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Thermal degradation of *N*-(*o*-carboxybenzoyl)-L-amino acids

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Abstract

The thermal degradation of *N*-(*o*-carboxybenzoyl)-L-phenylalanine (NCBPh), *N*-(*o*-carboxybenzoyl)-L-leucine (NCBL) and *N*-(*o*-carboxybenzoyl)-L-valine (NCBV) were studied by thermogravimetry and infrared spectroscopy. The results suggested that the loss of mass occurred by two main events which were defined as the imide formation and sublimation. The values of the activation energy (*E*) for the imide formation, considering both systems, were at the range 170–60 kJ mol^{-1} and the results indicate that thermal stability obeys the order: NCBPh > NCBV > NCBL. The apparent activation energies obtained for the sublimation process are consistent with the structure of the imides *N*-Phthaloylphenylalanine (PhtPh), *N*-phthaloylvaline (PhtV) and *N*-phthaloylleucine (PhtL). The values of *E* are in the order PhtPh > PhtV > PhtL. This higher energy for the sublimation of the phenylalanine derivative, may be associated to the presence of the phenyl group, which favours the crystalline packing. In a sealed tube at higher temperature, we detected the degradation of the imide, with the concurrent appearance of CO_2 (2370 and 2340 cm^{-1}), CO (2200–2100 cm^{-1}).

Keywords: Amino acids; Thermal degradation; Kinetic parameters

1. Introduction

A series of optically active *N*-(*o*-carboxybenzoyl)-amino acids were studied as simple models of aspartic proteinases in hydrolysis reactions [1,2]. The main interest in the study of this class of hydrolytic enzyme models is to find a proper mechanistic understanding of the role of the carboxyl groups in the intramolecular reactions of ester and amide hydrolysis. Indeed, the hydrolysis of the amide bond of phthalamic [3] and maleamic [4,5] acids and their derivatives [6,7] have been demonstrated to include neighbouring carboxyl group's participation.

Although there are various works related with the kinetic study of hydrolysis reactions of amino acids derivatives catalysed, in general, by enzymes, we can't find much information about the thermal stability of this compounds. Recently, some studies analysed the problem of the thermal stability of small biomolecules during their extraterrestial delivery [8,9]. The authors simulated a experiment in order to make rough estimates of the temperatures under which amino acids such as glycine, β-alanine, valine, leucine, proline, phenylalanine, etc., can survive during atmospheric entry heating of organics-bearing space bodies in inert atmosphere (nitrogen). The percentage of the recovery of the amino acids was determined by high-performance liquid chromatography (HPLC) and the results indicated at 400 °C values in the range 10–13% for β-alanine and valine, 3–4% for leucine and <1.5% for proline and phenylalanine. The thermal degradation of cytochrome c has been studied in presence of various concentrations of L-amino acids that are more hydrophobic than glycine [10]. The authors observed that arginine and histidine desestabilize the native protein, while isoleucine, leucine and phenylalanine have no effect on the thermal transition and valine and less hydrophobic amino acids stabilize the protein. Poly(amide imide)s based on bis(*p*-amidobenzoic acid)-*N*-trimellityimido-L-leucine was prepared and characterized by Mallakpour et al. [11]. The novel material was optically active and had moderate

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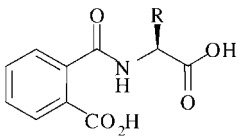
thermal stability. Thermal stability was used also for characterizing interactions of different domains in the secondary structure of human phenylalanine hydroxylase [12].

The aim of this work was to analyse the thermal stability of a series of optically active *N*-(*o*-carboxybenzoyl)-amino acids (I) with R equal to $-CH_2C_6CH_5$ (phenylalanine, NCBPh), $-CH_2CH(CH_3)_2$ (leucine, NCBL) and $-CH(CH_3)_2$ (valine, NCBV) in inert atmosphere by thermogravimetry (TG) and infrared spectroscopy (FTIR). The kinetic parameters, such as activation energy and pre-exponential factor, were determined by the Ozawa method [13,14].



