J Therm Anal Calorim (2014) 118:1033–1038
DOI 10.1007/s10973-014-3847-7

Thermal degradation of B-group vitamins: B_1, B_2 and B_6

Kinetic study

Adriana Fuliaş · Gabriela Vlase · Titus Vlase · Daniela Oneţiu · Nicolae Doca · Ionuţ Ledeţi

Received: 1 November 2013 / Accepted: 6 May 2014 / Published online: 22 June 2014

Abstract The thermal behaviour of vitamins B_1, B_2 and B_6 under non-isothermal conditions and dynamic air atmosphere was studied. According to our study, it was determined that the most stable compound is vitamin B_2. A kinetic analysis of the thermodegradation process using four different data processing methods (Friedman, Flynn–Wall–Ozawa, Kissinger–Akahira–Sunose and modified NPK) was performed. The NPK was the only method that made it possible to evaluate the contribution to the reaction rate of the temperature and conversion, respectively. The results obtained from kinetic analysis were corroborated with the molecular architecture of the studied compounds.

Keywords Thermal behaviour · Vitamin B · Kinetic analysis · Modified NPK method

A. Fuliaş · I. Ledeţi
Faculty of Pharmacy, University of Medicine and Pharmacy "Victor Babeş", Eftimie Murgu Square 2, Timisoara 300041, Romania

G. Vlase (✉) · T. Vlase · N. Doca
Research Centre for Thermal Analysis in Environmental Problems, West University of Timisoara, Pestalozzi Street 16, Timisoara 300115, Romania
e-mail: gabriela.vlase@cbg.uvt.ro

T. Vlase · N. Doca
SCIENT – Research Center for Instrumental Analysis (S.C. CROMATEC_PLUS S.R.L.), 18 Sos.Cotroceni, Bucharest 6 060114, Romania

D. Oneţiu
Faculty of Medicine, University of Medicine and Pharmacy "Victor Babeş", Eftimie Murgu Square 2, Timisoara 300041, Romania

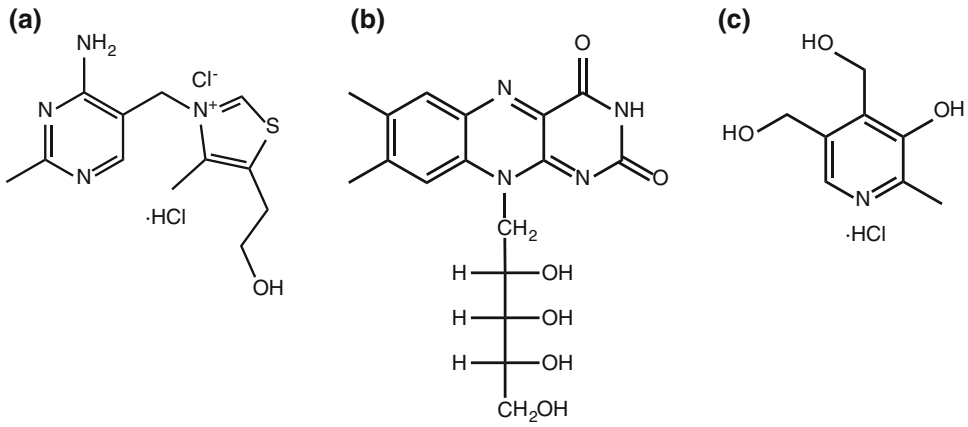
Introduction

Vitamins are organic compounds that are necessary for metabolism, generally in small amounts. Vitamins are usually classified by their biological and chemical activities and not their molecular structure. By now, there are thirteen vitamins universally recognized, namely nine water soluble (B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12} and C) and four liposoluble (A, D, E and K).

Vitamins of B-group play important roles in the human organism: vitamin B_1 plays a central role in energy production, vitamin B_2 is essential in Szent-Györgyi–Krebs cycle and beta-oxidation of fatty acids and vitamin B_6 is important for the metabolism of lipids and amino acids [1, 2]. The structural formulas of the analysed vitamins are presented in Scheme 1.

