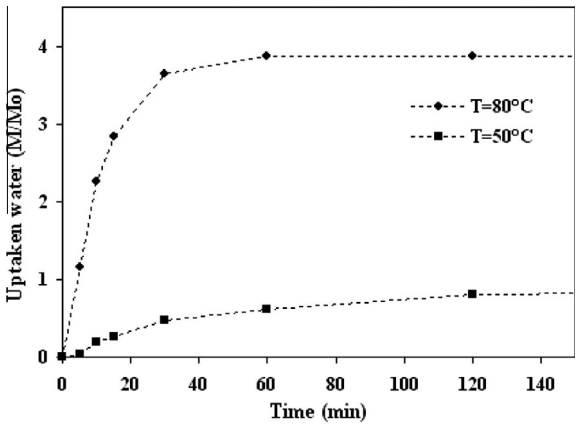

Figure 6 The rehydration curves of AZ50 and AZ80 in ambient conditions.

progressively, and was completed at 120 °C. This behaviour is consistent with a dehydration process leading to an isomorphic desolvate.

A rehydration test was carried out for the AZ50 and AZ80 samples. The two samples were exposed to the ambient air just after being removed from the oven. The corresponding curves are shown in Fig. 6. The AZ80's relative water uptake was nearly 4%. This corresponds approximately to two water molecules per molecule of azithromycin. The AZ80 was then the anhydrous form. The AZ50's relative water uptake was nearly 1%, which corresponds to a gain of less than one water molecule. In both cases, the rehydration rate was high. The AZ80 sample was rehydrated within 30 min. This behaviour suggested that AZ80 was the isomorphic desolvate of the dihydrate and that the crystalline lattice was not significantly altered by the dehydration in the oven. AZ50 corresponded to the dihydrate partially dehydrated.

3.5. Differential scanning calorimetry (DSC)

AZC was subjected to DSC with two pan types: sealed pan and open pan (Fig. 7).

In the case of a sealed pan, it was reported in the literature that AZ samples from different manufacturers exhibit variable thermal behaviour with either single or two DSC endotherms. Most of them exhibited two DSC endotherms while the USP (United States Pharmacopoeia) reference standard showed a single DSC endotherm. The DSC profile obtained in this study with the AZC sample consisted of two endotherms. The first endotherm in the temperature range 130–140 °C had an enthalpy of 62.03 J/g, which was in good agreement with the value reported by Ghandi et al. (2002). This endotherm corresponded to a dehydration step and could also involve a recrystallization step in case of a peritectic transition. A peritectic transition corresponds to the fusion of the dihydrate and the recrystallization of the anhydrous phase in a new crystalline lattice. No exotherm of recrystallisation could be detected between the two endotherms in Fig. 7. But its existence is not systematic. Thus, the DSC profile with sealed pan did not allow to distinguish between a peritectic transition and a dehydration leading to an isomorphic desolvate. The second endotherm was indicative of melting of an anhydrous

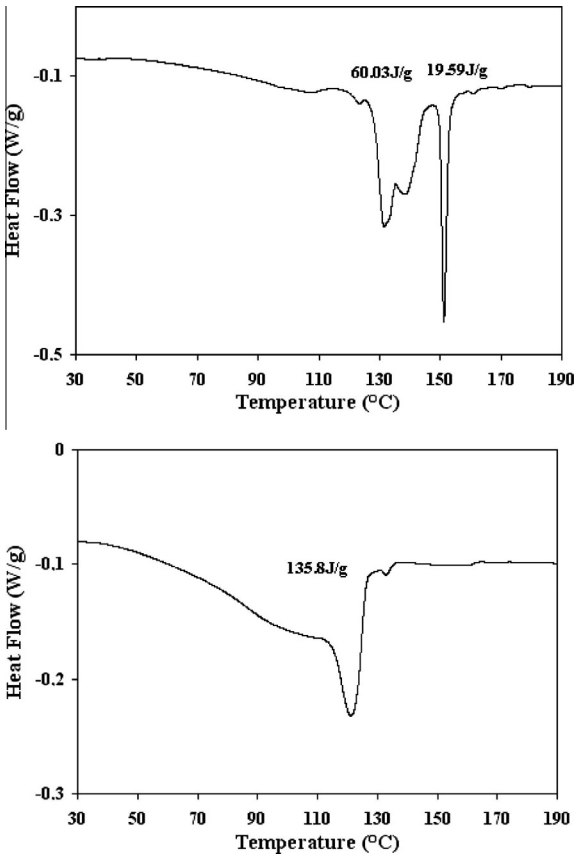

Figure 7 DSC thermograms of AZC for different pan types: sealed pan (top) and open pan (bottom).