R = $CH(CH_3)_2$ (NCBV)

= $CH_2CH(CH_3)_2$ (NCBL)

= $CH_2C_6H_5$ (NVBPh)

(I)

2. Experimental

The synthesis of the compounds *N*-(*o*-carboxybenzoyl)-L-phenylalanine (NCBPh), *N*-(*o*-carboxybenzoyl)-L-leucine (NCBL) and *N*-(*o*-carboxybenzoyl)-L-valine (NCBV) has been previously described [2,15].

DSC thermograms were obtained using a Shimadzu model DSC-50 equipment, heating from 100 to 200 °C at 10 °C min^{-1}. In order to eliminate the thermal history, in all measurements two scans were performed, using always the second to determine the transition values. The average sample size was 5 mg and the nitrogen flow rate 25 cm^3 min^{-1}.

Thermogravimetric measurements were carried out using a Shimadzu model TGA-50 equipment. Non-isothermal experiments were performed in the temperature range 25–500 °C at different heating rates (5, 10 and 20 °C min^{-1}) for each sample. The average sample size was 5 mg and the nitrogen flow was maintained at 25 cm^3 min^{-1}. The thermogravimetric data were analysed using the Ozawa method [13,14], and the parameters were determined using the associated TGA-50 software. The activation energy was derived from the slope of the dependence of the heating rate upon the reciprocal absolute temperature, at defined mass loss.

Infrared spectra (FTIR) were obtained on a Perkin Elmer, model 16PC, spectrometer using resolution of 4 cm^{-1}. For the FTIR analysis of the solid residues, samples (ca. 12 mg) submitted to degradation in the thermal gravimetric analyzer (TGA) at the required temperature were used. The solid residues were cooled to room temperature and the FTIR measurements carried out in KBr. For analysis of the evolved gas products, samples with ca. 200 mg were heated in a tubular oven connected to the FTIR in which the spectra were directly performed at different temperatures. In the tubular oven the nitrogen flow was maintained at 50 cm^3 min^{-1}.

