J Therm Anal Calorim (2012) 108:53–57
DOI 10.1007/s10973-011-1424-x

Analysis of phase transition and dehydration processes of nevirapine

George G. G. de Oliveira · Humberto G. Ferraz · Patrícia Severino · Eliana B. Souto

Received: 17 January 2011 / Accepted: 18 February 2011 / Published online: 11 March 2011

Abstract Solid-state characterization of crystalline drugs is an important pre-formulation step for the development and design of solid dosage forms, such as pellets and tablets. In this study, phase transition and dehydration processes of nevirapine have been studied by differential scanning calorimetry and thermogravimetry differential thermal analysis to overcome the problems of drug formulation, namely poor solubility and poor content uniformity. Phase solubility studies elucidated the mechanism of enhanced nevirapine solubility.

Keywords Nevirapine · Differential scanning calorimetry (DSC) · Thermogravimetry differential thermal analysis (TG-DTA) · Solid dosage forms

G. G. G. de Oliveira · H. G. Ferraz
Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo 05508-900, Brazil

P. Severino
Department of Biotechnological Processes, School of Engineering Chemical, University of Campinas, Campinas 13083-970, Brazil

P. Severino · E. B. Souto (✉)
Department of Pharmaceutical Technology, Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, 296, Office S.1, P-4200-150 Porto, Portugal
e-mail: eliana@ufp.edu.pt

E. B. Souto
Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, University of Trás-os-Montes e Alto Douro (IBB-CGB/UTAD), 5001-801 Vila Real, Portugal

Introduction

Nevirapine is the first antiretroviral member of non-nucleoside reverse transcriptase inhibitor approved by the Food and Drug Administration (FDA) for Human Immunodeficiency Virus (HIV). It is being an important component of Active Antiretroviral Therapy (HAART) being typically the primary choice for efficient viral suppression [1]. Considering the risk of physicochemical changes of drugs during pre-formulation and handling, it is necessary to identify the critical properties of the drug for the development of an efficient medicine [2]. Nevirapine is a poorly water soluble drug and presently available as tablet and paediatric oral suspensions, this drug shows three polymorphic forms [3, 4] and exists as solvates and as hydrates [5]. This may also have implications on the physicochemical behaviour of the drug during production and on its performance in vivo [5].

The tendency of pharmaceutical solids to exist in two or more crystalline forms with different molecular arrangement can be attributed to the complexity of their chemical structure [6]. Based on the internal structure, the drug crystal may exist as polymorph or as molecular adduct if its lattice consists of multicomponents and includes solvates and hydrates [7]. The term polymorph describes multiple crystal forms with the same molecular structure but having different free energies so that their physicochemical and biopharmaceutical properties are necessarily different. Solvates are pseudopolymorphs of different crystalline forms of the same compound with association of solvent within the crystal lattice in a stoichiometric or non-stoichiometric ratio. As a result, there are many different forms of polymorphs for the same drug molecule. This solid-state transformation of pharmaceutical solids to polymorphs or solvates has significant effect on the solubility and stability,

which in turn are key contributors for enhanced drug bioavailability [8, 9].

