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Crystal form conversion of nevirapine solvates subjected to elevated temperature and humidity: a qualitative study

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Some known nevirapine solvates have been reported to undergo solvent exchange in aqueous media to form a stable hemihydrate. This study aimed to determine the effects of atmospheric moisture on said nevirapine solvates and to gain insight into which factors determine the end product of transformation. Solvates were prepared by solvent recrystallisation and stored, together with the anhydrous and hemihydrate forms, in a climate chamber at 40 °C and 75% RH for a period of 28 days. Samples were analyzed using DSC, TGA, FT-IR, PXRD and Karl Fischer titration. Some solvates were observed to undergo desolvation to the anhydrous form of nevirapine (Form I), whilst others converted to the hemihydrate. It was found that water miscibility of the guest solvent determined the stable form of nevirapine, anhydrous or hemihydrate, to which each solvate eventually transformed. Transformation to the hemihydrate only occurred if the guest solvent was sufficiently water soluble to allow water molecules to enter solvent channels and displace the original guest. Solvates with hydrophobic guests desolvated to the anhydrous form. We concluded that, in the absence of a guest, solvent channels are lost during transformation to the monoclinic crystal system and space group $P2_1/c$ (Form I) so that water cannot enter after desolvation.

1. Introduction

Nevirapine is an anti-retroviral drug of the NNRTI (non-nucleoside reverse transcriptase inhibitor) class. It is used in multi-drug treatment regimes against HIV/AIDS and as single-drug treatment for the prevention of mother-to-child transmission of HIV (Beers et al. 1999; O'Neil et al. 2006).

Some forms of nevirapine (Pereira et al. 2007; Stieger et al. 2010a) have been reported to undergo solvent exchange in aqueous suspension to produce the known hemihydrate. Both the hemihydrate and anhydrous forms are considered stable under ambient conditions and are available in commercial dosage forms.

Two of the nevirapine forms in this study, used as references, were the well-known hemihydrate and anhydrous forms (Ayala et al. 2007; Caira et al. 2008; Stieger et al. 2010a). Other forms used included solvates isolated from: ethanol (Stieger et al. 2010a, b); 1,4-dioxane (Caira et al. 2008); ethyl acetate (Pereira et al. 2007; Caira et al., 2008); toluene (Caira et al. 2008); a series of solvates from primary alcohols $CH_3(CH_2)_nOH$ with n = 2–7 (Stieger et al. 2010b); and acetonitrile (ACN) (Caira 2009).

Although not previously published in a scientific journal, the ACN hemi-solvate has already been extensively characterized by Caira (2009) and Stieger (2009). The DSC trace of this form showed desolvation and fusion peaking at 91.5 °C and 244.1 °C, respectively. The thermogravimetrically determined mass loss indicated host:guest stoichiometry of 2:1 (Stieger, 2009). The PXRD pattern of the ACN hemi-solvate corresponded with those of the isostructural solvates isolated from primary alcohols (Stieger et al. 2010b). Single crystal data, data-collection details and parameters for refinement are listed in Table 1 (Caira 2009).

Table 1: Crystal data, data-collection details and refinement parameters for the acetonitrile hemi-solvate of nevirapine (Caira 2009)

Ratio	2 : 1 nevirapine : acetonitrile
Empirical formula	$C_{15}H_{14}N_4O \cdot (C_2H_3N)_{0.5}$
Formula weight	286.83
Temperature (K)	173 ± 2
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions	
a (Å)	7.7574(8)
b (Å)	8.4748(8)
c (Å)	12.3918(10)
a (°)	85.068(5)
b (°)	88.627(5)
g (°)	67.472(5)
V (Å^3)	749.68(12)
Z	2
Calculated density (Mg. m^{-3})	1.271
Absorption coefficient (mm^{-1})	0.084
Crystal size (mm^3)	0.13 x 0.22 x 0.23
***q* range for data collection (θ)**	3.00-25.3
Limiting indices	≤ 9 £ *h* £ 9
	≤ 10 £ *k* £ 10
	≤ 14 £ *l* £ 14
Reflections collected	7048
Independent reflections	2621 [R(int) = 0.040]
Observed data	1943
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2

