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DIFFERENTIAL SCANNING MICROCALORIMETRY STUDY OF THERMAL STABILITY OF NEVIRAPINE AND AZIDOTHYMIDINE MIXTURE

K. Michalik[1], *Zofia Drzazga*[1*], *Anna Michnik*[1] *and M. Kaszuba*[2]

[1]University of Silesia, A. Chełkowski' Institute of Physics, Department of Medical Physics,Ul. Uniwersytecka 4
40 – 007 Katowice, Poland,
[2]Medical University of Silesia, Department of Conservative and Peropdontology, 41-902 Bytom, Pl. Akademicki 17, Poland

A differential scanning calorimetry study of the thermal behavior of nevirapine and azidothymidine in water solution was carried out. For nevirapine scan rate dependent and irreversible endothermic peak were found. Thermal degradation of nevirapine as well as NVP – AZT mixture is relatively well described by the model involving only one irreversible step determined by a first-order rate constant. The estimated kinetic constants and activation energies indicate that the degradation process proceeds slower for nevirapine in presence of AZT ligands than without them.

Keywords: *azidothymidine, nevirapine, thermal analysis interaction*

Introduction

The antiviral agents azidothymidine (AZT) and nevirapine (NVP) are often used substances in Acquired immune deficiency syndrome (AIDS) treatment caused by the human immunodeficiency virus (HIV). Azidothymidine (3'-azido-3'deoxythymidine) being a nucleoside reverse transcriptase inhibitor of HIV was the first antiviral drug approved for the treatment of HIV infection. AZT belongs to 'prodrugs' and requires intercellular phosphorylation to active form [1]. Nevirapine (11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b:2',3'-e][1,4]diazepin- 6-one) is the non-nucleoside reverse transcriptase inhibitor. It binds directly to allosteric site on reverse transcriptase and inhibits both the RNA and DNA-dependent DNA polymerase activities [2]. AZT and NVP lead to slowing down of virus HIV reproduction. Unfortunately both these drugs and their degradation products have great potential for causing many serious side effects. Therefore therapeutic drug monitoring is necessary for optimal therapy for HIV infected patients [1, 3]. The therapeutic strategy regimes require the combination of antiretroviral drugs due to the rapid rate of virus HIV mutation and successive anti-HIV drug resistant during the long therapy [4]. One of such promising combination is mixture containing AZT and NVP that has been shown to reduce mother-to -child transmission of HIV without increasing side-effects [5, 6].

The anti-HIV drugs have been intensively investigated [1–10] but relatively few studies have been done by means microcalorimetry technique [11, 12]. A thermal analysis and kinetics studies of drugs have been carried out to find out their stability [13–15].

The aim of the present work is to study thermal stability of AZT and NVP in aqueous solution and an influence of the NVP and AZT interaction on mixed solution stability. Moreover analysis of the DSC data is performed basing on the kinetic model.

Materials and methods

Azidothymidine and nevirapine were purchased from GlaxoSmithKline and Boehringer Ingelheim Pharmaceuticals Inc. respectively. Azidothymidine (reference standard) (Lot112K3485) and thymine (Lot 123K0719) were received by courtesy of Sigma. Potatoes starch was purchased from POCH (1331-427-9940). Aqua pro injection was used as a solvent in all experiments.

DSC curves for $0.7\cdot10^{-3}$–$1.4\cdot10^{-3}$ M L^{-1} nevirapine water solution were performed using the VP DSC ultrasensitive microcalorimeter (Microcal Inc., Northampton, MA) with cell volumes 0.5 mL. Azidothymidine and thymine were added to obtain final concentration ratio 2:1, 1:1 and 1:2.

Heat capacity *vs*. temperature profiles for studied samples were obtained for scanning rates of 40, 60, 90°C h^{-1} in the temperature range 25–120°C at constant pressure of about 1.8 atm over the liquids in the cells.