crystalline form at 151 °C (i.e. the isomorphic desolvate or a new crystalline anhydrate) and the enthalpy of fusion was 19.59 J/g. The pressure inside the sealed pan increased and the water evaporation could not occur. The desorbed/eliminated water was still in liquid state inside the pan. The heat of fusion of the dihydrate was the sum of the two enthalpies and was then equal to 81.62 J/g. It could be noted that this value was higher than the heats of solution obtained from the different solubility curves. However, the two enthalpies did not correspond to the same phenomena since the heat of solution also involved the heat of mixing.

In the case of the open pan, the thermogram (Fig. 7) exhibited a single endotherm of 135.8 J/g. This enthalpy was higher than that obtained with the sealed pan. In addition to the enthalpy of dehydration of water (ΔH_d) and the enthalpy of fusion of the anhydrate (ΔH_f), this enthalpy included the enthalpy of vaporization (ΔH_v) of water. ΔH_d, ΔH_v and ΔH_f could not be measured separately because the three steps (dehydration, vaporization and fusion) could not be separated. It should be also mentioned that the fusion occured at 120 °C in the open pan, while the temperature of fusion was equal to 151 °C in the sealed pan. This was surprising since the temperature of fusion of a solid is very lightly affected by the pressure but similar behaviour had already been reported in literature (Ghandi et al., 2002). Besides, the sealed pan curves were not reproducible. Because of the high pressure reached inside the pan, the pan could undergo a deformation and could leak. The open pan curves were reproducible.

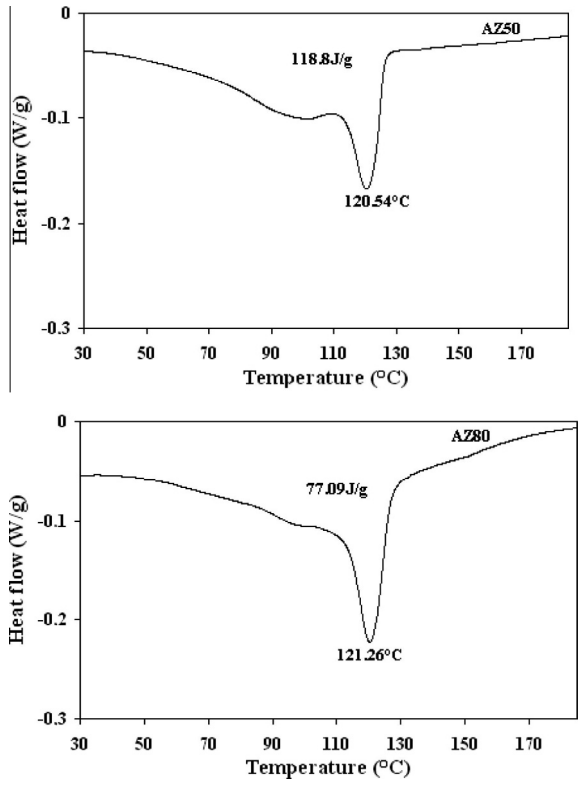

Figure 8 DSC thermograms of AZ50 (top) and AZ80 (bottom) in open pan.

AZ50 and AZ80 were subjected to DSC in open pans only (Fig. 8). In this case (open pan) the desorption and evaporation of water were progressive until 110 °C. The remaining water was completely evaporated in the temperature range of 120 °C and 130 °C. All DSC thermograms presented one singular endotherm at 120 °C corresponding probably to the melting of the dehydrated crystals. The endotherm and its corresponding enthalpy decreased from AZC to AZ80 because the product contained less water. For AZ80, the pan was filled directly in the oven, which was used to prepare the AZ80 sample. However, the AZ80 sample tested in the DSC was probably not completely anhydrous because of its high hygroscopicity. Besides, the different DSC profiles were all consistent with a dehydration process leading to an isomorphic desolvate.

