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Polymorphism and pseudopolymorphism: influencing the dissolution properties of the guanine derivative acyclovir

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Abstract

In this work we established that acyclovir exists in hydrated form and that the ratio between acyclovir and water molecules in the crystal structure is 3:2. The anhydrous crystalline form of acyclovir was also prepared. Both crystalline forms were examined by means of thermal analyses, X-ray powder diffraction, infrared spectroscopy, solubility and dissolution rate studies. The differences in almost all tested parameters between the acyclovir hydrated and anhydrous forms were observed. They were explained by different crystal forms of the substances examined. It was found, that besides hydrate, two anhydrous forms of acyclovir are present: the unstable one, obtained at a drying temperature below 150°C (which converts to the hydrate almost immediately in the atmosphere), and the stable one, obtained at drying temperatures above 150°C (which shows, on heating to 172°C, the solid–solid transition). It was thus postulated that acyclovir can exist as a pseudopolymorphic and polymorphic solvate.

Keywords: Polymorphic solvate; Pseudopolymorphic solvate; Acyclovir; Anhydrous acyclovir; Dissolution behavior; Solid–solid transformation

Many organic and inorganic compounds are able to exist in more than one crystalline form having different physical properties. In some cases these states are the result of solvate formation. It is known that crystalline solvated and nonsolvated forms exhibit different dissolution patterns (Shefter and Higuchi, 1963; Fung and Nealon, 1974; Yalkowsky, 1981). The dissolution patterns are also different when using various dissolution media, the fastest rate of dissolution was found in solutions which interact with the solvent of sol-

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vated form (Fung and Nealon, 1974; Yalkowsky, 1981).

Previously we reported that acyclovir (ACV), 9-(2-hydroxyethoxymethyl) guanine, a potent antiviral agent, exists in crystalline solvated form (Kristl et al., 1989), probably due to the precipitation process used in the final crystallization (Matsumoto et al., 1988; Štimac and Kobe, 1990; Wells, 1988). It was also proposed, that ACV molecules are probably in hydrated form. In this work we describe the nature and the structure of the ACV solvated form. The nonsolvated form of ACV was also prepared and compared with the solvated one in terms of dissolution and other physical properties.

ACV was synthesized at the National Chemical Institute, Ljubljana, Slovenia (Štimac and Kobe, 1990). All other reagents used were of analytical grade.

Differential scanning calorimetry (DSC) was performed by DSC Perkin Elmer 4 and 7 in an Al-pan at a heating rate of 10 K/min in a dynamic nitrogen atmosphere (40 ml/min). Thermogravimetric analyses (TGA) were carried out by TGA (Perkin Elmer 7) instrument under the same conditions as DSC. Water content in the ACV crystals was determined by the Karl Fischer method, using a Methrom apparatus and by TGA. The anhydrous form of ACV was prepared by drying the hydrate in the atmosphere at temperatures at or higher than 150°C for a few hours.

The aqueous solubility of both ACV forms was determined by stirring an excess of the compound in demineralized water for 24 h at 23 ± 0.1°C. After filtration and appropriate dilution the concentration was determined by a Perkin Elmer Lambda 15 UV–VIS spectrophotometer at 251 nm.

The rotating paddle method was used for determination of the dissolution profile and the rate respectively. The apparatus used was the same as described in USP XXII under Apparatus 2 (Erweka DT-D). The untreated substance (50 mg) was introduced into 1 l of thermostated demineralized water ($T = 23.0 \pm 0.1$°C). Rotation speed of the paddle was adjusted to 100 RPM. The 2 ml samples were withdrawn at different time intervals (after 0.5, 1, 2, 5, 10, 15, 20 and 30 min) and filtered (pore diameter 0.22 μm) to remove solid particles. The concentrations of the dissolved drug were determined by UV–VIS spectrophotometry.

For the determination of the intrinsic dissolution rate (IDR) 250 mg of the substance was compressed slowly in a 13 mm infrared punch and die set to 11 000 psi (pounds per square inch) to ensure minimal or zero porosity and a long dwell time to improve compaction. The compressed disk was fixed to the special paddle holder (Tehtnica, Slovenia) so that only one surface of the disk (1 cm^2) was accessible to the water. The holder was mounted in a beaker containing 1000 ml of demineralized water and the temperature was maintained at 23.0 ± 0.1°C. The rotation speed of the holder was adjusted to 100 rpm. The 2 ml samples were withdrawn at different times (after 5, 10, 20, 30, 60, 120 and 180 min), filtered and the concentrations of dissolved drug were determined spectrophotometrically. The experiments for each solubility determination as well as for the dissolution rate studies were repeated at least three times.

