Journal of Thermal Analysis and Calorimetry, Vol. 88 (2007) 2, 401–404

THERMAL STABILITY STUDY OF THE PROTEASE INHIBITORS Nelfinavir mesylate and atazanavir sulfate

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A thermal and kinetic analysis of two protease inhibitors: nelfinavir mesylate and atazanavir sulfate, were carried out to find their thermal stability. DSC curves of both drugs showed exothermic transition. This observed process resulted in two steps. Obtained apparent activation energy pointed at low stability of studied protease inhibitors in water solutions.

Keywords: *atazanavir sulfate, DSC, nelfinavir mesylate, thermal analysis*

Introduction

The decomposition reactions of drugs possess very important practical significance. The knowledge about behavior of medical substances at various temperatures is indispensable for the prediction of their physico-chemical and pharmacological properties. The determination of the temperature range, in which a given drug substance is stable both in its structure and pharmacotherapeutic action, is crucial from the point of view of drug storage, its technological transformations, technology of drug formulation and what is most important behavior in human organism [1, 2]. Some drugs and their decomposition products are often very toxic, therefore stability specificity, has great importance.

Nelfinavir mesylate and atazanavir sulfate (Table 1) belong to the anti HIV drug class called protease inhibitor (PIs). HIV protease enzyme is required for post-translational cleavage of Gag and Gag-Pol polyproteins into smaller functional proteins. PIs block this enzyme, leading to non-infectious immature virion formation [3]. These substances are associated with metabolic abnormalities that may increase risk of atherosclerotic vascular disease, including dyslipidemia, insulin resistance, and central obesity [4].

Thermal analysis was utilized as a tool for the rapid evaluation of drug stability. Kinetic analysis allows the determination of the knowledge about the decomposition rate, the suitable mechanism, half-life at any temperature, and the activation energy values of pharmacological compounds; thus getting deeper insight into thermal behavior [5].

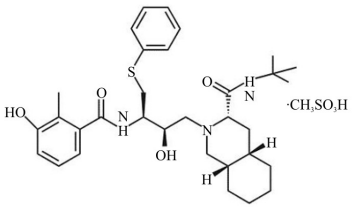
Experimental

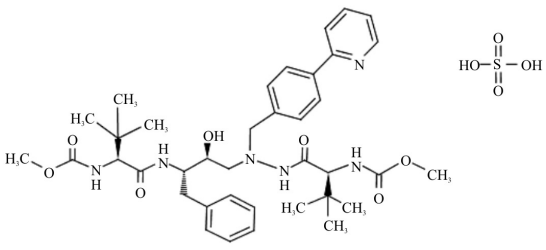
Materials

Atazanavir sulfate and nelfinavir mesylate were purchased respectively from Bristol Myers Squibb

Table 1 Nelfinavir mesylate and atazanavir sulfate description

Name	Nelfinavir mesylate	Atazanavir sulfate
Chemical formula	C_{32}–H_{45}–N_3–O_4–S·C–H_4–O_3–S	C_{38}–H_{52}–N_6–O_7·H_2–S–O_4
Molecular mass	663.90	802.9
Structural formula		





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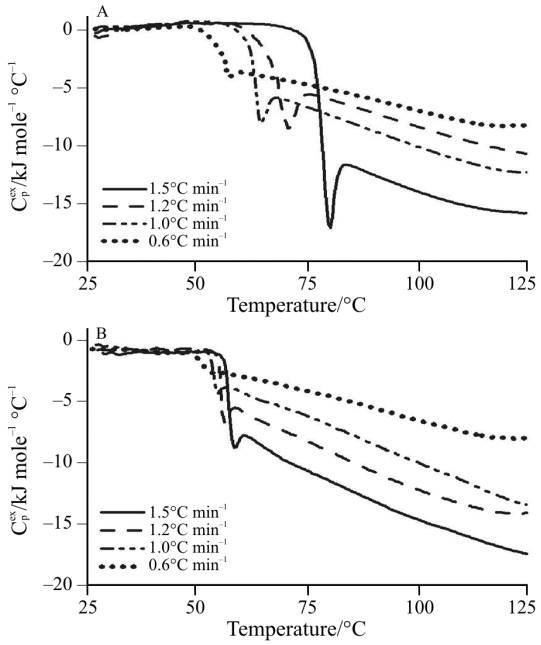

Fig. 1 Excess heat capacity of atazanavir sulfate (Fig. 1A) and nelfinavir mesylate (Fig. 1B) *vs.* temperature obtained at four different scanning rates (1.5, 1.2, 1.0 and 0.6°C min^{-1}). The concentration of the solution was 1.36 mM L^{-1} and pH was 5.0 for atazanavir sulfate and 7.0 for nelfinavir mesylate

and Roche. Aqua pro injection was used as a solvent in all experiments.