Ratio	2 : 1 nevirapine : acetonitrile
Data/parameters	2621/195
***R* indices**	$R_1 = 0.0699$, $wR_2 = 0.1980$
Largest diff. peak and hole (e. Å^{-3})	0.95 and -0.40

The crystal asymmetric unit (Fig. 1) comprises one molecule of nevirapine and an acetonitrile molecule with site-occupancy factor 0.5. The well-known 'butterfly' conformation is adopted by the drug molecule, as observed both in the monoclinic polymorph of nevirapine and in isostructural nevirapine solvates.

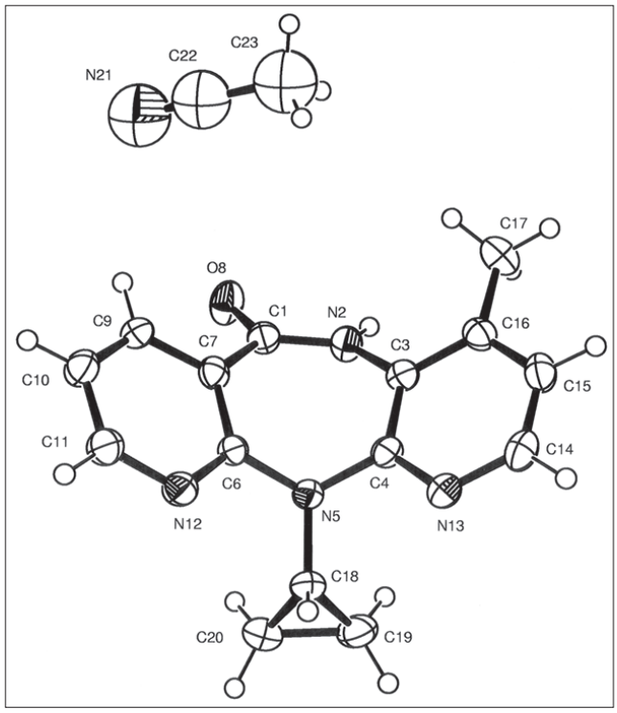

Fig. 1: ORTEP diagram of the asymmetric unit in the nevirapine hemi-acetonitrile solvate. Thermal ellipsoids for the atoms of the drug molecule are drawn at the 50% probability level (Caira, 2009).

The aim of this study was to investigate the effect of high moisture levels and increased temperature on 13 nevirapine forms with known crystal structure. Storage conditions of 40 °C and 75 % RH (relative humidity) were chosen because they closely match the atmospheric conditions of equatorial Africa. We anticipated to gather more insight into the processes of desolvation, solvate formation, solvent exchange, as well as the associated phase transformations to one or the other stable crystal structures.

2. Investigations and results

Table 2 gives a summary of guest solvent properties (Reichard 2003; O'Neil et al. 2006; Murov 2017) and the changes observed for the associated nevirapine forms exposed to 40 °C and 75 % RH. The commercially available forms of nevirapine, anhydrous and hemihydrate, were impervious to the test conditions and no changes were observed. All solvates converted, either fully or in part, to one of these stable forms under the same conditions. Those that had partially converted would surely have completed the transformation given more time. It should be noted that the hemihydrate is stable at ambient or higher moisture levels, but can be dehydrated at lower humidity levels or by applying heat or vacuum.

In the absence of moisture, when heated in an inert N_2 atmosphere, all forms (including the hemihydrate) converted to anhydrous nevirapine.

Figures 2 and 3 show the initial DSC traces for the 13 nevirapine forms tested and Fig. 4 gives relevant PXRD patterns in stacked format. Only one representative PXRD pattern is shown for the isostructural solvates (i.e. all except the 1,4-dioxane product) (Caira 2009; Stieger 2009; Stieger et al. 2010b). Figure 4 also includes patterns illustrating partial conversion of isostructural solvates to the hemihydrate form, as well as the partial desolvation observed for 1-octanol.

