Solid State Stability of Cholesterol

Preliminary kinetic analysis

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Kinetic analysis is a powerful tool of characterization for degradative processes which involve bioactive compounds. In this study, we set our goal in the preliminary kinetic analysis for the decomposition processes of cholesterol under non-isothermal conditions, by employing two methods proposed by Kissinger: the classical Kissinger method vs. isoconversional Kissinger-Akahira-Sunose method. The obtained results by employing the two methods suggest the superiority of the isoconversional method. The mean value for activation energies were obtained as follows: 82.7 kJ/mol by using Kissinger method vs. 100.8±3.1 kJ/mol by using KAS method. A discussion for the differences between these values is presented.

Keywords: kinetic study, cholesterol, thermal analysis, isoconversional method, Kissinger

Cholesterol (CH) is an endogenous sterol with a great importance in the human body, since it is a precursor for the biosynthesis of bile acids, steroid hormones and vitamin D. The structure of cholesterol is presented in figure 1.

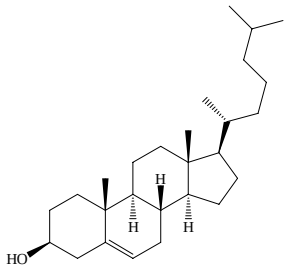

Fig. 1. The structure of cholesterol

CH can't travel freely through the bloodstream due to its very pour water solubility. Thus, in order to insure its delivery to cells and tissues, the cholesterol molecules are enclosed in lipoprotein complexes made of hydrophobic core consisting of the lipid molecules that are being transported surrounded by a surface monolayer of polar lipids and proteins. According to their density, these complexes are classified as chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). The body has particular mechanisms that regulate the transport and amount of cholesterol present [1].

Without diminishing the huge importance of cholesterol in maintaining the proper functions of the human body, it is essential to mention that high levels of this lipid are markers for a number of illnesses such as atherosclerosis, hypertension, coronary disease, a possible myocardial infarction, nephrosis, diabetes, but also for the formation of gallbladder stones [2-3].

For their proper functioning, body cells only require a small amount of cholesterol. Considering that the exogenous cholesterol it's a combination between a diet high in fats and a sedentary lifestyle, usually the cells can't consume it all. The remaining excess may deposit in the arterial walls causing the start of the atherosclerosis process [4].

The formation of the atheromatous plaque it is believed to be caused by a chronic inflammatory response due to macrophage and white blood cells accumulation promoted by LDL (the so called „bad cholesterol") without the right amount of HDL ("good cholesterol") that lead to the removal of cholesterol and fats by macrophage cells [5]. The inflammatory reaction all too often can lead to the destruction of the arterial wall and a thrombotic vascular occlusion that tends to result in the infarction of the tissues supplied by the affected vessel [6].

The high level of cholesterol in the bloodstream is a modifiable risk factor. By increasing daily physical activity, dietary changes, weight' control and drug therapy or by combining some or all of these, cholesterol levels can be lowered [7].

The importance of the kinetic studies is due to the fact that the obtained values for activation energy (E_a), reaction order (n) and pre-exponential factor (A) can lead to obtaining some details regarding the decomposition mechanism and thermal stability of bioactive and potential bioactive molecules [8-11].

Experimental part

Cholesterol anhydrous (CH) was a commercial product supplied by Sigma (C8667) and used as received, without preliminary purification. Producer indicates purity higher

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than 99%, with a melting point in the 147-149 °C temperature range.

UATR-FTIR spectrum of solid CH was drawn on a Perkin Elmer SPECTRUM 100 device, without any a priori preparation of the samples.

For carrying the kinetic study, TG/DTG/HF measurements were realized on a Perkin-Elmer DIAMOND TG/DTA instrument, by using 5-6 mg of CH which was weighted into an open aluminum crucible. The temperature was programmed to increase under non-isothermal conditions from 35 up to 500 °C, linearly at heating rates β = 5, 7, 10, 12 and 15 °C min^{-1}. The experiments were completed in oxidative conditions (synthetic air atmosphere) at a flow rate of 100 mL min^{-1}. All the protocols were repeated in duplicate and the results were practically identical.

Results and discussions

FTIR spectroscopy was employed only as a confirmation technique for the purity analysis of the CH sample. The determined spectrum was compared to the one in the spectrometer's database, and the confirmation of purity was confirmed. The main observed bands were noticed at the following wavenumbers (in cm^{-1}): 3412, 2931, 2900, 2867, 2848, 1671, 1465, 1436, 1376, 1364, 1333, 1254, 1235, 1190, 1169, 1131, 1107, 1083, 1022, 985, 955, 926, 882, 840, 799, 740 and 700 (fig.2.)