An accurately weighed amount of drugs was dissolved in aqua pro injection. The solutions were prepared with the concentration range from 0.07 to 1.36 mM L^{-1}. pH of the solution was from 5 to 3 for atazanavir sulfate and from 5.5 to 7 for nelfinavir mesylate.

Instrumental methods

DSC scans were performed using the VP DSC ultrasensitive microcalorimeter (MicroCal Inc., Northampton, MA) with cell volumes at 0.5 mL. Heat capacity *vs.* temperature profiles were obtained for scanning rates of 0.6 1.0, 1.2, 1.5°C min^{-1} in temperature range 25–125°C. Additionally constant pressure of about 1.8 atm was applied over the liquids in the cells. Samples were degassed immediately before loading the cells.

UV VIS spectra of the samples were recorded in the wavelength range of 190–800 nm on JASCO V-530 spectrophotometer with 2 nm band-width.

Analysis

The calorimetric data were corrected for the instrumental baseline water – water. A cubic baseline was used to obtain the excess apparent molar heat capacity (C_p^{ex}). DSC curves were analyzed with MicroCal Origin software. The fit of the curves of the theoretical model to the experimental data was achieved by the non-liner Levenberg-Marquardt method.

Results and discussion

Figure 1 showed an original DSC apparent heat capacity (C_p^{ex}) profiles for thermal degradation of atazanavir sulfate (Fig. 1A) and nelfinavir mesylate (Fig. 1B) water solution (concentration 1.36 mM L^{-1}) after subtracted water–water scan and concentration normalization. Curves were obtained at four different scanning rates v=1.5, 1.2, 1.0 and 0.6°C min^{-1}. In all experiments concentration of the samples was corrected with use of UV VIS method (extinction coefficient for atazanavir sulfate was 7489±318 and for nelfinavir mesylate 175±8 mol dm^{-3} cm^{-1} and not depend on pH in studied concentration range).

For both drug water solutions exothermic transitions were observed that were not reproducible on reheating sample (not shown). Moreover, effect of scanning rate was much stronger for atazanavir sulfate than for nelfinavir mesylate. In both cases transition temperature T_m shifted toward lower temperatures with scan rate decreasing. Peaks became less intense. The enthalpy change ΔH estimated as the area under the peak increased with scan rate increasing (Table 2). Scan rate dependence and the fact that the calorimetric traces were found to be irreversible indicated kinetically controlled processes [6].

Table 2 Thermodynamic parameters (±SEM) of nelfinavir mesylate and atazanavir sulfate obtained from DSC scan at different scan rate. The concentration of the solution was 1.36 mM L^{-1} and pH was 5.0 for atazanavir sulfate and 7.0 for nelfinavir mesylate

v/°C min^{-1}	T_m/°C		ΔH/kJ mol^{-1}	
	Nelfinavir mesylate	Atazanavir sulfate	Nelfinavir mesylate	Atazanavir sulfate
0.6	53.1±0.2	57.0±0.4	8.7±0.4	7.5±5.1
1.0	54.9±0.3	63.9±0.5	10.8±0.8	19.2±4.1
1.2	56.2±0.1	70.5±0.2	18.4±1.2	25.1±1.2
1.5	58.1±0.6	79.7±0.2	25.5±0.5	56.9±4.4

The effect of the drug concentration on the position of the transition temperature T_m gave some information about the changes in molecularity occurring during the thermal degradation process [7]. It was important to check if the calorimetric profiles for PIs were concentration dependent. Figure 2 illustrates the effect of drug concentration on DSC curves. For atazanavir sulfate shift of T_m toward higher temperatures with concomitant decrease in heat capacity minimum C_p^{ex} was observed after drug titration (Fig. 2A). ΔH increased with concentration (from 20.1±1.2 to 56.9±4.4 kJ mole^{-1}). Different situation for nelfinavir mesylate was observed (Fig. 2B). T_m was practically independent of drug concentration (the average T_m value for nelfinavir mesylate was 57.5°C±0.5°C) as well as ΔH. However, DSC traces underwent changes. Pretransition shoulder became longer and posttransition successively fell down with concentration decreasing, what was not observed for atazanavir sulfate solution. DSC curves of PIs obtained for concentration lower than 1.0 mM L^{-1} were broad, and no sharp, exothermic peak was observed. However, observed processes for concentration in the range 0.07–1.0 mM L^{-1} were not recurrent especially for nelfinavir mesylate. The change of exothermic transition shape after PIs titration resembled the shape of protein aggregation curves [8]. It was also observed that after pulling out drug solution from DSC cells, sample was cloudy and