The calorimetric data were corrected for the instrumental baseline and for the difference in heat ca-

* Author for correspondence: drzazga@us.edu.pl

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

pacity between the initial and final state by using a linear baseline. DSC curves were analyzed with MicroCal Origin software. The fit of the theoretical curves to the experimental data was achieved by the non-linear Levenberg – Marquardt method.

Results and discussion

Overall characteristic of AZT and NVP thermal transitions and their mixtures

Figure 1A presents DSC curve for AZT drug in water solution after subtracted water – water scan. Endothermic peak in temperature range 60–73°C is observed (at scanning rate 90°C h^{-1}). It was reported that in the case of AZT powder samples the DSC curves revealed a sharp endothermic peak corresponding to melting in the range 120–124°C [11] and the thermogravimetric method indicated thermal decomposition in melt at 140–201°C [12]. It should be noted (Fig. 1B) that water solution of AZT (reference standard) as well as thymine show no thermal transitions in the studied temperature range 25–120°C. The observed endothermic peak in AZT drug solution (Fig. 1A) seems to be connected with one of excipients, probably with the starch (Fig. 1C) [16].

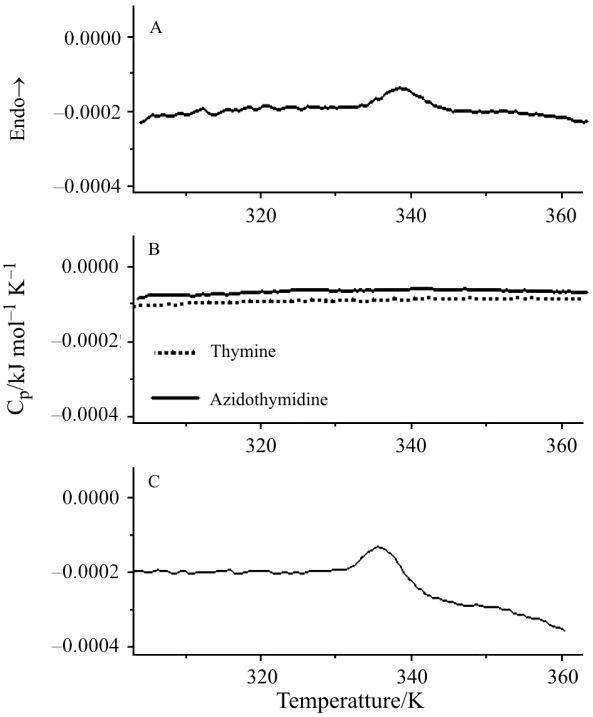

Fig. 1 DSC curves of the relative heat capacity *vs.* temperature for A – azidothymidine, B – thymine and azidothymidine reference standard and C – potato starch (scan rate 90°C h^{-1})

Table 1 Transition temperature T_m and enthalpy change ΔH (mean ± SEM) for nevirapine water solution at different concentration (scan rate 90°C h^{-1})

Concentration/mM L^{-1}	T_m/°C	ΔH/kJ mol^{-1}
0.7	75.5±1.5	7.5±0.4
1.0	85.0±1.2	8.7±2.8
1.4	92.0±1.0	12.5±1.4

Nevirapine unlike AZT shows the wide endothermic peak (Fig. 2). The transition temperature T_m depends markedly on concentration (Fig. 2A). The area under the peak (the enthalpy change ΔH) as well as T_m increase with increasing drug concentration (Table 1).

It follows from Fig. 2B that nevirapine DSC curves are weakly scan rate dependent. Change of scan rate from 90 to 40°C h^{-1} causes some decrease of enthalpy change ΔH and shift of T_m from 85°C±1.2 to 82.5°C±2.0. The trends observed in the DSC scans suggest that the studied transition is kinetically controlled.

The reversibility of the thermal transition for NVP is tested by reheating experiment which is illustrated on Fig. 3. If the first heating is carried out to 120°C the process is irreversible (curve a and e). However, in another experimental variant if heating is stopped at lower chosen temperatures from the transition region (69, 80, 84°C) the DSC curves (b, c, d) prove that observed decomposition process is only partly irreversible.