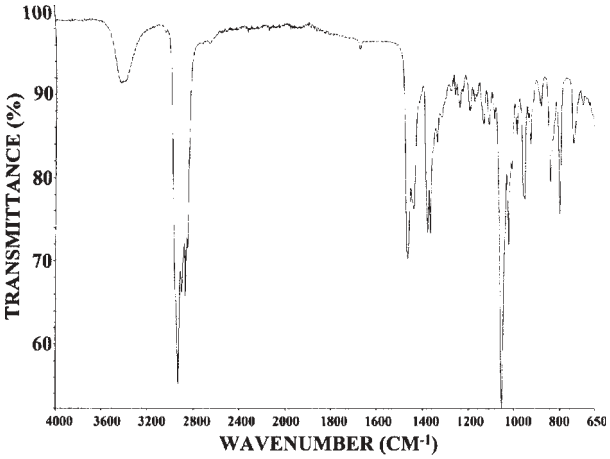

Fig.2. UATR-FTIR spectrum of Cholesterol

The kinetic analysis was performed using the thermogravimetric data obtained in air atmosphere for the first decomposition step. The thermoanalytical curves TG/DTG/DTA for CH sample under heating (β =7 °C x min^{-1}) in dynamic air atmosphere are presented in figure 3. TG and

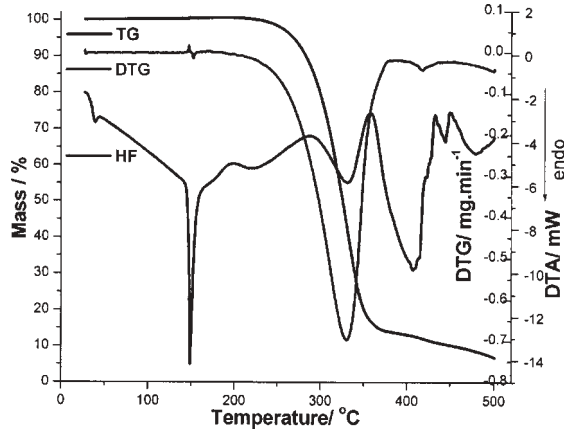

Fig.3. The thermoanalytical curves TG/DTG/HF for CH in air at a heating rateβ=7 °C'"min^{-1}

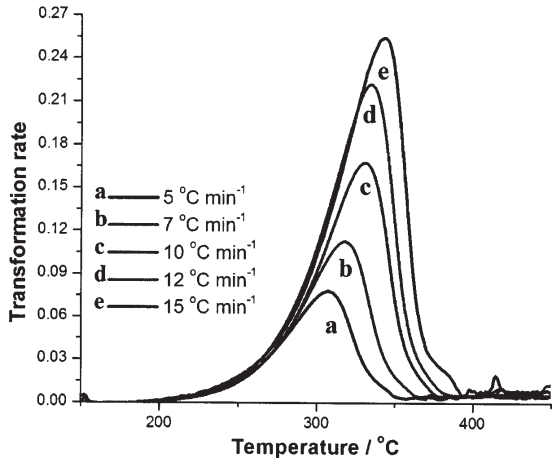

Fig.4. The plotting of transformation rates *vs.* temperature for CH in air at selected heating rates

DTG curves reveal that CH is thermally stable up to 224 °C. With the increase of temperature, the degradation process advances, as suggested by the DTG curve, and confirmed by the thermal events from the HF curves. The main decomposition step was investigated by kinetic means, *i.e.* the process revealed by the thermoanalytical curves in the 200-400 °C temperature range.

For a better illustration of the kinetic-investigated process, the transformation rate curves *vs.* temperatures are presented in figure 4. As it was expected, the maximum reaction rate was observed at higher temperatures as the heating rate increased, all the degradation processes occurring in temperature range 200-400°C. These temperatures where the maximum reaction rate was observed were used in the preliminary estimation of the kinetic parameters using the classical Kissinger (K) method.

The classical Kissinger method (K) is based on the fact that for the Arrhenius-type dependence of the rate constant vs. temperature, a linearized model can be obtained, as shown in eq.1:

$$\ln\left(\frac{\beta}{T_{max}^2}\right) = \ln\frac{AR}{E_a} + \ln[n(1-\alpha_{max})^{n-1}] - \frac{E_a}{R \cdot T_{max}} \quad (1)$$

where:

-β is the heating rate;

-T_{max} is the temperature where the maximum of the reaction rate is observed;

-A is the pre-exponential factor;

-R is the universal gas constant,;

- n is the reaction order;

-α is the conversion degree;

- E_a is the activation energy.