Thermal analysis is a group of techniques that allows evaluating the physical properties of a drug and/or its reaction products, whilst the drug is subject to a controlled temperature programming [10, 11]. The main use of thermal analysis focuses on the study of transition temperature (melting, boiling, presence of solvates, liquid crystals, glass transitions and isomerization), heat of transition, purity, compatibility and decomposition kinetics of polymorphic transition [12, 13, 14]. The advantages of thermal analysis to check the effect to the heating treatment on the materials rely on great precision, accuracy and sensitivity, being used as a permanent record [15]. The transition from a solid to a liquid state of pure material should produce a sharp peak, but in reality this does not occur because impurities or defects in the crystal structure results in a peak with a large base and dislocation of the melting point [16]. The extent of displacement of the melting point of a sample is often used to determine its purity [17]. For the application of this technique, some requirements are needed. The melting point should be a triple point as gas phase, liquid and solid must be in balance and have the same vapour pressure at this temperature. Change in free energy (ΔG) must be zero to maintain balance throughout the merger process. This means that the sample should be subject to small and low heating rate (β). The impurity should not form a solid solution with the drug, but instead an eutectic solution during melting should be obtained. Therefore, thermal analysis is not used for samples that undergo degradation, volatilization or interact during melting. The amount of impurities should be less than 2% [18]. The potential of thermal analysis in the pharmaceutical field is considered not only for the study of polymorphism, but also for their versatility in other applications. These encompass characterization of polymorphs, inclusion complexes and solid dispersants, studies of drug/drug and drug/excipients compatibility at the preformulation level, determination of chemical purity, study of reactions in solid-state (thermal stability and kinetic parameters) analysis of solid dosage forms, quality control of drugs and medicines (as in the determination of the contents of ash and humidity, amongst others), the evaluation of isomers and polymorphs, the analysis of raw materials (drugs and excipients) as well as finished dosage forms (emulsions, suppositories, microspheres, pills, liposomes, powders, creams and gels) [19]. Discontinuous solid solutions occur when there is a limited solubility and therefore the differences in size and shape of molecules that allow the replacement of the crystalline structure occurs only to a limited extent. This is required because if a solid solution is present, no displacement of the melting peak will be observed. The presence of compounds with different characteristics in the solid state is clearly depicted in the differential scanning calorimetry (DSC) curves [20].

The aim of this article was to analyse the phase transition and dehydration processes of nevirapine using DSC and thermogravimetry differential thermal analysis (TG-DTA).

Materials and methods

Nevirapine was provided by the pharmaceutical company Cristália (Itapira/Brazil). Other reagents were used as received if not stated otherwise. For the DSC analysis a TA-2920-TA Instruments (California, USA) was used.

DSC

Thermal behaviour of lipid matrices was assessed accurately weighted; 3 mg of nevirapine was loaded into an aluminium pan and sealed hermetically, in inert atmosphere (N_2). The analysis was performed from 25 to 300 °C at a heating rate of 10 °C/min, following cooling at the rate of 5 °C/min down to 10 °C.

The assessment of drug purity and other thermodynamic parameters were performed by the program-TA Universal Analysis®.

Differential thermogravimetric analysis (TG-DTA)

For TG-DTA analysis, accurately weighted 10 mg of nevirapine was measured in a 2950-TA (Instruments, California, USA). The heating rate was 10 °C/min in the temperature range 25–600 °C. Recorded data were processed with TA-Universal Analysis® (California, USA).

Results and discussion

The curve (Fig. 1) shows that nevirapine has only one thermal event with an onset at 244.48 °C, peaking at 246.79 °C and having an enthalpy of $\Delta H = 130$ J/g. Above 300 °C, no other thermal event was observed, and, the thermo analytical curve was also used for the assessment of drug purity. According to the recorded data, the dried drug has purity greater than 99.0%. The adequacy of the method for determination of purity depends on the correction values and in this case it was too low (<10%) to be used to provide reliable data.

The TG-DTA profile is shown in Fig. 2, where the loss of the drug total mass occurs in one single event standing at 200 °C with the onset temperature at 271.41 °C and extends up to 320 °C. Mathematical modelling clearly shows the degradation of nevirapine, occurring in two distinct steps.