3.6. X-ray diffractometry (XRD)

The X-ray diffractograms for AZC, AZ50 and AZ80 samples are shown in Fig. 9. On all diffractograms the same positions of the peaks were found out (even for low θ values, no significant gaps were observed) with noticeable intensity differences. The same peak positions in the three diffractograms revealed that they corresponded to the same crystalline lattice. The rehydration curve had showed that AZ50 and AZ80 were highly hygroscopic. Thus, they probably uptook some water during the preparation of the sample and the XRD measurement. However, the partially rehydrated solid presented the same diffractogram as that of the dihydrate. This means that the successive dehydration and rehydration steps did not alter the crystalline skeleton of azithromycin. The dihydrate and the anhydrous had the same crystalline structure. Thus, azithromycin was a typical example for isomorphic desolvation.

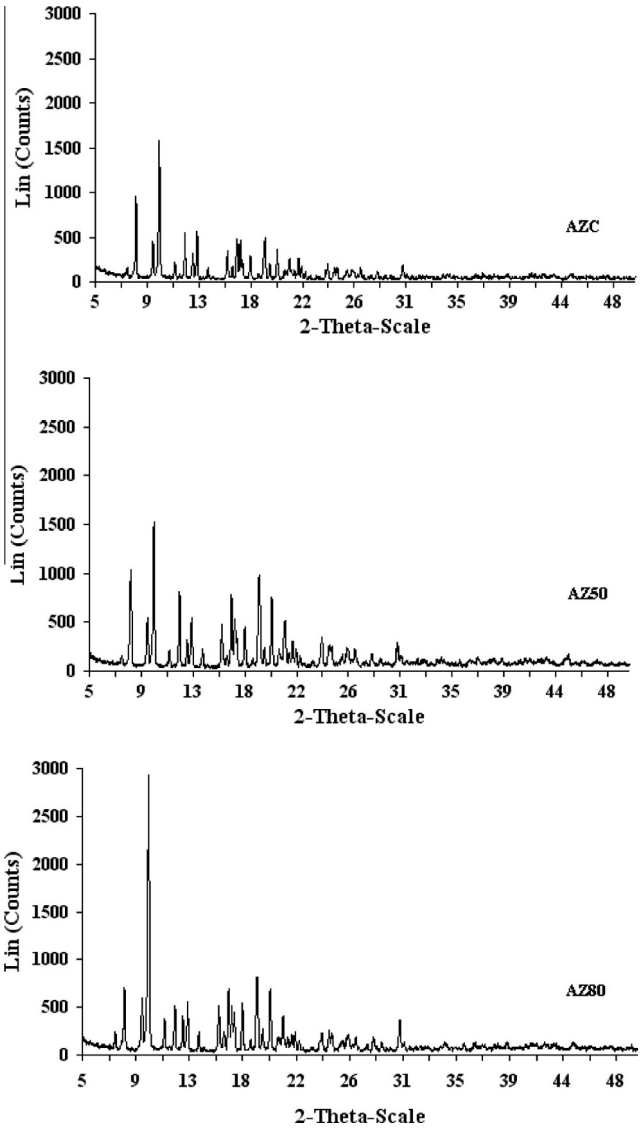

Figure 9 X-ray diffractograms of AZC, AZ50 and AZ80.

The differences in peak intensities from AZC to AZ80 could be attributed to a change in particle sizes or to preferred orientations of the crystals in the samples. Indeed, photos in Fig. 1 showed that the size and the habits of the crystals were modified by the dehydration. Thus, the dehydration/re-hydration cycle was not completely reversible.

4. Conclusion

Azithromycin was found to convert from dihydrate to anhydrous form when heated up to 80 °C. The dehydration occurred without modification of the crystalline lattice and the anhydrate corresponded to an isomorphic desolvate. However, the size and the habit of the crystals were strongly modified by the dehydration. The anhydrous form, in the presence of moisture and at ambient temperature, converted rapidly into the more stable dihydrate form. It is thus very important to

control the moisture levels during the drying process and during the various operations involved in the formulation. Moreover, it is necessary to ensure that the excipients do not have an influence on the moisture content of azithromycin, which in turn can induce an inter-conversion of one form into another (dihydrate to anhydrate or vice versa).

Acknowledgment

The authors acknowledge the ERCROS Company for providing the material.

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