For the sake of application of the AZT and NVP complex in therapy the thermal decomposition of AZT and NVP mixture is also studied. Figures 4A and B illustrate the effect of azidothymidine and thymine concentration on DSC curves of nevirapine, respectively. A small tendency to shift T_m toward lower temperatures with increasing concentration is observed (see also Table 2). The shift of peak maximum indicates that interaction between NVP and AZT drugs takes place. It is reported [17] that change of T_m in DSC experiment can be understood as a ligand binding effect. The ligand binding constant K_b can be calculated by the following equation:

$$K_b = [L]^{-1}\left(\exp\left\{\left(\frac{1}{T_m}-\frac{1}{T_{mL}}\right)\frac{\Delta H}{R}\right\}-1\right) \qquad (1)$$

where T_m and T_{mL} are transitions temperatures in absence and presence of ligands (in our case AZT and thymine), ΔH is the enthalpy of nevirapine decomposition, R is the gas constant and $[L]$ is free ligand concentration. The obtained values of K_b are presented in Table 2. One can see that estimated values of K_b are relatively low and depend a little on AZT (or thymine) content in mixture.

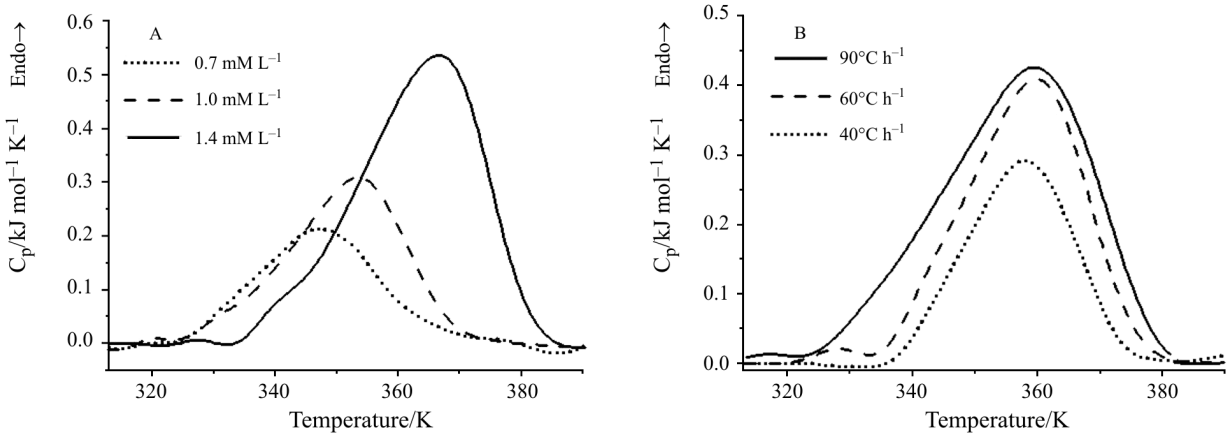

Fig. 2 DSC curves for nevirapine water solution (1 mM L^{-1}) at A – different concentration and B – different scan rate

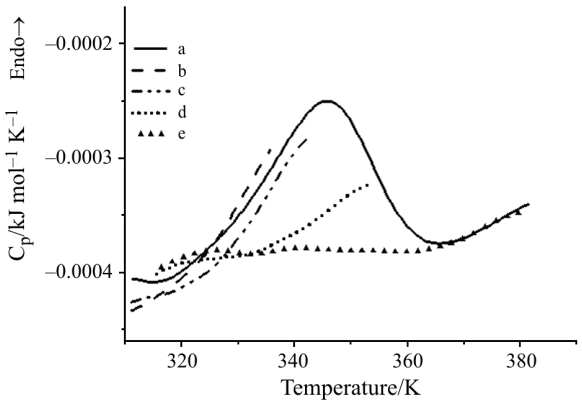

Fig. 3 The raw DSC profiles for nevirapine water solution heated to a given temperature (a – 120, b – 69, c – 80, d – 84 and e – 120°C) and reheated to higher after the cooling from the previous run (scan rate 90°C h^{-1})