High concentration of vitamin B_1 is found in yeast, wheat germ, pork, brown rice, vegetables, potatoes, liver and egg yolk. Generally, manufactured food presents a lower quantity of thiamine [2]. The main sources for vitamin B_2 are dairy products (milk and cheese), fruits and vegetables (bananas, green beans and leafs), almonds, etc. and for vitamin B_6 (as pyridoxine) are soybeans and beans, bananas, walnuts, brown rice, potatoes, etc. Rucker et al. described extensively the biosynthesis, occurrence in food, absorption and transport, diseases associated with deficiency of these vitamins, and the effect of exposure to high doses [2]. Even if the influence of several factors such as pH, moisture, oxidative atmosphere/oxygen, radiation/light and temperature over the stability of vitamins was described, a study regarding the thermal behaviour and kinetic analysis of the decomposition process was not realised, according to our knowledge. The vitamin degradation can occur during food or pharmaceutical product processing and formulation, but as well during storage under

Scheme 1 Structural formulas of the analysed vitamins: **a** thiamine hydrochloride (vitamin B_1), **b** riboflavin (vitamin B_2) and **c** pyridoxine hydrochloride (vitamin B_6)



impropriate conditions. According to these premises, we set our goal in the study of thermal behaviour of three vitamins from B-class, namely B_1, B_2 and B_6, and as well for the analysis of their kinetic degradative process.

The study of thermal behaviour of pharmaceutical active substances is essential for the estimation of the possible interactions that can occur in solid dosage forms [3–5]. These estimations can be obtained by various methods, but of great importance and reproducibility are the methods that are based on kinetic data, including the ones obtained under non-isothermal conditions.

In our previous studies [6–13] we realised both kinetic analysis regarding the thermal stability of pharmaceuticals and compatibility studies of active pharmaceutical ingredients (API) with different excipients. The differences obtained for the kinetic parameters were easily correlated with the differences in the molecular architecture of API.

In the present paper, we applied a similar strategy for the kinetic analysis, but on significantly different molecular structures (see Scheme 1), even if the physiological role of these compounds are comparable.

Materials and methods

The vitamins are commercial products and used as received without further purification. The vitamins B_1 (purity >99 %) and B_6 (purity >99 %) were obtained from Acros Organics, Geel, Belgium and vitamin B_2 (purity >98 %) was obtained from Fisher Bioreagents, Geel, Belgium. The samples were kept in a desiccator during the study to avoid moisture absorption.

TG/DTG/HF measurements were performed on a Perkin-Elmer DIAMOND TG/DTA instrument. The experiments were carried out using about 7 mg of sample which was weighted into an open aluminium crucible. The furnace temperature was programmed to rise under non-isothermal conditions from ambient temperature to 550 °C linearly at a heating rate of 10 °C min^{-1}. The experiments were completed in a synthetic air atmosphere at a flow rate of 100 mL min^{-1}. For kinetic analysis, the TG/DTG/HF data obtained at heating rates $\beta = 5$, 7, 10, 12 and 15 °C min^{-1} were used.

Results and discussion

1) Thermoanalytical data

Figure 1a–c present the thermoanalytical curves TG, DTG and HF.

Thermal decomposition of the studied vitamins in air atmosphere occurs at temperatures higher than 200 °C with $DTG_{peak} = 246$ °C for B_1, 296 °C for B_2, and 212 °C for B_6, respectively. For all the three vitamins, the thermodegradation process is an endothermic one with mass loss of 43.9 % for B_1, 42.4 % for B_2 and 21.6 % for B_6, respectively.

Kinetic analysis of the non-isothermal data

The reaction rate r defined by

$$r = \mathrm{d}\alpha/\mathrm{d}t \quad (1)$$

becomes

$$r = \beta(\mathrm{d}\alpha/\mathrm{d}T) \quad (2)$$

in non-isothermal conditions, where β is the heating rate and the temperature T depends on time (t) by $T = T_0 + \beta t$. In Eq. (2), $\beta(\mathrm{d}\alpha/\mathrm{d}T)$ represents the value from the DTG curve at the temperature T.