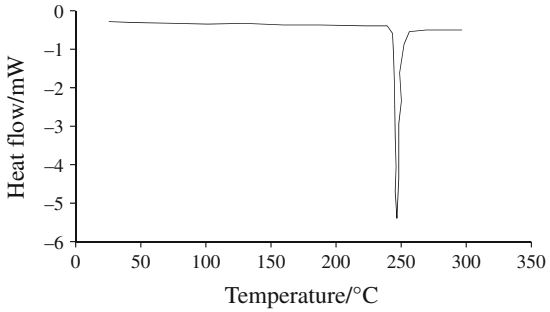

Fig. 1 DSC curve of nevirapine at a heating run of 10 °C/min

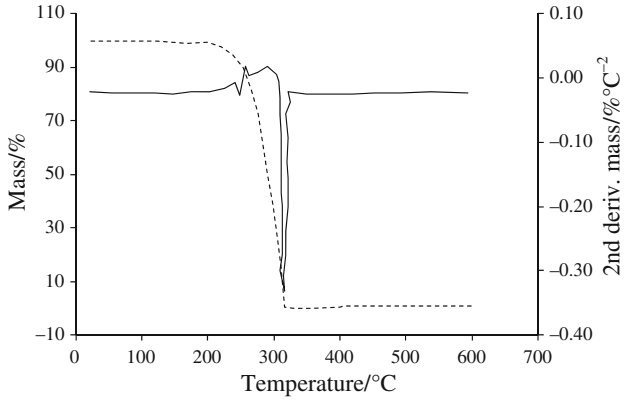

Fig. 2 TG-DTA curve of nevirapine and derivative (*dashed line* mass/% and *solid line* 2nd derivative, mass/%/°C^{-2}), in inert atmosphere (N_2), 50 mL/min, mass 10.0 ± 0.1 mg and following heating at the rate of 10 °C/min

The first peak shows the onset temperature at 238.47 °C and a residual mass of 94.59% with a peak at 246.98 °C extending to 256.88 °C with residual mass of 87.63%. The second onset temperature occurred at 306.30 °C at residual mass of 20.87% and having a maximum at 315.69 °C extending to 323.74 °C with 0.02% residual mass.

Comparing both diffractograms TG-DTA and DSC, the first partial degradation of the drug is shown within the same temperature range, and no changes occur on the thermal properties or on the baseline. Examination of the platinum crucible after the test using a TG-DTA balance indicates that there is a waste, raising the possibility that the degradation of the drug promotes the formation of volatile chemicals, however, without forming residues. Thus, the use of sealed crucibles prevents the degradation, which is completely shifted to the formation of intermediates. In fact, the heating process during TG-DTA leads to saturation of the gases present in the microenvironment that continuously prevents degradation so that equilibrium is shifted towards the reagents.

To confirm that saturation of the environment is causing a shift on the degradation, thermal properties of nevirapine were evaluated using a sealed aluminium crucible with a perforated lid (pin hole). The test conditions were modified so that the event was tackled continuously by TG-DTA since previous data reveal that thermal event did not occur below 200 °C. For this analysis, the sample was heated from 170 up to 250 °C with a heating rate of 2.5 °C/min, following a heating run up to 300 °C a rate of 10 °C/min.

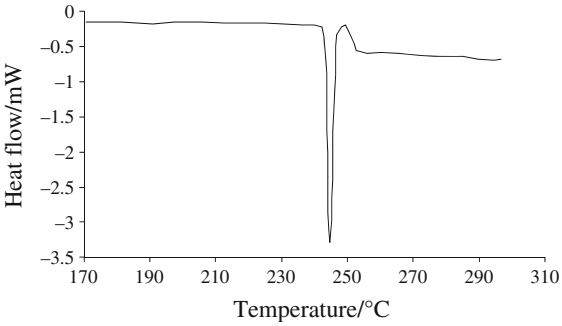

Fig. 3 DSC thermal analysis curve of nevirapine following a second heating run from 170 up to 250 °C

Figure 3 shows that the event on the thermal properties of nevirapine is present, but with decreasing heating rate and the change of vapour pressure with the presence of the hole in the crucible. The thermal event was attributed to the melting of the drug and started at 240 °C showing the onset temperature at 243.55 °C, and the peak at maximum of 244.57 °C and extending to approximately 250 °C with a melting enthalpy of 142.2 J/g. The change in baseline (not previously observed) became evident above 250 °C. The peak observed between fusion and degradation was attributed to the changes of the heating rate (i.e., from 2.5 to 10 °C/min) and stabilization of the system is not related to thermal events of the sample.

By overlaying both DSC and TG-DTA curves, the observed peak between 240 and 260 °C occurs in the same temperature range, as that observed for nevirapine. Thus, the melting event related to nevirapine also occurs, suffering partial mass loss. One of the groups present in the molecule that might be associated with this mass loss is the initial cluster cyclopropane. The diagram representing the reaction involved is shown in Fig. 4.