The DSC curve of the ACV solvated form (Fig. 1) exhibits a broad endothermic peak from about 70–130°C. A similar situation is shown by TGA analysis (Fig. 1), indicating that under these conditions the loss of mass (5.15%) occurs in the temperature range about 60–130°C.

To find out whether the ACV solvate is a hydrate or not we determined the water content in the crystals by the Karl-Fischer method. It was

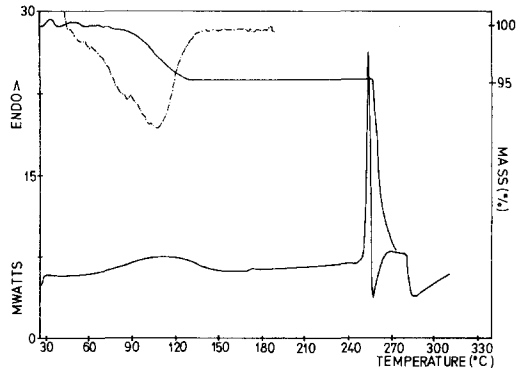

Fig. 1. DSC and TGA curves of hydrated ACV. 1st derivative (- - -) of the TGA curve is also shown.

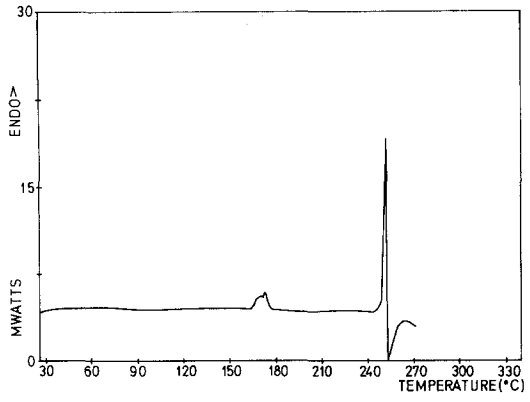

Fig. 2. DSC curve of anhydrous ACV.

found, that in the solvated crystalline form of ACV 5.10% of water is present while in the non-solvated (dried) form only traces of water could be determined. The content of water in the solvated form is practically the same as the mass loss determined by TGA. It can be thus concluded that the ACV solvated form is a hydrate and that on one drug molecule about 0.67 of a water molecule is bound. This result is in accordance with a previously reported study (Birrnbaum et al., 1981), that three molecules of ACV and two molecules of water form an asymmetric crystal unit. Additionally, the shape of the first derivative curve of the TGA thermogram given in Fig. 1 also shows that all the water molecules in the ACV hydrate are of the same origin (i.e. bound to the drug molecules).

From the DSC curve in Fig. 1 one can also observe a small peak at approximately 170°C and a sharp melting peak at 252°C followed by decomposition of the substance.

The DSC thermogram of the nonsolvated form of ACV (prepared by drying at $T = 150$°C, in the atmosphere) is presented in Fig. 2. In this thermogram the endothermic peak at about 170°C is evident. It was shown by TLC and HPLC analyses that no impurities, due to decomposition or chemical reaction of ACV during drying at very high temperatures, were present in the ACV anhydrous form. The endothermic peak at 170°C therefore represents not an impurities melting peak but probably a solid–solid transformation. The observation of Figs. 1 and 2 also clearly shows the difference in the magnitude of the peak at 170°C indicating, that this endothermic change (i.e. the solid–solid transformation) is time dependent (the sample must be maintained at very high temperatures—i.e. 150°C—for a few hours). The time dependency of this phenomenon was also shown by performing DSC experiments under different conditions: when the sample of ACV hydrate was treated in DSC with a heating rate of 1°C/min a small peak at 172°C can be observed ($\Delta H = 3.0$ J/g). If this sample is cooled down from 180°C to room temperature and then re-heated the peak at 172°C enlarges ($\Delta H = 11.0$ J/g). Further heating and cooling of the sample did not change the shape and the magnitude of the peak at 172°C; it was reversible.

We also found that ACV dried at these high temperatures (after cooling to room temperature) does not bind water molecules from the atmosphere. Even after a long period (at least 6 months) of keeping dried ACV in the atmosphere the DSC thermogram was the same as shown in Fig. 2.

X-ray patterns (not shown) for hydrated and anhydrous forms of ACV exhibit quite big differences. IR spectra of both crystalline forms (not shown) also differ markedly. These observations strongly indicate that hydrated and anhydrous forms of ACV represent different crystal forms.

Additionally some isothermal TG analyses were performed. It was found that even at as low a temperature as 50°C, after approximately 140 minutes drying, the anhydrous form of ACV can be obtained. However, after less than 2 min almost the same amount of water (4.80%) was adsorbed back into the ACV sample from the atmosphere. These results indicate that the stable anhydrous form of ACV can be obtained only by drying ACV hydrate at very high temperatures (i.e. above 150°C).