According to the generally accepted hypothesis, the reaction rate can be expressed as a product of two separable functions, one depending on temperature, $k(T)$, another on conversion, $f(\alpha)$, respectively:

$$r = k(T)f(\alpha). \quad (3)$$

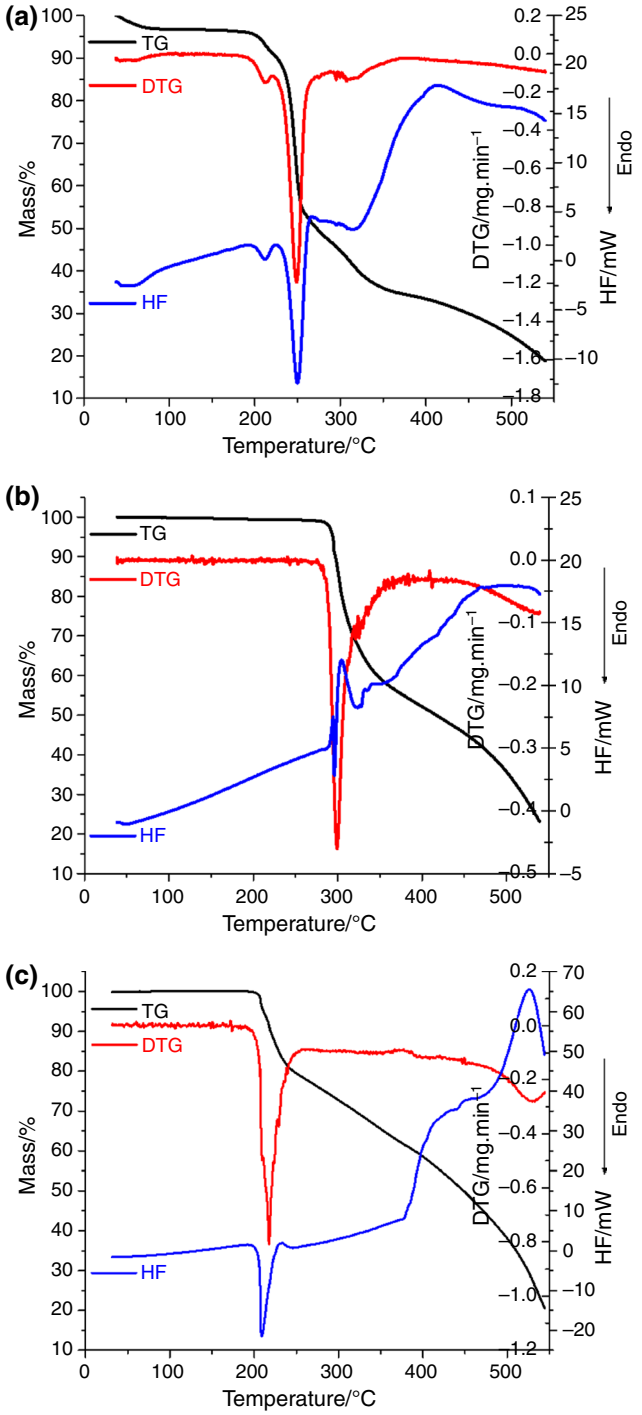


Fig. 1 TG/DTG/heat flow obtained in air at 10 °C min^{-1} for **a** vitamin B_1; **b** vitamin B_2; **c** vitamin B_6

With Eq. (2), it becomes

$$\beta(\mathrm{d}\alpha/\mathrm{d}T) = k(T)f(\alpha). \tag{4}$$

Accepting an Arrhenius dependence on temperature, Eq. (4) can be rewritten as

$$\ln[\beta(\mathrm{d}\alpha/\mathrm{d}T)]_\alpha = \ln[Af(\alpha)] - E/RT. \tag{5}$$

This is the Friedman (FR) method [14] for determining the activation energy by plotting the left member of Eq. (5) versus $1/T$, at different values of conversion degree (α). So the Friedman method is a differential isoconversional model-free method ('model free' because the conversion function $f(\alpha)$ is not explicit described).

The results are presented in Fig. 2. The standard deviation from the mean value does not exceed 10 %, this being an indication of a single-step process.

If Eq. (4) is integrated, it becomes

$$\beta g(\alpha) = \int k(T)\mathrm{d}T \tag{6}$$

with

$$g(\alpha) = \int \mathrm{d}\alpha/f(\alpha). \tag{7}$$

Equation (6) is a starting point for a series of isoconversional integral and model-free methods. The analytical forms depend on the approximation used for solving the temperature integral ($\int k(T)\mathrm{d}T$). We used two well-known methods:

- The Fynn–Wall [15] and Ozawa [16] method (FWO) based on the equation:

$$(\ln\beta)_\alpha = \ln[AE/(Rg(\alpha))] - 5.331 - 1.052E/(RT). \tag{8}$$

- The Kissinger–Akahira–Sunose [17, 18] method (KAS), with the equation:

$$\ln(\beta/T^2) = \ln[AE/(Rg(\alpha))] - E/(RT). \tag{9}$$

Figures 3 and 4 present the results obtained by applying these methods.