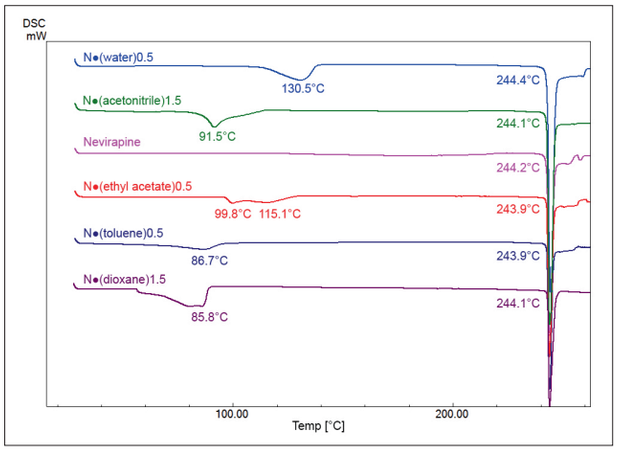

Fig. 2: Initial DSC traces 1 - 6 of the 13 nevirapine forms. *"N" represents nevirapine in the notation used to indicate solvate stoichiometry.

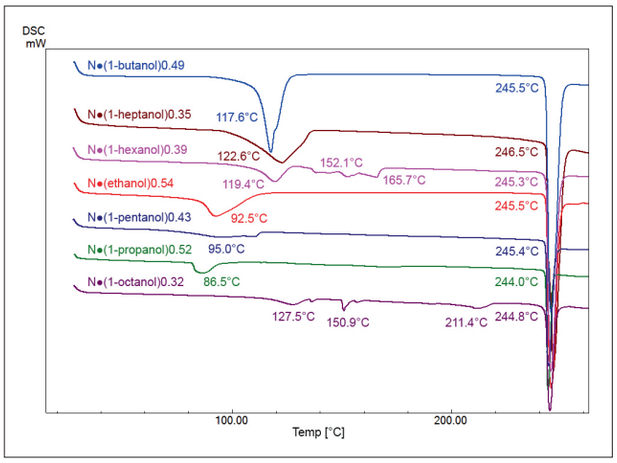

Fig. 3: Initial DSC traces 7 – 13 of the 13 nevirapine forms.*"N" represents nevirapine in the notation used to indicate solvate stoichiometry.

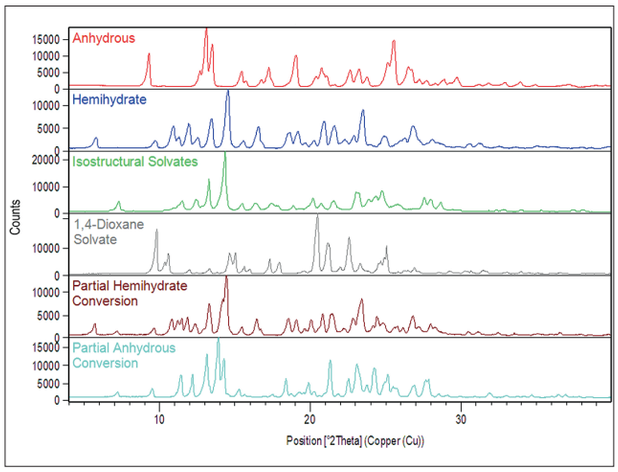

Fig. 4: PXRD patterns for nevirapine's anhydrous, hemihydrate, 1,4-dioxane solvate and isostructural hemi-solvate forms. Also shown are the partial form conversions observed during testing.