On the basis of these findings the following scheme for different ACV crystalline forms can be proposed (Scheme 1):

ACV hydrate $\rightarrow$ ($T <$ cca.150°C) $\rightarrow$

ACV anhydrous (unstable) form

→ (T > cca.150°C) →

ACV anhydrous (stable) form 1 → (T > 172°C)

→ ACV anhydrous form 2

The dissolution properties of the hydrated and anhydrous forms were also investigated. Determined solubilities in demineralized water were 1.61 ± 0.05 and 1.55 ± 0.07 g/l for the hydrated and anhydrous forms of ACV respectively. Only slight and insignificant differences exist in solubility values between these two forms.

The dissolution rate profiles (Fig. 3) exhibit great differences between these two forms. It is obvious that the hydrated form of ACV is dissolved almost immediately (in a few minutes), while on the other hand the anhydrous form (stable form 1) dissolves relatively slowly. These dissolution rate studies are in contradiction with the literature data. It was found for numerous substances (namely cholesterol, theophylline, caffeine, glutethimide and succinyl sulfathiazole (Shefter and Higuchi, 1963) that the dissolution rate of the anhydrous form was much greater than that of the corresponding hydrates. Even supersaturation was reached with these anhydrous forms. These findings were explained by thermodynamic differences, i.e. the hydrates usually possess smaller thermodynamic activity and are therefore in a more stable state than their anhydrous forms (Yalkowsky, 1981).

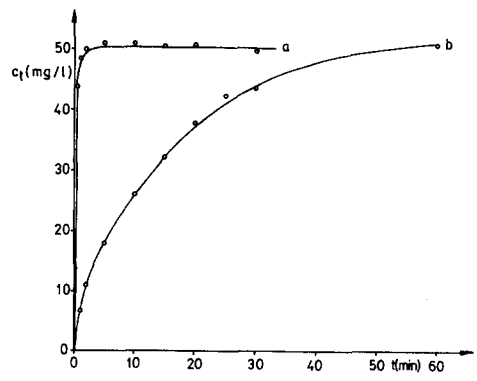

Fig. 3. Dissolution rate profiles of (a) hydrated ACV and (b) its anhydrous form.

Table 1
The intrinsic dissolution results of hydrated and anhydrous forms of ACV

Concentration (mg/l cm^2)							
min	5	10	20	30	60	120	180
Anhydrous ACV	0.4	0.5	1.1	1.6	2.4	3.2	4.0
ACV hydrate	0.6	0.9	1.6	2.3	4.2	6.7	9.4

To examine the differences in dissolution behaviour the intrinsic dissolution rate (IDR) was also determined. IDR is namely the parameter, which is independent of formulation parameters, disintegration, deagregation, the size and the shape of the particles and others; it measures only the intrinsic properties of the drug as a function of the dissolution media (Wells, 1988). The results of the IDR experiments are given in Table 1 and are in accordance with the dissolution rate profiles. It is evident, that the anhydrous form of ACV under specified conditions dissolves almost twice as slowly as its hydrated form. These contradictions in dissolution behaviour could be explained by proposed different crystal forms of ACV. A different crystal structure of ACV anhydrous (stable) form 1 and therefore a slower dissolution rate are the consequences of heating ACV hydrate at very high temperatures (i.e. 150°C and more). It is also clear (from Scheme 1) that the unstable anhydrous form (obtained at temperatures lower than cca. 150°C) is almost immediately transformed to the hydrated form, which has the same dissolution pattern as shown in Fig. 3a. On the basis of these results one can say that during heating of ACV hydrate at high temperatures (T > 150°C) structural changes in the crystal lattice take place. The consequence of these changes is greater thermodynamic stability and therefore also a slower dissolution rate and no hygroscopic tendencies.

Bryn (1982) classified the solvates into two groups: solvates that transform to another crystal form (different X-ray powder diffraction pattern) upon desolvation are polymorphic solvates, while the solvates that remain in the same crystal form (similar X-ray powder diffraction pattern) are pseudopolymorphic solvates. An important difference between these two classes is that pseudopoly-

morphic solvates are readily resolvated, while polymorphic solvates are resolvated only after a phase transformation (Bryn, 1982). One could thus conclude that the hydrated form of ACV represents, depending on the drying temperatures, a pseudopolymorphic or a polymorphic solvate (hydrate).

Overall we can say that ACV is normally present in hydrated form (3 ACV molecules bind 2 molecules of water). The stable anhydrous form can be obtained by drying hydrated ACV at temperatures above 150°C; but it possesses, in contradiction to the known literature data about dissolution rates of solvated and unsolvated forms, worse dissolution properties than the hydrated form of ACV.

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