By both the integral methods, the standard deviation of E versus α does not exceed 10 %, this being an indication of a single-step process in these cases, too.

The non-parametric kinetics (NPK) method [19–23] represents a special approach for processing the kinetic data. The method introduces a new point of view in kinetic analysis which is based on the single-step kinetics approximation. The experimental values of reaction rates are arranged in a matrix which is expressed as a product of two vectors containing information on $k(T)$ and $f(\alpha)$. As a matter of fact, this mathematical model is a consequence of Eq. (3). The most important feature of the method is that it enables to decouple the submatrix related to the temperature (V) and conversion functions (U), without the need of any assumptions about their functionality. The data were obtained by analysing the vector u (the first column of U) in respect to a kinetic model suggested by Šesták and Berggren [24]: $f(\alpha) = \alpha^m(1-\alpha)^n$, respectively, the vector v (the first column of V) for an Arrhenius type T dependence. The value of the explained variance, λ, expresses the contribution of each simultaneous steps to the whole

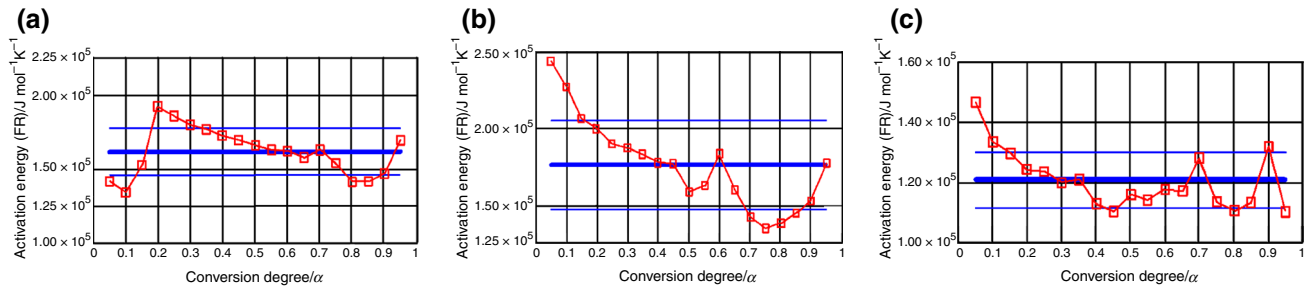


Fig. 2 Activation energy (E) versus α by Friedman's method for vitamins: **a** B_1, **b** B_2 and **c** B_6

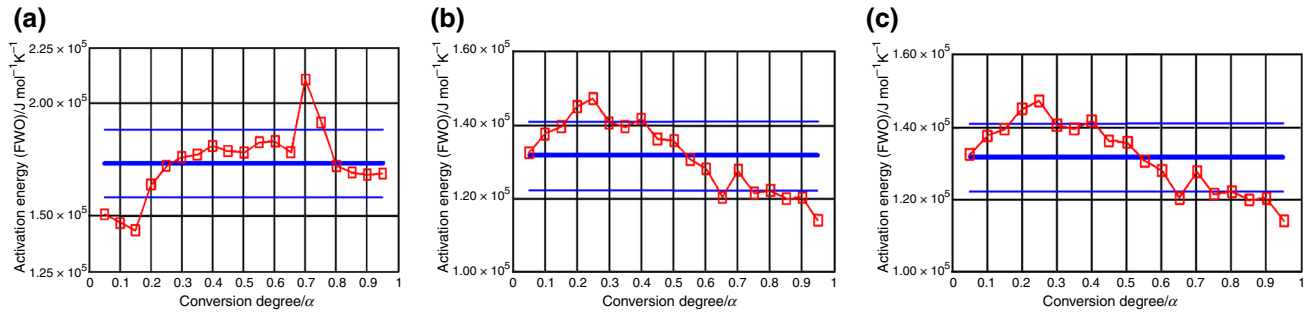


Fig. 3 Activation energy (E) versus α by FWO method for vitamins: **a** B_1, **b** B_2 and **c** B_6