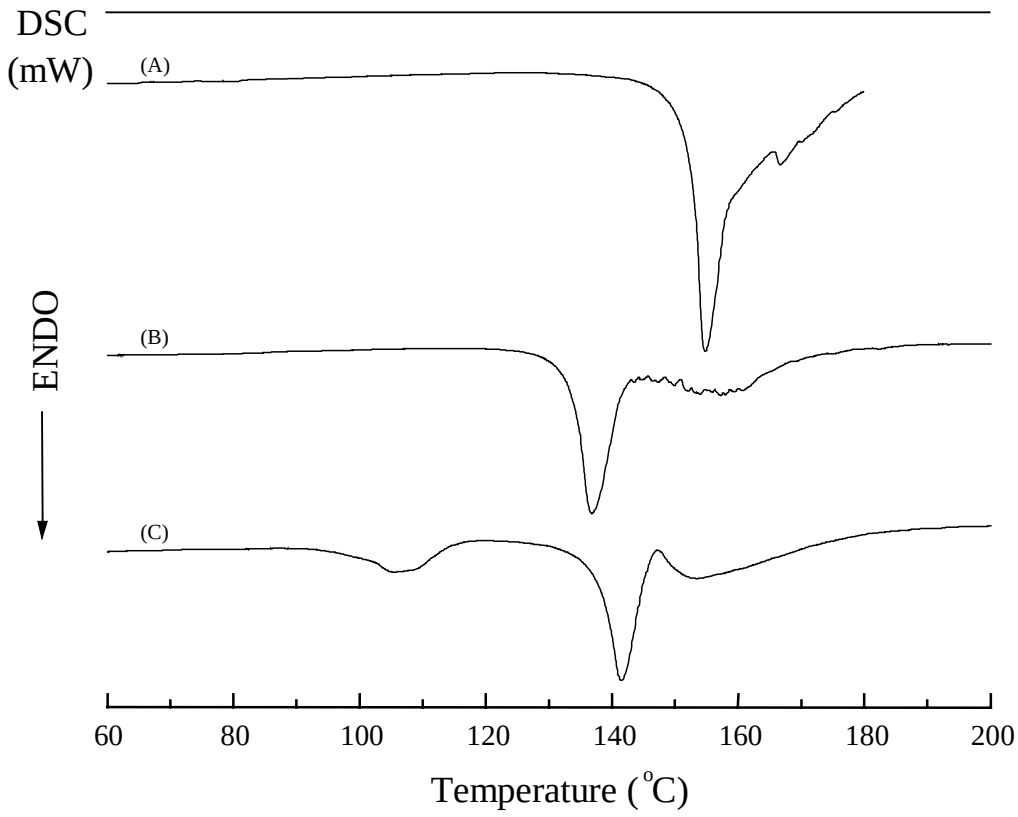


Fig. 1. DSC curves for (A) NCBV, (B) NCBL and (C) NCBPh obtained at a heating rate of 10 °C min^{-1}.

3. Results

3.1. Thermal analysis behaviour

The DSC curves of samples of the *N*-(*o*-carboxybenzoyl)-L-amino acids NCBV, NCBL and NCBPh are shown in Fig. 1. A similar behaviour was observed for both NCBL and NCBV, showing only one endothermic peak that corresponds to the melting temperatures (T_m) of NCBL and NCBV at 137 and 146 °C, respectively. For the NCBPh, however, two endothermic peaks are in evidence, one at 100 °C which corresponds to hydration water and other at 140 °C associated with the melting temperature. The presence of water in the crystalline NCBPh sample is fully consistent with the elemental analysis reported by Onofrio et al. [15]. The profile of the DSC curves for both amino acids above the T_m indicated that the thermal degradation begins, in agreement with the thermogravimetric analysis discussed below.

The thermogravimetric curves (TG) obtained at a heating rate of 10 °C min^{-1} and the corresponding derivatives (DTG) for the *N*-(-o-carboxybenzoyl)-L-aminoacids are shown in Fig. 2. Two stages of mass loss were observed for NCBV and NCBL, and the maximum temperatures of mass loss were found to be 182 and 293 °C for the NCBV sample and 168 and 305 °C for the NCBL. In general, the presence of more than one stage of mass loss is associated to changes of the mechanism during thermal degradation. For the NCBPh sample, however, three stages of mass loss were observed and the corresponding values of T_{max} were found to be 124, 190 and 352 °C. The mass loss stage with $T_{max} = 124$ °C is consistent with the loss of the hydration water which was confirmed as present by elemental analysis [15] and by DSC in this work. Similarly to the results described for NCBV and NCBL, two stages of mass loss at values of T_{max} of 190 and 352 °C were obtained for the NCBPh derivative. The residual mass at 400 °C was in the range 2–4% for all the amino acid derivatives studied in the present work (Table 1). Our results are consistent with the data reported by Douda and Basiuk [9], which determined by pyrolysis studies 3.5% of amino acid recovery for pure leucine at 400°C and in nitrogen atmosphere. The same authors determined for pure valine 13% of amino acid recovery at the same conditions.

Table 1
Thermogravimetric and kinetic parameters for the sublimation of the phthaloyl aminoacids

Phthaloyl aminoacid	T_{max} (°C)[a]	E (kJ mol^{-1})[b]	log Z	Residue (%)[c]
NCBV	293	90.2	8.0	4
NCBL	305	68.7	5.8	2
NCBPh	352	107.4	8.8	4

[a] Determined from the DTG of the Fig. 2.
[b] Average values considering the α range 0.2–0.8 from Fig. 5. The standard deviation was lower than 2%.
[c] Values determined at 400 °C in a heating rate of 10 °C min^{-1}.