The data recorded for nevirapine are in agreement with the literature [21, 22]. Other studies, however, report a melting point of 244.0 °C loss in a single event [23, 24]. However, our DTG curves depict two events (Fig. 2). Comparing the conditions employed in both studies, the difference in methodology is the mass of sample to be tested for TG analysis i.e., 4.0 mg were weighted, and this may have been one of the factors that influenced the non-occurrence of the event between 240 and 250 °C related to the partial loss of mass.

For the assessment of drug purity, the Van't Hoff equation describing the cryoscopic lowering for solutions

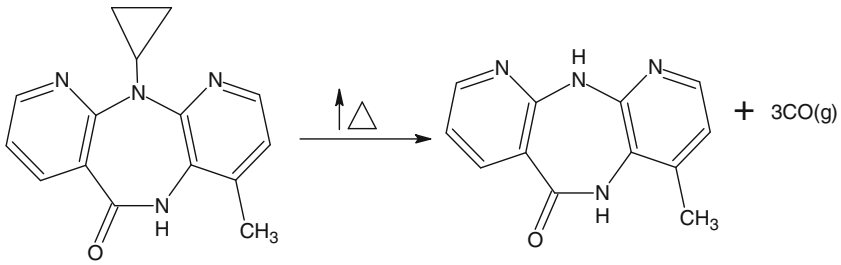

Fig. 4 Reaction of nevirapine exposed to heat releasing cyclopropane and gaseous carbon compounds

containing solids was used. There are nevertheless some difficulties in identifying the point at which fusion occurs because eutectic mixtures can happen upon drug fusion if impurities are present. This is due to non-equilibrium conditions of dynamic heating, and consequently formation of a solid solution. Therefore, the equation is not a straight line and, thus a correction factor must be employed. For the calculation of purity using this approach, the correction factor should be below 5%. The behaviour observed for nevirapine during the melting process meets the requirement to employ this methodology to assess drug purity. In a previous report using a correction factor >20%, the conditions used were not appropriate for the calculation of purity, since there was a proper linearization of the Van't Hoff curve. Thus, different tests were undertaken using different heating runs seeking to reduce the observed correction factor. The data are shown in Table 1.

Table 1 DSC parameters recorded at different heating runs of nevirapine

β/°C/min	T_{onset}/°C	T_{peak}/°C	ΔH/kJ/mol	FC[a]	Purity/%
10.0	244.40	246.46	46.53	20.000	98.97
5.0	244.61	245.62	36.57	0.8961	99.95
2.0	244.45	244.68	57.72	7.0040	99.73
1.0	244.59	245.04	32.68	0.4414	99.94
0.5	243.48	243.89	31.03	6.3470	99.88

[a] Adjusting factor

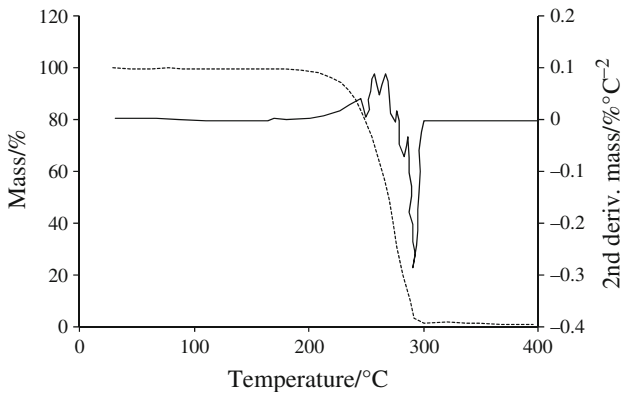

Fig. 5 TG-DTA curves of nevirapine and derivative (*solid line* mass/% and *dashed line* 2nd derivative, mass/%/°C^{-2}), in inert atmosphere (N_2), 50 mL/min, mass 10.0 ± 0.1 mg and following heating at the rate of 5 °C/min