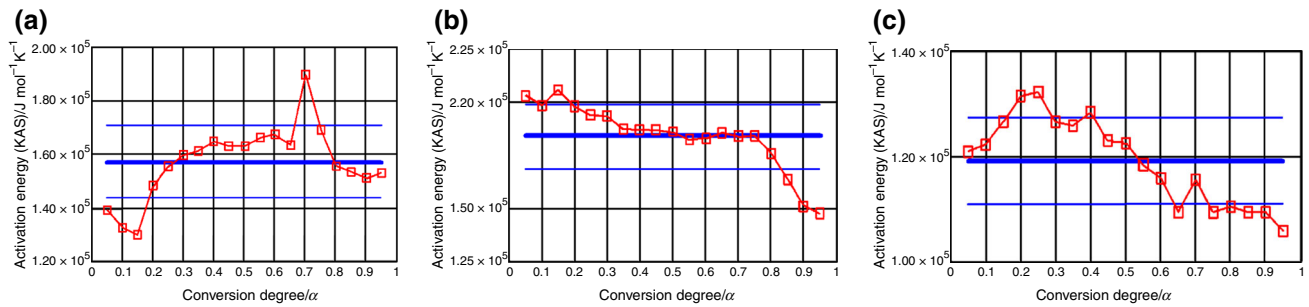


Fig. 4 Activation energy (E) versus α by KAS method for vitamins: **a** B_1, **b** B_2 and **c** B_6

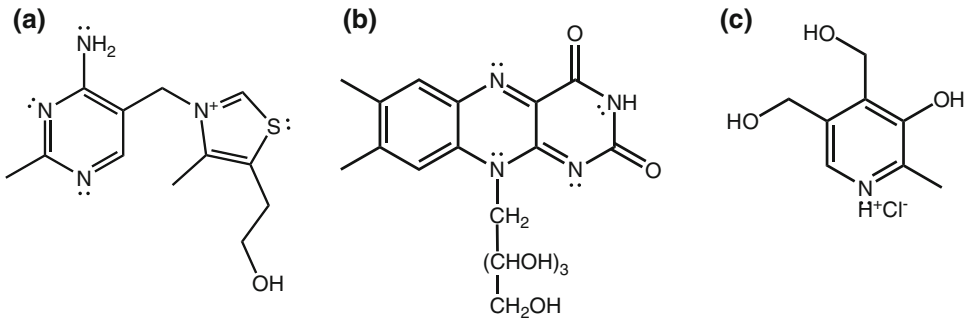
Table 1 Kinetic analysis for the three vitamins, the NPK method

Vitamin	Process	λ/%	E/kJ mol^{-1}	A/s^{-1}	n	m	Šesták–Berggren eq.	$\bar{E}$/kJ mol^{-1}
B_1	1	92	154.6 ± 11.1	2.99×10^{16}	1	–	$(1-\alpha)$	156.3 ± 10.4
	2	8	175.4 ± 1.9	4.54×10^{18}	–	1	α	
B_2	1	87.8	179.7 ± 13.0	2.12×10^{16}	2/3	–	$(1-\alpha)$	181.8 ± 14.1
	2	12.2	197.8 ± 13.7	9.10×10^{17}	–	2	α^2	
B_6	1	65.1	119.2 ± 3.8	1.38×10^{13}	4/5	1/3	$(1-\alpha)^{4/5} \cdot \alpha^{1/3}$	120.0 ± 9.7
	2	33.4	127.1 ± 2.34	8.58×10^{13}	–	2	α^2	

The data systematized in Table 2 are useful for discussions on the results of the kinetic analysis

thermodegradation process, so that $\Sigma\lambda_i = 100$ %. If $\lambda < 10$ %, we consider that the discussed step can be neglected.

The results on NPK method are systematized in Table 1. The data indicate that the differences in the thermal behaviours of the three vitamins are due to both the different temperature dependences (A and E values), and the different conversion dependences (m and n parameters). Also, different thermal behaviours were observed: by B_1 it is clear that there is only one significant process depending



Scheme 2 The aromaticity evaluation of the analysed vitamins: **a** thiamine hydrochloride (vitamin B_1), **b** riboflavin (vitamin B_2) and **c** pyridoxine hydrochloride (vitamin B_6)

Table 2 The main values of the activation energy obtained by the four kinetic methods

Method	$\bar{E}$/kJ mol^{-1}		
	B_1	B_2	B_6
FR	161.3 ± 3.7	179.4 ± 6.6	131.5 ± 2.2
FWO	172.8 ± 3.6	206.2 ± 5.2	121.5 ± 2.1
KAS	157.0 ± 3.2	207.2 ± 5.5	130.1 ± 2.4
Modified NPK	156.3 ± 10.4	181.8 ± 14.1	120.0 ± 9.7

on the reactant $(1 - \alpha)$, in case of B_6 there are two parallel processes, with 65 and 33 % contributions to the observed thermal behaviour.