Table 2: Summary of results, with solvents sorted according first to water miscibility and second to molecular mass

Guest solvent	Solvent properties				Nevirapine form		
	BP (°C)	M (g/mol)	Solubility in Water at 25°C (g/100 g)	Relative polarity	Initial	7 days	28 days
None	-	-	-	-	Anhydrous	Anhydrous	Anhydrous
Water	100.0	18.02	Miscible[1]	1.000	Hemihydrate	Hemihydrate	Hemihydrate
Acetonitrile[4]	81.6	41.05	Miscible	0.460	Solvate 1:0.5	Hemihydrate	Hemihydrate
Ethanol[4]	78.5	46.07	Miscible	0.654	Solvate 1:0.54	Hemihydrate	Hemihydrate
1-Propanol[4]	97.2	60.10	Miscible	0.617	Solvate 1:0.52	Hemihydrate	Hemihydrate
1,4-Dioxane	101.1	88.11	Miscible	0.164	Solvate 1:1.5	Hemihydrate	Hemihydrate
Ethyl acetate[4]	77.0	88.11	8.7	0.228	Solvate 1:0.5	Partial HH[2] conversion	Hemihydrate
1-Butanol[4]	117.6	74.12	7.7	0.586	Solvate 1:0.49	Partial HH conversion	Hemihydrate
1-Pentanol[4]	137.5	88.15	2.2	0.568	Solvate 1:0.43	Partial HH conversion	Hemihydrate
1-Hexanol[4]	157.0	102.17	0.59	0.559	Solvate 1:0.39	Partial HH conversion	Hemihydrate
1-Heptanol[4]	176.5	116.20	0.17	0.549	Solvate 1:0.35	Partial AH[3] conversion	Anhydrous
1-Octanol[4]	194.4	130.23	0.096	0.537	Solvate 1:0.32	Solvate	Partial AH conversion
Toluene[4]	110.6	92.14	0.05	0.099	Solvate 1:0.5	Anhydrous	Anhydrous

[1] Miscible = fully soluble in each other at any ratio
[2] HH = Hemihydrate
[3] AH = Anhydrous
[4] Isostructural solvates

Thermal microscopy was performed on the solvates to visualize desolvation under silicone oil. With those samples having sufficiently large individual crystals, desolvation was observed to be accompanied by conversion to pseudomorphs, indicating a change in crystal structure (Fig. 5).

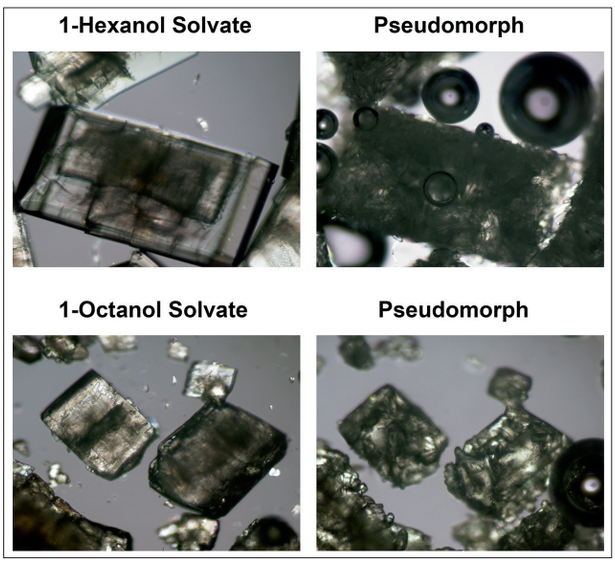

Fig. 5: Example of two solvates showing clear pseudomorphic transition associated with desolvation.

3. Discussion

It might be assumed that water could, in theory, displace any of the solvents used in this study from solvent channels, due to its high polarity and implied ability to form stronger intermolecular interactions. Experimental results disproved this theory. Table 2 shows that relative polarity does not seem to be a major factor here. When one compares the properties of the solvents to the preferred crystal form to which their nevirapine solvates convert, it is clear that solvent/water miscibility is the factor which ultimately determines the end product of transformation (Table 2). Fully water-miscible solvents will be displaced by water to form nevirapine hemihydrate. The rate at which this occurs is influenced by the guest's boiling point and molecular weight which both affect the ease by which it is displaced from the crystal.