Table 2 TG-DTA data obtained from nevirapine

Nevirapine	T_{onset}/°C	T_{peak}/°C	Δm/°C/%	T_{endset}/°C	Δm/%[a]
Loss 1	243.60	248.77	0.036	254.58	25.71
Loss 2	254.58	259.75	0.049	266.21	44.83
Loss 3	266.21	273.32	0.016	276.55	68.29
Loss 4	276.55	281.72	0.070	285.59	87.82
Loss 5	285.59	291.40	0.280	297.86	100.0

[a] $\%\Delta m$ variation of mass loss

The best condition focusing on the DSC curve for the calculation of purity of nevirapine is the employment of test samples (3 mg) under an inert atmosphere (N_2) 50 mL/min and a heating rate of 5 °C/min in sealed aluminium crucible. According to Grangeiro et al. [25], nevirapine has a single step mass loss observed by TG-DTA. However, the use of different sample mass under different heating ratios indicates that the degradation of drug occurs in consecutive steps at speeds of different mass loss (Fig. 5). Respective thermal parameters are depicted in Table 2.

Conclusions

A drug substance can exist in various forms, such as an amorphous form as hydrate or solvate according to conditions of the manufacturing processes, e.g., drying, grinding and compressing. Depending on the drug form the key factors to consider in the design of a dosage form are its physical properties, bioavailability, stability, dissolution rate and moisture. Therefore, it is important to analyse the characterization of the bulk substance and to select the suitable form for formulation. This study allowed us to characterize nevirapine, to be further processed in pharmaceutical dosage forms. Nevirapine is sufficiently stable to undertake thermal stress currently in use in several production processes.

Acknowledgements The authors wish to acknowledge the sponsorship of the FAPESP (*Fundação de Amparo a Pesquisa*) and CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*).