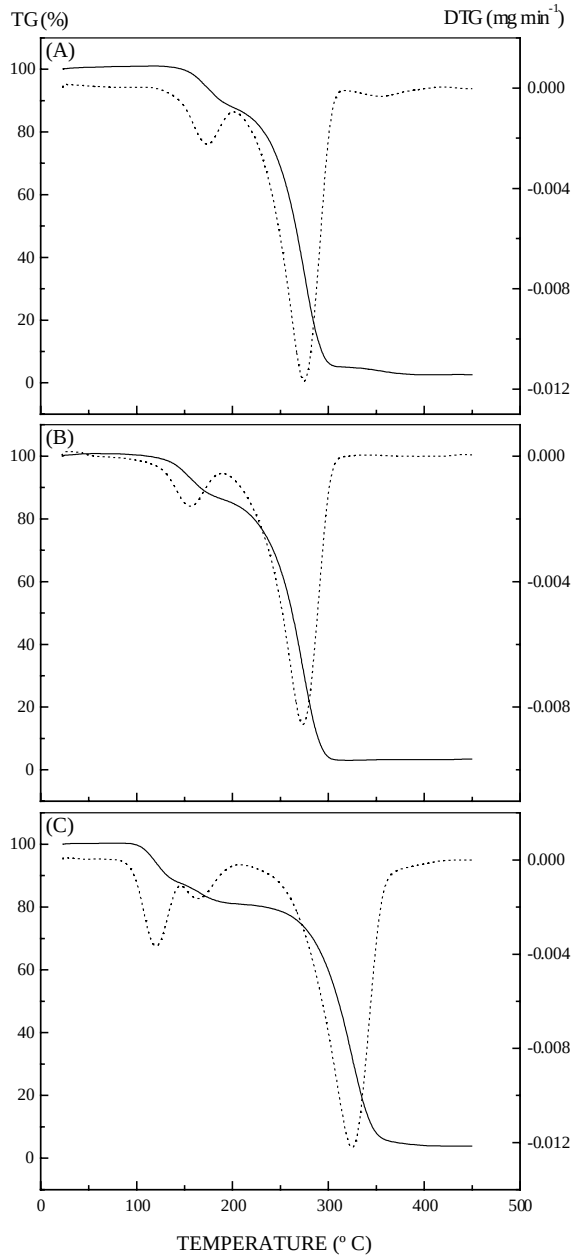


Fig. 2. TG (—) and DTG (···) curves for (A) NCBV, (B) NCBL and (C) NCBPh obtained at a heating rate of 10 °C min^{-1}.

3.2. Spectroscopic data

The FTIR and NMR spectra for samples of the studied *N*-(*o*-carboxybenzoyl)-L-amino acids heated at different temperatures showed identical behavior. For this reason, are shown in this work only the spectra of the residues and gas products at different heating temperatures for *N*-(*o*-carboxybenzoyl)-L-leucine (NCBL) (Figs. 2 and 3, respectively). The spectral data for NCBL obtained at room temperature, and the corresponding assignments, were summarized in Table 2.

As shown in Fig. 3, at 100 °C the IR spectra was identical to that observed at room temperature (Table 2). At 180 °C