By plotting of $\ln(\beta / T^2_{max})$ *vs.* $1000/T_{max}$ for the five measurements carried out at different heating rates, the value of E_a can be determined [12].

The obtained value for the activation energy using the classical Kissinger method is E_a= 82.65 kJ mol^{-1} (fig. 5)

In order to correlate and compare the obtained results by this preliminary method, in the last part of the study we employed an isoconversional method, namely the integral method of Kissinger-Akahira-Sunose (KAS). As a clear advantage of the KAS method, it worth mentioning that is a model free method which can reveal the complex nature of the degradation process, by allowing the estimation of variation of E_a *vs.* α. It is generally considered that a variation of the E_a value around the mean in the limit of ±10% suggest a single-step process, while a greater variation clearly indicate a complex pathway of decomposition. The

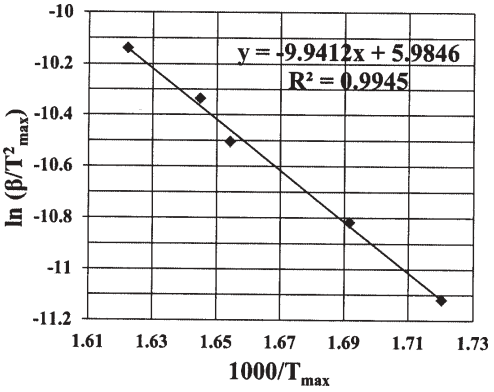

Fig.5. The linear plot $\ln(\beta/T^2_{max})$ *vs.* $1000/T_{max}$ corresponding to Kissinger method for CH

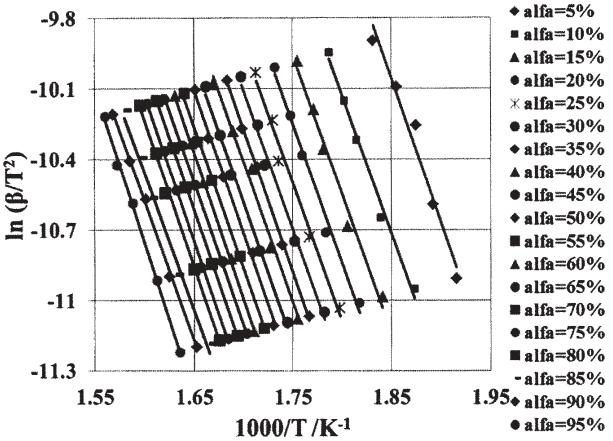

Fig.6. Plotting of the data according to KAS method for degradation of CH

Sample and kinetic method		Conversion degree α										E_a/ kJ mol^{-1}
		0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5	
CHOLESTEROL	KAS	101.4	97.6	97.7	98.3	97.6	98.2	100.9	99.3	99.8	101.4	100.8±3.1
		Conversion degree α										
		0.55	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95		
		100.8	103.9	100.8	103.2	103.9	103.7	97.4	99.1	109.6		
	K											82.7

Table 1
THE E_a vALUES OBTAINED BY KINETIC METHODS FOR CH

mathematical model of the KAS method [14-15] is showed in eq.4.

$$\ln\frac{\beta}{T^2} = \ln\frac{A\cdot R}{E_a\cdot g(\alpha)} - \frac{E_a}{R\cdot T} \qquad (2)$$

Plotting of the data according to KAS method is presented in figure 6. The estimation of E_a values was realized from the slopes of those lines, for $0.05 \le \alpha \le 0.95$, with a α variation step of 0.05. The obtained results are presented in table 1, for each case determination coefficients were higher than 0.972, indicating good linear correlations.

By the analysis of E_a *vs.* α values, it can be said that the isoconversional method of KAS suggest a single-step degradation, since the variation of each estimated value of E_a falls within the range of variation of ±10% around the mean. This method should be considered the most accurate one, since it allows the estimation of activation energies without knowing the mathematical model for the conversion function, but with the main inconvenient in the inability of separating parallel pathways to the degradation.

Conclusions

In this study, we employed two different types of kinetic methods for the analysis of the degradative mechanism of cholesterol in the temperature range 200-400 °C. The classical Kissinger method suggested a mean value for the activation energy of 82.7 kJ/mol.

The most accurate method was the isoconversional integral method of Kissinger-Akahira-Sunose, which allowed the estimation of mean value for E_a using five different heating rates at certain conversion degrees. This method suggested a mean value for E_a around 100.8 kJ/mol and an independent mechanism of decomposition to temperature. Only in a future study, employing more isoconversional methods and as well the non-parametric kinetic method, a concrete separation of the parallel processes that are contributing to the degradation of CH can be achieved.

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