References

1. Ramana LN, Sethuraman S, Ranga U, Krishnan UM. Development of a liposomal nanodelivery system for nevirapine. J Biomed Sci. 2010. doi:10.1186/1423-0127-17-57.
2. Capparelli EV, Aweeka F, Hitti J, Stek A, Hu C, Burchett SK et al. Chronic administration of nevirapine during pregnancy: impact of pregnancy on pharmacokinetics. HIV Med. 2008. doi: 10.1111/j.1468-1293.2008.00553.x.
3. Reguri BR, Chakka R. Crystalline forms of nevirapine. US Patent 0183738 A1. 2006. http://www.patentstorm.us/applications/20060183738/description.html. Accessed 17 Aug 2006.
4. Reguri BR, Chakka R. Novel crystalline forms of 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (Nevirapine). US Patent 0059653A1 2005. http://www.freepatentsonline.com/y2005/0059653.html. Accessed 17 Mar 2005.
5. Chadha R, Arora P, Saini A, Jain DS. Solvated crystalline forms of nevirapine: thermoanalytical and spectroscopic studies. AAPS Pharm Sci Tech. 2010. doi:10.1208/s12249-010-9511-z.
6. Willart JF, Descamps M. Solid state amorphization of pharmaceuticals. Mol Pharm. 2008. doi:10.1021/mp800092t.
7. Giron D. Applications of thermal analysis in the pharmaceutical industry. J Pharm Biomed Anal. 1986. doi:0731-7085(86)80086-3.
8. Xia D, Cui F, Piao H, Cun D, Jiang Y, Ouyang M et al. Effect of crystal size on the in vitro dissolution and oral absorption of nitrendipine in rats. Pharm Res. 2010. doi:10.1007/s11095-010-0200-0.
9. Park YJ, Xuan JJ, Oh DH, Balakrishnan P, Yang HJ, Yeo WH et al. Development of novel itraconazole-loaded solid dispersion without crystalline change with improved bioavailability. Arch Pharm Res. 2010. doi:10.1007/s12272-010-0812-2.
10. Soares-Sobrinho JL, de La Roca Soares MF, Lopes PQ, Correia LP, de Souza FS, Macedo RO et al. A preformulation study of a new medicine for Chagas disease treatment: physicochemical characterization, thermal stability, and compatibility of benznidazole. AAPS Pharm Sci Tech. 2010. doi:10.1208/s12249-010-9495-8.
11. Park J, Park HJ, Cho W, Cha KH, Kang YS, Hwang SJ. Preparation and pharmaceutical characterization of amorphous cefdinir using spray-drying and SAS-process. Int J Pharm. 2010. doi: 10.1016/j.ijpharm.2010.06.032.
12. Passerini N, Calogera G, Albertini B, Rodriguez L. Melt granulation of pharmaceutical powders: a comparison of high-shear mixer and fluidised bed processes. Int J Pharm. 2010. doi: 10.1016/j.ijpharm.2010.03.013.
13. Paluch KJ, Tajber L, McCabe T, O'Brien JE, Corrigan OI, Healy AM. Preparation and solid state characterisation of chlorothiazide sodium intermolecular self-assembly suprastructure. Eur J Pharm Sci. 2010. doi:10.1016/j.ejps.2010.08.013.
14. Kumar S, Chawla G, Sobhia ME, Bansal AK. Characterization of solid-state forms of mebendazole. Pharmazie. 2008. doi: 10.1691/ph.2008.7168.
15. Murdande SB, Pikal MJ, Shanker RM, Bogner RH. Solubility advantage of amorphous pharmaceuticals: I. A thermodynamic analysis. J Pharm Sci. 2010. doi:10.1002/jps.21903.
16. Ruscica R, Bianchi M, Quintero M, Martinez A, Vega DR. Solid-state forms of zoledronic acid: polymorphism in hydrates. J Pharm Sci. 2010. doi:10.1002/jps.22223.
17. Yoshida MI, Gomes EC, Soares CD, Cunha AF, Oliveira MA. Thermal analysis applied to verapamil hydrochloride characterization in pharmaceutical formulations. Molecules. 2010. doi: 10.3390/molecules15042439.
18. Kobayashi K, Fukuhara H, Kawamoto I, Hata T, Sekine A, Uekusa H et al. Physicochemical analyses of phase transition and dehydration processes of a new oral 1beta-methylcarbapenem antibiotic agent, CS-834. Chem Pharm Bull (Tokyo). 2002. doi: 10.1248/cpb.50.1570.
19. Araújo AAS, Bezerra MdS, Storpirtis S, Matos JdR. Determination of the melting temperature, heat of fusion, and purity analysis of different samples of zidovudine (AZT) using DSC. Brazilian J Pharm Sci. 2010. doi:10.1590/S1984-82502010000100005.
20. Wojnarowska Z, Grzybowska K, Adrjanowicz K, Kaminski K, Paluch M, Hawelek L et al. Study of the amorphous glibenclamide drug: analysis of the molecular dynamics of quenched and cryomilled material. Mol Pharm. 2010. doi:10.1021/mp100077c.
21. Grob PM, Wu JC, Cohen KA, Ingraham RH, Shih CK, Hargrave KD et al. Non nucleoside inhibitors of HIV-1 reverse transcriptase: nevirapine as a prototype drug. AIDS Res Hum Retroviruses. 1992. doi: 10.1089/aid.1992.8.145.
22. Koup RA, Merluzzi VJ, Hargrave KD, Adams J, Grozinger K, Eckner RJ et al. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by the dipyridodiazepinone BI-RG-587. J Infect Dis. 1991. doi:10.1093/indis/163.5.966.
23. Grangeiro A, Teixeira L, Bastos FI, Teixeira P. Sustainability of Brazilian policy for access to antiretroviral drugs. Rev Saude Publica. 2006. doi:S0034-89102006000700009.
24. Grangeiro A, Ferraz D, Barbosa R, Barreira D, Veras MA, Villela W et al. UNGASS-HIV/AIDS: a review of the Brazilian response, 2001–2005. Rev Saude Publica. 2006. doi:S0034-89102006000700002.
25. Grangeiro A, Strattmann RR, Alburquerque MM, Araújo AAS, Matos JR, Neto PJR. In vitro evaluation of dissolution profiles and thermal properties of some commercial formulations of nevirapine tablets. Acta Farm Bonaerense. 2006. http://www.latamjpharm.org/trabajos/25/1/LAJOP_25_1_1_12_96B013S1LJ.pdf. Accessed 17 Dec 2010.