Thermal Characterization of Cholesterol in Air vs. Nitrogen Atmosphere

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In this paper, we describe a preliminary study regarding the thermal behavior and solid-state characterization of pure cholesterol (CH) samples in oxidative air atmosphere vs. nitrogen atmosphere. The study was also completed by the analysis of CH samples heated at 50, 100, 150, 200, 250 and 300 °C in air atmosphere, employing FT-IR spectroscopy.

Keywords: cholesterol, thermal analysis, FTIR, stability study, oxidative and inert atmosphere

Cholesterol (abbreviated CH, fig. 1) continues to attract the attention of physicians, cell biologists and biochemists due to its importance not only in the human body's physiology but in its pathology as well [1]. It plays a fundamental role in the structure of the cell membrane, the reproduction process, regulating cellular functions, salt and water balance and the absorption of nutrients. Moreover, it is also a precursor of vitamin D, bile acids and steroid hormones. All this considering, it is only natural that there are over 100 genes devoted to its synthesis, transport, metabolism and regulation [2-3].

The membrane of every human cell contains cholesterol molecules that are required to bond with chains of phospholipid fatty-acids. This interaction increases membrane packing which in its turn reduces membrane fluidity. The plasma membrane permeability to protons, sodium ions and neutral solutes it is also reduced by the presence of CH due to the bonds that form between the polar ends of sphingolipids and phospholipids and the hydroxyl group of CH molecules [4].

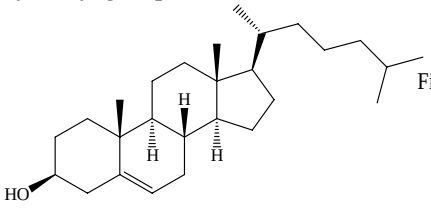

Fig.1. Structural formula of cholesterol (CH)

CH synthesis takes place in the liver, it is transported in the blood stream with carrier lipoproteins due to its liposolubility and after fulfilling its role, it is recycled. From a total content of 35 g, the human body produces about 1 g of CH daily. This lipid suffers an important transformation in the liver, converting into bile acids, which are then stored in the gallbladder [5]. This stage in the CH' metabolism is considered a major route for its removal from the body. After the bile acids are synthesized, they enter the intestinal lumen through the bile duct when a meal is consumed to stimulate solubilization and absorption of dietary lipids and fat soluble vitamins [6]. A very large part of the bile acids is reabsorbed, transported back to the liver where after the CH molecules are recuperated, new bile acids are formed (enterohepatic circulation) [7].

Instrumental analysis, consisting in different techniques like FTIR spectroscopy [8], thermal analysis [9-11] and PXRD [12-14] are important tools in characterization of biological active molecules [15-18] or potential bioactive ones [19-20], but as well of great importance in preformulation studies in pharmaceutical technology [21-22].

Following these considerations regarding the importance of CH in anatomy and physiology, and the corroboration of this study with our previous reported ones in the field of analyzing gallbladder stones [23] and other solid human concretions, we set our goal in this paper in the comparative analysis of thermal stability of CH in air *vs.* nitrogen atmosphere, under dynamic heating. Also, the study was completed with spectroscopic analysis (FTIR) for CH samples subjected for established period of times (5 or 15 min) to different temperatures (from 50 to 300 °C, with 50°C heating step), in air atmosphere.

Experimental part

Materials and methods

Pure sample of Cholesterol (3 β-Hydroxy-5-cholestene, 5-Cholesten-3β-ol, CAS 57-88-5) was obtained from Sigma (C8667) and used as received, without further purification (purity >99%, melting point 147-149 °C, boiling point 360 °C). The sample was kept in sealed vial under ambiental temperature until use.

The thermoanalytical TG/DTG/DTA curves were drawn up in a dynamic air or nitrogen atmosphere under non-isothermal conditions at a heating rate $\beta = 10\ °C \cdot min^{-1}$ using a Perkin-Elmer DIAMOND equipment. Samples of mass in

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the range of 4-5 mg were put into aluminum crucibles and heated by increasing temperature from ambient up to 500 °C (TG analysis). In order to evaluate the accuracy of the measurements, two repetitions were realised with this experimental protocol for the samples and the obtained results were practically identical.

The thermal treatment of CH samples at different temperatures was carried out using a Perkin-Elmer DIAMOND equipment and was realised as follows: the samples were put into aluminum crucibles, heated with 10°C·min^{-1} up to desired temperature (i.e. 50 , 100 150, 200, 250 and 300 °C) and maintained in isothermally conditions for 5 min. A CH sample heated at 300 °C were also kept in isothermally conditions for 15 min.

The FTIR spectra of the solid samples were obtained on Perkin Elmer SPECTRUM 100 device using the U-ATR technique, without further preparation of the samples.

Reesults and discussions

FTIR spectroscopy

Cholesterol samples were analyzed by UATR-FTIR Spectroscopy technique, in order to determine any structural modification of the molecule under thermal treatment. All the discussions are carried out in comparison with the FTIR spectra of pure CH, thermally untreated. Literature data [23-24] presents the main FTIR bands identified in the spectrum of CH, as well their attributions to functional moieties: large band between 3600-3200 cm^{-1} is due to stretching vibrations of the O-H bond (peak at 3412 cm^{-1}). This band is also present in the spectrum of thermally treated cholesterol (up to 300°C), suggesting that the analysed sample consist in anhydrous CH, without any adsorbed or crystallization water. These results are also in good agreement with the ones obtained by thermal analysis, since no mass loss occurs up to 235 °C.