Whatever used kinetic method would be analysed, the values of the activation energy indicate that vitamin B_2 presents the higher thermal stability by the three studied vitamins. The use of certain value determined for activation energy in the estimation of the thermal stability is risky. Even if the standard deviation of E values obtained by certain methods exceed 15 % from the mean value for each compound, it is reasonable to avoid any quantitative comparisons using only the model-free methods. In our case, it is certain that the thermal stability decreases in order:

$$B_2 > B_1 > B_6.$$

The NPK method assures the obtaining of realistic kinetic parameters, even if the decomposition process is a complex one, due to the fact that all kinetic parameters (A, E, m and n) were obtained without any approximations regarding the temperature and the conversion integral.

The high thermal stability observed for vitamin B_2 was somehow unexpected due to the presence of the carbohydrate moiety in the molecular structure, although the increased stability can be explained by the presence of *N*-heterocyclic aromatic benzo[*g*]pteridine moiety, which is known to be a stabilizing structure. Furthermore, the degree of aromaticity of vitamin B_2 is considerably higher than that of B_1 and B_6, respectively. It is known that double bonded nitrogen and/or oxygen can also be part of a conjugated structure, as well as single bonded due to the lone pair orbital which can become part of an extended conjugated aromatic system. It can be noticed that in the case of vitamin B_2, a conjugated system containing 16 atoms is formed. As a further simple proof for the extended conjugation and high aromaticity of this vitamin is the fact that it absorbs light complementary to yellow in the visible spectrum.

For the case of vitamin B_1, a lower degree of aromaticity is expected. This vitamin is formed by two aromatic moieties, namely 4-amino-2-methyl-pyrimidin-5-yl and methyl-thiazolyl nuclei. The extended conjugation is, however, interrupted by the presence of connecting methylene 'bridge', determining a lower stability comparative to the one of vitamin B_2. The simplest structure, the one of pyridoxine, consists of a classic Hückel aromatic system. For the formation of hydrochloride, pyridinic nucleus is protonated forming a positively charged aromatic polyatomic ion. By kinetic analysis, it is confirmed that the degree of aromaticity of vitamin B_6 is lower than that of vitamin B_1 (Scheme 2).

Conclusions

A study on the thermal behaviour of B_1, B_2 and B_6 vitamins was performed. All the three compounds present a relatively high thermal stability, the most stable being vitamin B_2.

A kinetic analysis based on four different methods confirms the higher thermal stability of vitamin B_2. The individual values of the activation energy obtained by isoconversional methods differ in range of 15 %, so an estimation using only one method is risky, especially by such cases of compounds with significant different molecular architectures.

A way for avoiding this difficulty is the use of Non-Parametric Kinetic method, which allows a separation between the temperatures and the respective conversion dependence on the reaction rate, without any a priori hypothesis. The data processing strategy offers a complete description of the reaction rate of the thermally induced degradation of the vitamins. In order to realise a correlation between structure and stability of the analysed vitamins, the aromaticities of these compounds were analysed.

Acknowledgements This work was supported by a grant from the University of Medicine and Pharmacy 'Victor Babeş' Timişoara (Grant PIII-C1-CFI-2014/2015-03 to A. F. and I. L.).