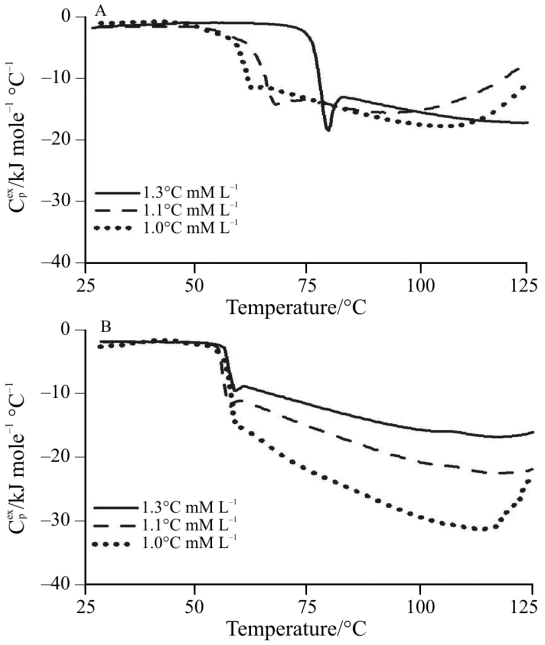

Fig. 2 Effect of drugs concentration: A – atazanavir sulfate and B – nelfinavir mesylate on DSC curves obtained at constant scan rate 1.5°C min^{-1} and concentration range 1.0–1.3 mM L^{-1}. pH of the solution was from 5 to 3 for atazanavir sulfate and from 5.5 to 7 for nelfinavir mesylate

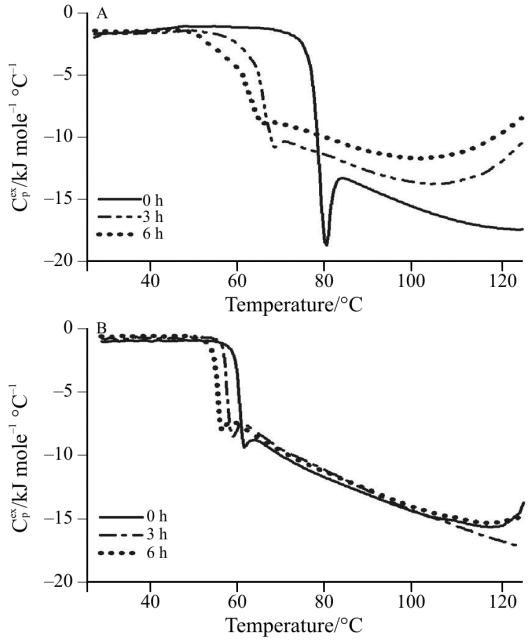

Fig. 3 Ageing process of A – atazanavir sulfate and B – nelfinavir mesylate water solution obtained at constant scan rate 1.5°C min^{-1} and concentration 1.36 mM L^{-1}. The pH of the solution was 5.0 for atazanavir sulfate and 7.0 for nelfinavir mesylate

not homogenous what could probably confirm aggregation process.

It is noteworthy that examined solutions changed relatively fast in time what was visible on DSC curves obtained for samples stored 3 and 6 h in room temperature (Fig. 4). Ageing of sample caused decrease of T_m and ΔH (Table 3). Observed changes were much more significant for atazanavir sulfate than for nelfinavir mesylate. It could indicate that atazanavir sulfate was more sensitive to water presence and it lost stability faster than nelfinavir mesylate.

Analysis

The strong scan rate dependence and irreversibility of the thermal transition suggested that degradation process of atazanavir sulfate and nelfinavir mesylate could be treated as kinetically controlled. In the first approach, the simplest one step kinetic model of irreversible drug degradation was considered. However our results didn't fulfilled the criteria of this model validity discussed by Kurganov *et al.* – if the one step model is valid, the plot $1/T$ *vs.* $\ln[\nu C_p^{ex}(\Delta H - \Delta H_T)^{-1}]$ should be linear (ΔH_T is the heat evolved at given temperature T) [9]. Therefore, the two-step Lumry – Eyring model with fast equilibrating first step and the irreversible second was tested. It was possible to calculate apparent activation energy E_{app} from the following Eq. (1) [10]:

Table 3 Thermodynamic parameters (±SEM) obtained from DSC scan of nelfinavir mesylate and atazanavir sulfate for different time storage. The concentration of the solution was 1.36 mM L^{-1} and pH was 5.0 for atazanavir sulfate and 7.0 for nelfinavir mesylate

t/h	T_m/°C		ΔH/kJ mol^{-1}	
	Nelfinavir mesylate	Atazanavir sulfate	Nelfinavir mesylate	Atazanavir sulfate
0	58.1±0.6	79.7±0.2	25.5±5.0	56.9±4.4
3	57.0±0.1	64.0±0.1	22.2±4.1	24.2±0.9
6	55.8±0.2	64.5±0.2	19.2±3.7	22.6±0.4

$$C_p^{ex} = \frac{\Delta H E_{app}}{R T_m^2} \exp\left(\frac{E_{app}\Delta T}{R T_m^2}\right) \cdot \left\{1+\frac{(1-\mu)}{\mu}\exp\left(\frac{E_{app}\Delta T}{R T_m^2}\right)\right\}^{\frac{1}{\mu-1}} \quad (1)$$

where E_{app} is apparent activation energy, R is gas constant, μ is the change in molecularity and $\Delta T = T - T_m$.

Minimization of the sum of the squared deviations between experimental and calculated C_p^{ex} with use of Origin software let us to find E_{app} and μ for which the best fit to the experimental data was achieved. The fits of the heat capacity profiles obtained are shown in Fig. 4.

Calculated E_{app}: 1.3±0.1 kJ mol^{-1} for atazanavir sulfate and 3.6±0.2 kJ mol^{-1} for nelfinavir mesylate pointed at low stability of the samples. Obtained values of μ were comparable for both drugs (≈0.13±0.04).

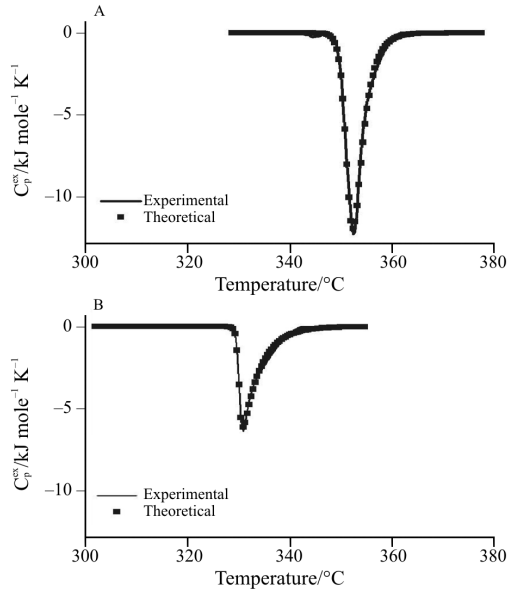

Fig. 4 Results of fitting of Eq. (1) to the experimental DSC profiles of A – atazanavir sulfate (E_{app}=1.3 kJ mol^{-1}, μ=0.14) and B – nelfinavir mesylate (E_{app}=3.6 kJ mol^{-1}, μ=0.13). The solid line is experimentally obtained DSC curve and the dotted line is its fit. The concentration of the solution was 1.36 mM L^{-1} and pH was 5.0 for atazanavir sulfate and 7.0 for nelfinavir mesylate

Knowing values of E^{app} it was possible to calculate apparent rate constant k_{app} and half life time τ taking as a reference temperature of the human body=37°C.

Obtained values of k_{app} and τ amount to: 0.2±0.1 min^{-1} and 1.1±0.7 min respectively for atazanavir sulfate and 0.6±0.2 min^{-1} and 2.8±0.7 min respectively for nelfinavir mesylate. Kinetic parameters of nelfinavir mesylate were somewhat higher than for atazanavir sulfate, what suggest its larger stability in water solution.

Conclusions

The DSC studies of protease inhibitors: atazanavir sulfate and nelfinavir mesylate showed that during heating solutions underwent irreversible, exothermic process in studied temperature range. Thermal degradation of studied PIs was scan rate and concentration dependent especially marked in the case of atazanavir sulfate. These processes were well-described by the two-step Lumry–Eyring model with fast equilibrating first step and the irreversible second. Obtained values of apparent activation energy and rate constant pointed at low stability of studied drugs. Results indicate caution in storage of studied drugs and in planning treatment.

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DOI: 10.1007/s10973-006-8128-7