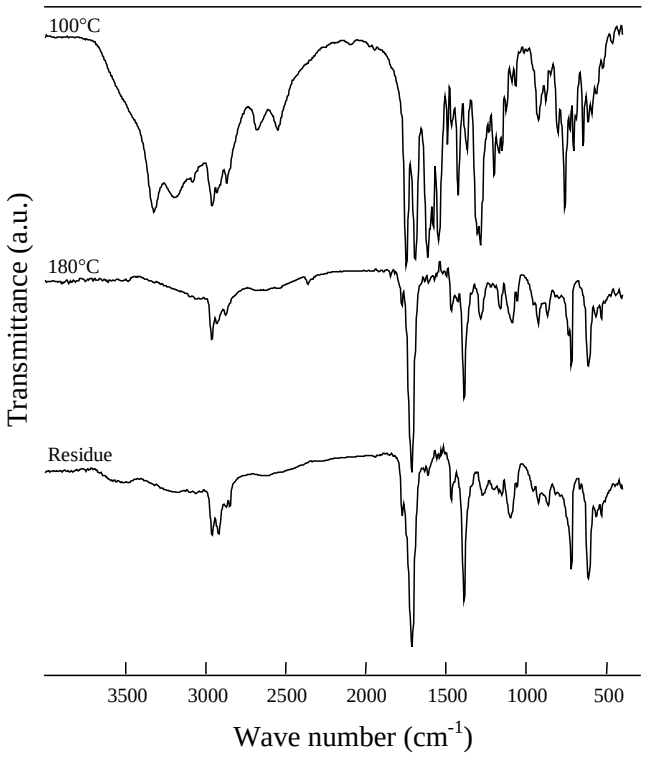


Fig. 3. FTIR spectra for the residues of NCBL during thermal degradation at indicated temperatures.

the bands corresponding to the NCBL derivative disappeared and the observed IR and NMR signals were identical with those of an authentic cyclic imide, *N*-phthaloylleucine [2,15]. Cyclic imides were described, for example, by

Table 2
Spectroscopic data for NCBL at room temperature

IR (KBr), ν_{max} (cm^{-1})	3300 (br NH), 3200–2500 (OH), 1750 and 1690 (CO_2H), 1616 (CONH)
^1H NMR (DMSO-d_6, 200 MHz), δ (ppm)	12.80 (1H, br s, CO_2H, exchange with D_2O), 8.63 (1H, d, *J* 8.00 HZ, N*H*, exchange with D_2O), 7.78-7.39 (4H, m, ArH), 4.40-4.30 (1H, m, NC*H*), 1.70 (3H, m, C*HCH*$_2$), 0.98 (6H, d, *J* 6.37 Hz, (C*H*$_3$)$_2$)
^{13}C NMR (DMSO-d_6, 50.3 MHz), δ (ppm)	174.07, 168.31, 167.89 (C=O), 138.05, 131.04, 130.80, 129.18, 129.06, and 127.76 (Ar), 50.51 (CHNH), 39.79 (CH_2); 24.16 (CH), 23.01 and 21.24 ((CH_3)$_2$)

Levchik et al. [16] as a products in the heated process of polycaprolactam (polyamide 6). The authors suggested that the polyamide 6 formed cyclic oligomers in the melt. Practically, the same absorption bands observed at 180 °C remained in the spectra at 320 °C. This fact suggested that the chain scission and consequently the formation of evolved products and lower hydrocarbons occurred only at temperatures above 320 °C, as confirmed by the evident CO_2 formation, showed in the FTIR spectra at 350 °C in Fig. 4.

The infrared spectra for the gas products evolved from the degradation reaction of the NCBL are shown in Fig. 4. Practically no gas products were observed up to 300 °C, confirming that the first stage of degradation (T_{max} =168 °C) was associated with the formation of the cyclic structure (imide) by loss of water (not detected in the spectra) followed by sublimation (see above). At 350–450 °C, two absorption bands corresponding to CO_2 (2370 and 2340 cm^{-1}), associated with the decarboxylation of the *N*-phthaloylleucine initially