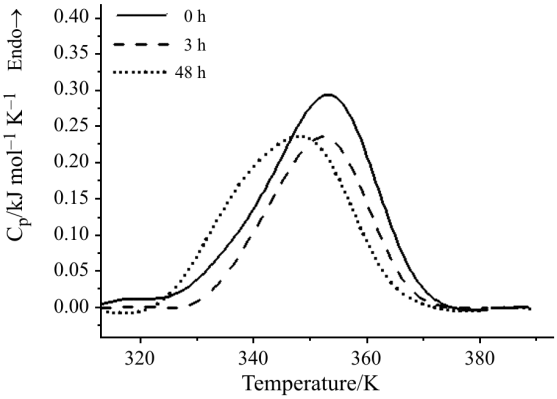

Fig. 4 DSC curves of nevirapine water solution stored for different times (scan rate 90°C h^{-1})

Table 2 Binding constant K_b and difference between transition temperature in absence (T_m) and presence (T_{mL}) of azidothymidine or thymine (scan rate 90°C h^{-1})

Sample	Concentration ratio	T_m–T_{mL}/ °C	K_b/M^{-1}
NVP+AZT	2:1	2.0±0.4	56.1±19.5
NVP+AZT	1:1	2.0±0.6	22.8±10.0
NVP+Thymine	2:1	3.2±0.8	87.7±23.4
NVP+Thymine	1:1	3.4±0.5	37.7±6.3

Calorimetric analysis in kinetic model

The scan rate dependence, only partial reversibility indicate that degradation process of nevirapine could be treated as kinetically controlled [18]. The analysis of DSC data in such case should begin with checking whether experimental data satisfy the one-step model $A \xrightarrow{k} B$ where A is the initial state, B is the final state and k is a first-order kinetic constant. If process is one step the plot $1/T$ *vs*. (V – scan rate, C_p^{ex} excess heat capacity, Q_t – total heat of process (equivalent to ΔH), Q – heat evolved at given temperature T) should be linear [19]. Figure 6 shows that the experimental points describing main endothermic peak can be approximated by a straight line for nevirapine as well as for mixture NVP – AZT (not shown). It follows that in first approximation the degradation process of nevirapine can be interpreted in terms of a simple first-order one-step kinetic process. The activation energy of this process can be calculated with the use of three different ways [17] :

- from the equation

$$k = VC_p^{ex}(Q_t - Q)^{-1} \quad (2)$$

and the slope of the Arrhenius plot, ln$k$ *vs*. $1/T$.

- from the dependence

$$\ln[\ln Q_t (Q_t - Q)^{-1}] = \frac{E_a}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right) \quad (3)$$

and the plot of $\ln[\ln Q_t (Q_t - Q)^{-1}]$ *vs*. $1/T$,

- using the coordinates of the maximum of the calorimetric peak (T_m, C_p^m) according to:

Table 3 The activation energies E_a, first order rate constant k and half life time $\tau_{1/2}$ for nevirapine water solution in absence and presence of azidothymidine or thymine

Sample	Concentration ratio	E_a/kJ mol^{-1}	$\tau_{1/2}$/days		$k\cdot10^{-4}$/min^{-1}	
			25°C	37°C	25°C	37°C
NVP	–	99±8	3.0±1.0	0.6±0.1	1.4±0.1	7.5±0.1
NVP+AZT	2:1	132±16	12.0±2.5	4.5±5.5	0.4±0.3	2.7±5.0
NVP+AZT	1:1	124±14	7.5±0.7	1.2±0.1	0.6±0.1	4.2±0.3
NVP+Thymine	2:1	120±13	9.0±2.1	1.2±0.3	0.6±0.2	4.0±1.0
NVP+Thymine	1:1	122±11	7.5±1.3	1.1±0.1	0.6±0.1	4.3±0.6