References

1. Fattal-Valevski A. Thiamine. J Evid Based Complement Altern Med. 2011;16:12–20.
2. Rucker RB, Suttie JW, McCormick DB, Machlin LJ. Handbook of vitamins. 3rd ed. New York: Marcel Dekker Inc.; 2001.
3. Fulias A, Ledeti I, Vlase G, Vlase T. Physico-chemical solid-state characterization of pharmaceutical pyrazolones: an unexpected thermal behaviour. J Pharm Biomed. 2013;81–82:44–9.
4. Fulias A, Vlase T, Vlase G, Doca N. Thermal behaviour of cephalexin in different mixtures. J Therm Anal Calorim. 2010;99(3):987–92.
5. Fulias A, Vlase G, Grigorie C, Ledeti I, Albu P, Bilanin M, Vlase T. Thermal behaviour studies of procaine and benzocaine: part 1. Kinetic analysis of the active substances under non-isothermal conditions. J Therm Anal Calorim. 2013;113(1):265–71.
6. Anghel M, Vlase G, Bilanin M, Vlase T, Albu P, Fulias A, Tolan I, Doca N. Comparative study on the thermal behavior of two similar triterpenes from birch. J Therm Anal Calorim. 2013; 113(3):1379–85.
7. Ledeti I, Simu G, Vlase G, Săvoiu G, Vlase T, Suta L-M, Popoiu C, Fulias A. Synthesis and solid-state characterization of Zn(II) metal complex with acetaminophen. Rev Chim–Bucharest. 2013; 64(10):1127–30.
8. Ledeti I, Fulias A, Vlasei G, Vlase T, Bercean V, Doca N. Thermal behaviour and kinetic study of some triazoles as potential anti-inflammatory agents. J Therm Anal Calorim. 2013; 114:1295–305.
9. Fulias A, Vlase G, Vlase T, Soica C, Heghes A, Craina M, Ledeti I. Comparative kinetic analysis on thermal degradation of some cephalosporins using TG and DSC data. Chem Cent J. 2013; 7(1):70.
10. Bodescu AM, Sirghie C, Vlase T, Doca N. Kinetics of thermal decomposition of natrium oxalato-oxo-diperoxo molibdate. J Therm Anal Calorim. 2012;110(3):1243–7.
11. Bodescu AM, Sirghie C, Vlase T, Doca N. Comparative kinetics studies of thermal decomposition of kalium, respectively natrium oxalato-oxo-diperoxo molibdate. J Therm Anal Calorim. 2013; 113(3):1431–5.
12. Albu P, Bolcu C, Vlase G, Doca N, Vlase T. Kinetics of degradation under non-isothermal conditions of a thermooxidative stabilized polyurethane. J Therm Anal Calorim. 2011;105(2): 685–9.
13. Vlase T, Vlase G, Chiriac A, Doca N. Decomposition of organic salts of some d and f metals: non-isothermal kinetics and FT-IR studies. J Therm Anal Calorim. 2003;72(3):839–45.
14. Birta N, Doca N, Vlase G, Vlase T. Kinetic of sorbitol decomposition under non-isothermal conditions. J Therm Anal Calorim. 2008;92:635–8.
15. Friedman HL. Kinetics of thermal degradation of char-foaming plastics from thermogravimetry: application to a phenolic resin. J Polym Sci. 1965;6C:183–95.
16. Flynn JH, Wall LA. A quick, direct method for the determination of activation energy from thermogravimetric data. Polym Lett. 1966;4:323–8.
17. Ozawa T. A new method of analyzing thermogravimetric data. Bull Chem Soc Jpn. 1965;38:1881–6.
18. Kissinger HE. Reaction kinetics in differential thermal analysis. Anal Chem. 1957;29(11):1702–6.
19. Akahira T, Sunose T. Joint convention of four electrical institutes. Research Report Chiba Institute of Technology. Sci Technol. 1971;16:22–31.
20. Serra R, Nomen R, Sempere J. The non-parametric kinetics. A new method for the kinetic study of thermoanalytical data. J Therm Anal Calorim. 1998;52:933–43.
21. Serra R, Sempere J, Nomen R. A new method for the kinetic study of thermoanalytical data: the non-parametric kinetics method. Thermochim Acta. 1998;316:37–45.
22. Vlase T, Vlase G, Doca N, Bolcu C. Processing of non-isothermal TG data. Comparative kinetic analysis with NPK method. J Therm Anal Calorim. 2005;80:59–64.
23. Vlase T, Vlase G, Doca N, Ilia G, Fulias A. Coupled thermogravimetric-IR techniques and kinetic analysis by non-isothermal decomposition of Cd^{2+} and Co^{2+} vinyl-phosphonates. J Therm Anal Calorim. 2009;97:467–72.
24. Šesták J, Berggren G. Study of the kinetics of the mechanism of solid-state reactions at increasing temperatures. Thermochim Acta. 1971;3:1–12.