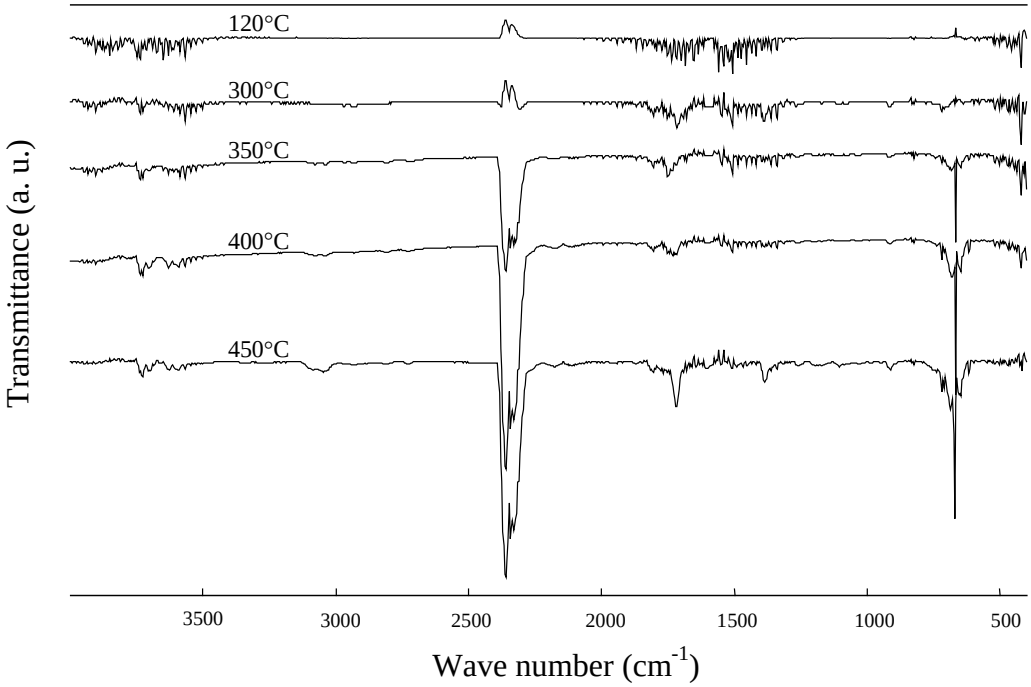


Fig. 4. FTIR spectra for the volatile products of NCBL during thermal degradation at indicated temperatures.

formed from NCBL, were observed. Data from the literature indicated the formation of several carboxylic acids, primary and secondary amides in the pyrolysis of leucine and valine [9,17]. In the present work, the analysis of the FTIR spectra shown in Fig. 4, indicated that the major evolved products were CO_2 and hydrocarbons.

4. Discussion

The kinetic parameters (activation energy and pre-exponential factor) for the thermal degradation of the compounds in the solid state were determined from the thermogravimetric curves by the Ozawa method, which relates the Arrhenius equation with the weight loss in the thermal degradation (Eq. (1)). In this equation α represents the weight

$$\frac{d\alpha}{dt} = Z\,e^{-ERT}(1-\alpha)^n \quad (1)$$

loss fraction, t is the time, E is the activation energy, Z is the pre-exponential factor, R the gas constant, and n the reaction order.

The experimental values of E for the compounds NCBV, NCBL and NCBPh versus the weight-loss fraction (α) are shown in Fig. 5. For all amino acids, two different behaviours in the E curves, were observed. Initially, for low values of α, up to ≈ 0.2, the values of E varied approximately in the range 170–110 kJ mol^{-1} for the NCBPh, 120–80 kJ mol^{-1} for the NCBV and 100–60 kJ mol^{-1} for the NCBL. These values are associated with the thermodinamically favoured dehydration reaction stages which showed $T_{max} = 190\,^{\circ}C$ (NCBPh), $T_{max} = 182\,^{\circ}C$ (NCBV) and $T_{max} = 168\,^{\circ}C$ (NCBL), and resulted in the formation of the corresponding imides. These results are in agreement with the cyclization of the aminoacid derivatives in acid solutions. Indeed, the intramolecular cyclization reaction of *N*-(*o*-carboxybenzoyl)-L-Leucine results in the formation of the *N*-phthaloylleucine, and the reaction depends strongly on the acidity of the solution, becoming important and linearly dependent on the acidity for Ho < -1 (Ho is known as the Hammett acidity function and is used to define the acidity of strong acids-water mixtures) and the dominant reaction in the high acidity region (Ho < -3). The differences of reactivity between the amides were attributed to the structural changes [2,15]. On the other hand, in the region of α between 0.2 and 0.8, which corresponds to the main stage of mass loss, the values of E were practically constant for each amino acid derivative. The observation of constant E-values, in this α-range, is indicative of systems which follow adequately Eq. (1) and where n and Z are constant in the above described range. Thus, the reaction order determined by the Ozawa method, considering the Eq. (1), was constant and equal to the unity for the compounds NCBL, NCBV and NCBPh.