Partially water-miscible guests were also displaced by water, albeit at a slower rate. The conversion was slower for all of these solvates. Where the guests were practically immiscible with water, the product of conversion was anhydrous nevirapine (Form I). Again, the higher the boiling point and the larger the solvent molecules, the longer the timespan for the process to take place.

Transformation to the hemihydrate occurs only if the guest solvent is sufficiently water soluble to allow water molecules to enter solvent channels and displace the guest. Solvates with hydrophobic guests desolvate to the anhydrous form. We conclude that, in the absence of a guest, the solvent channels and sites are lost during transformation to the monoclinic crystal system and space group $P2_1/c$ so that water cannot enter after desolvation.

A pharmaceutically relevant implication of these results is that Form I cannot undergo transformation to the hemihydrate through solvent-mediated transformation. Only solution-mediated transformation can yield the hemihydrate (Aucamp et al. 2015). Given

the hydrophobicity of nevirapine, this is likely to be a very slow process if pure water is used. Therefore, nevirapine hemihydrate is best prepared from a co-solvent system containing water (Stieger et al. 2010a).

4. Experimental

Anhydrous nevirapine was purchased from Cipla (Mumbai, India, batch number: 1001003). The hemihydrate form was prepared by recrystallisation from a 30:70 ethanol:water solvent system. All solvents used were of analytical grade. Water used was prepared with a Millipore™ MilliQ® Ultrapure Water Purification System (USA).

Recrystallisation procedure was as follows: Saturated solutions of nevirapine were prepared for each solvent by heating to just below boiling point and continuous stirring on Heidolph (Germany) MR3001K magnetic stirrers. Once clear, solutions were covered and left to cool. The recrystallisation products were left under mother liquor and only dried briefly on filter paper prior to analysis.

A portion of each crystal form, in open Petri dishes, was placed in a climate chamber set to 40 °C and 75% RH for a period of 28 days. These were tested after 0, 7 and 28 days.

Equipment and experimental procedures:

Binder KBF 112 (Switzerland) constant climate chamber set to 40 °C and 75% RH.

Differential scanning calorimetry (DSC): Shimadzu DSC-60A (Japan) with TA60 version 2.11 software. Approximately 2-4 mg of each sample was weighed and heated in closed aluminum crucibles. Samples were heated at 10 K/min in an inert nitrogen atmosphere.

Thermogravimetric analysis (TGA): Shimadzu DTG-60 (Japan) with TA60 version 2.11 software. Samples were heated from 25 °C to 300 °C at 10 K/min, in open aluminum crucibles. Nitrogen gas was used as inert atmosphere.

Fourier transform infrared spectroscopy (FTIR): Shimadzu IR Prestige-21 (Japan), using a Pike Multi-Reflectance ATR accessory, with Shimadzu IRsolution version 1.40 software. Spectra were recorded over a range of 400 - 4000 cm^{-1}. KBr was used as background. The sample was dispersed in a matrix of powdered potassium bromide and, through diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), the IR-spectrum was measured in a reflectance cell.

Powder X-ray diffraction (PXRD): Philips XPert-Pro (Netherlands).

Karl Fischer titration: Metrohm 870 KF Titrino Plus (Switzerland) autotitrator. The titrator was calibrated using a predetermined mass of water (25 – 30 µl) and Hydranal® water standard 10.0. Approximately 50 mg of each sample was used for the moisture determination.

Thermal microscopy: Nikon Eclipse E400 and Leitz Wetzlar hot stage, equipped with a Nikon DS-Fi1 camera. Image capturing software: NIS-Elements F 2.30.

Single crystal X-ray structure determination was performed using the same procedures reported earlier (Caira et al. 2008).

Where relevant, experiments were performed in triplicate to ensure accuracy and precision.

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