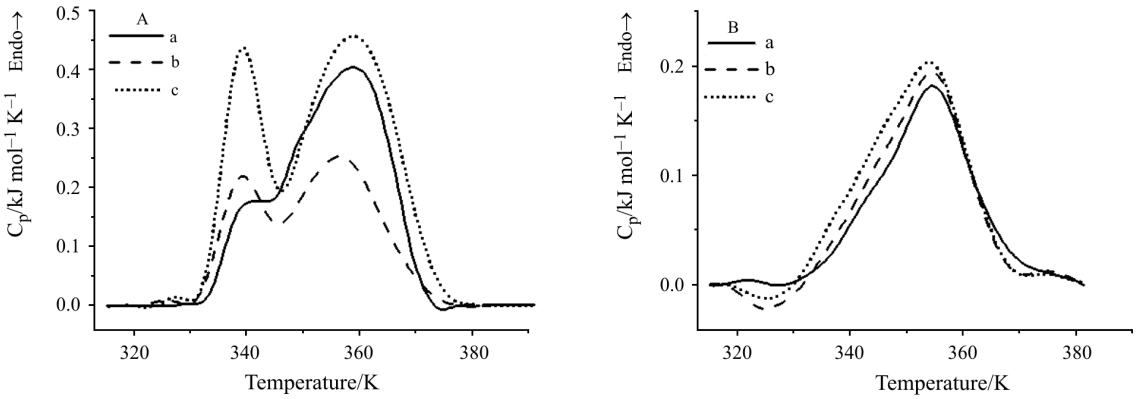

Fig. 5 Effect of A – azidothymidine and B – thymine concentration on DSC curves on nevirapine water solution (concentration ratio a – 2 : 1, b – 1 : 1, c – 1 : 2; scan rate 90° C h^{-1})

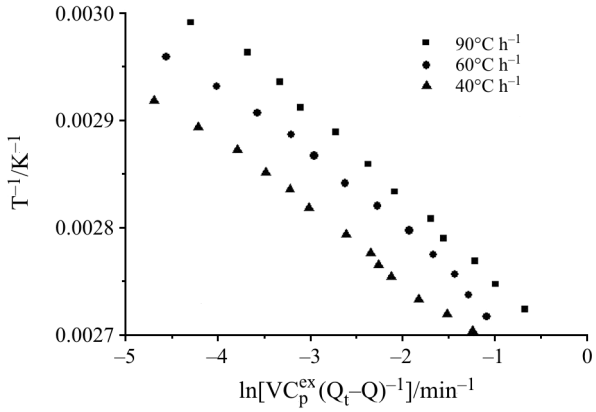

Fig. 6 Dependence of T^{-1} on ln[$VC_p^{ex}/(Q_t–Q)$] for nevirapine water solution

$$E_a = 2.718RC_p^m T_m^2 Q_t^{-1} \quad (4)$$

The results do not depend on the calculation way. Values of activation energy obtained with use of Eq. (2) are presented in Table 3. The activation energy for NVP is about 100 kJ mol^{-1} and increases with AZT or thymine addition.

The Eq. (2) allows to estimate the values of the rate constant k and half-life times at chosen temperatures which are listed in Table 3. The half-life time for nevirapine is found to be 3 and 0.6 days at 25 and 37°C, respectively. The half-life times of NVP – AZT (NVP – thymine) solutions are several times longer than of pure NVP one. It follows that application of AZT – NVP mixture improves the stability of solution regarding NVP water solution.

Conclusions

In the studied temperature range up to 120°C nevirapine show broad endothermic transition unlike azidothymidine and thymine. The degradation processes of nevirapine is markedly concentration dependent and weakly scan rate dependent. Moreover it is found to be partly irreversible.

Thermal degradation of nevirapine as well as NVP – AZT mixture is relatively well-described by the model involving only one irreversible step determined by a first-order rate constant. The estimated kinetic constants and activation energies indicate that the degradation process proceeds slower for nevirapine in presence of AZT ligands than without them. However estimated binding constant of NVP with AZT is relatively weak in studied solutions.

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