In an independent experiment, we trapped the compounds responsible for the mass loss, and infrared spectra of the trapped compounds showed to be identical to the corresponding imides and to the spectra of the mass residue that remains in the thermogravimetric experiment. Thus, clearly the observed phenomena corresponds to the sublimation of the imides formed in the dehydration step. The kinetic parameters determined during the main stage of mass loss are

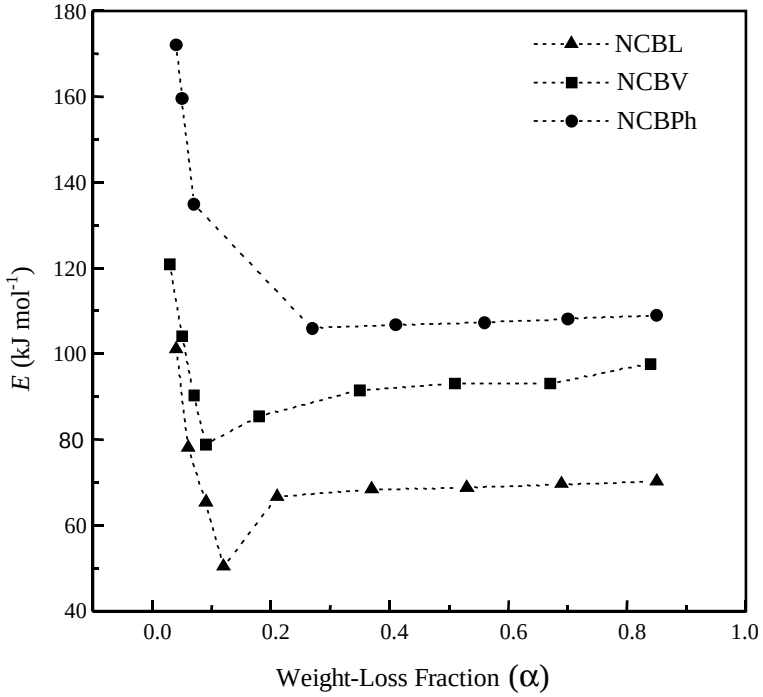


Fig. 5. Plots of activation energy vs. weight-loss fraction for the studied *N*-(*o*-carboxybenzoyl)-L-amino acids.

summarized in Table 1, and the reported E values corresponds to the average calculated in the α range 0.2–0.8. In all cases, values of E lower than 110 kJ mol^{-1} were obtained, which are consistent with those expected for sublimation phenomena. The apparent activation energies obtained for the sublimation processes are consistent with the structure of the imides and values of E are in the order PhtPh > PhtV > PhtL. This higher energy of sublimation for the phenylalanine derivative, may be associated to the presence of the phenyl group, which favours the crystalline packing. Curiously, these results are also in agreement to the solution kinetic studies (hydrolysis reaction) in which the difference in terms of the rate constant was attributed to the structural changes [2]. The pre-exponential factor Z follows a similar trend to that of the E values and Z decreases in the order PhtPh > PhtV > PhtL.

5. Conclusions

The thermal stability studies of the *N*-(*o*-carboxybenzoyl)-amino acids showed two main mass loss stages which were defined as a dehydration reaction to form the corresponding *N*-phthaloyl aminoacids, which was followed by sublimation of the cyclic imides. The imide formation occurred at low temperatures (ca. 200 °C) for all systems, and in agreement to the thermogravimetric curves, the *E*-values indicated the following order of thermal stability: NCBPh > NCBV > NCBL. The *E*-values, in each case, were higher than those obtained for the sublimation process, which were lower than 110 kJ mol^{-1}.

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