Other bands were observed, as follows: 2930 and 2901 cm^{-1} (CH_2 and CH_3 asymmetric stretching), 2867 and 2848 cm^{-1} (CH_2 and CH_3 symmetric stretching), 1464 and 1436 cm^{-1} (CH_2 and CH_3 bending) and 1050 cm^{-1} (C-C stretching). Also, the recorded FTIR spectra at the CH samples subjected to selected temperatures (fig.2), suggest that thermal treatment in isothermal conditions, for 5 min, up to 300 °C doesn't drastically modify the composition of the sample.

However, the CH sample subjected to 300 °C for 15 min is different in both aspect (brown powder), but as well as spectroscopic behavior. This observation is in good agreement with the thermoanalytical data, when a heterogeneous degradation of CH occurs.

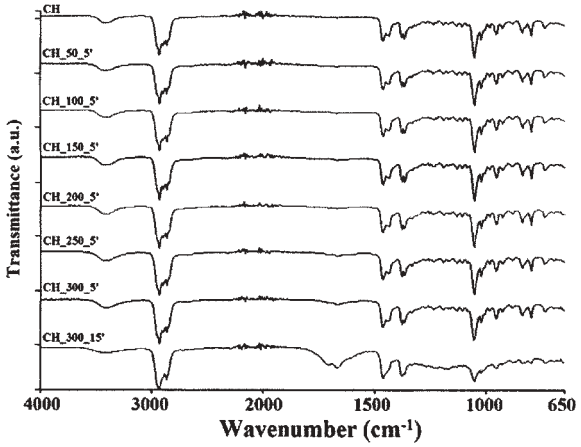

Fig.2. UATR-FTIR spectra of thermally treated CH samples (sample abbreviation as follows: CH_x_y', where x=temperature in °C and y-time in min) vs. untreated sample (CH)

For the CH sample treated at 300 °C for 15 min, the FTIR spectrum is modified: the broad band between 3600-3200 cm^{-1} is dramatically diminished, suggesting that the degradative process occur involve the destruction of the CH skeleton near this functional moiety. Two of the four sharp peaks between 2930-2848 cm^{-1} associated with stretching of methyl and methylene groups disappeared from the spectrum, so only the bands around 2930 and 2867 cm^{-1} are still present in the spectrum. These observations are the proof that the degradation of the CH skeleton occurs in the lateral aliphatic moiety, as well.

Comparative thermal analysis

TG Analysis

The superimposed TG curves for pure CH in both dynamic oxidative (air) atmosphere and inert (nitrogen) are presented in figure 3. CH is thermally stable up to 228 °C in air and 197 °C in nitrogen, respectively. Up to these temperatures, no mass losses occur. With the increasing of temperature, the rapid mass loss takes place up to 367 °C in air (Δm_{air}=85.1%) and 344 °C in nitrogen ($\Delta m_{nitrogen}$=97.5%), respectively. In the temperature ranges 367-500 °C in air, respectively 351-500 °C in nitrogen, the mass loss is considerable lower (Δm_{air}=4.6%, $\Delta m_{nitrogen}$=2.4%).

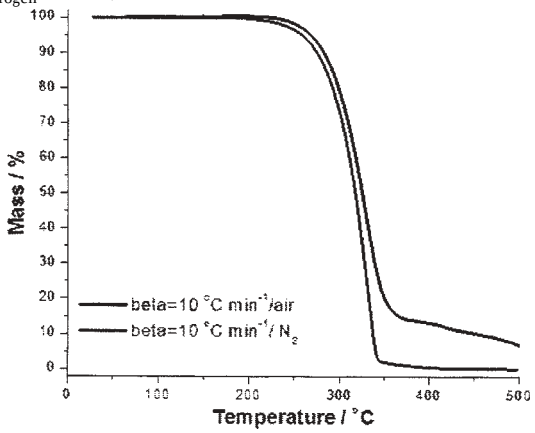

Fig.3. The superimposed TG curves obtained for CH in dynamic air *vs.* nitrogen atmosphere

The mass losses suggest that the degradative mechanism is different and dependent of surrounding atmosphere, fact that was expected, since in air, the most probable mechanism is an oxidative one. However, the TG data are better understood after corroborating the information with the ones from DTG curve, where the processes are better individualized and separated.

DTG Analysis

DTG curve recorded at a heating rate β=10 °C·min^{-1} suggest a thermal stability up to 222°C in air atmosphere and 200°C in nitrogen atmosphere, respectively (fig.4). The first degradative process in air presents a maximum at 331 °C in air, respectively 333 °C in nitrogen. The processes are well individualized. The main degradative process that occur in air atmosphere is followed by another one, with an considerable smaller amplitude in the temperature range 381-500 °C, with a maximum of 418°C. This process is not observed when the heating takes place in nitrogen.

DTA Analysis

Anhydrous cholesterol undergoes a polymorphic crystalline transition at 39°C (endothermal event), which is in god agreement with already reported data of Loomis, Shipley and Small [25], as presented in figure 5. This polymorphic transition takes place in nitrogen atmosphere

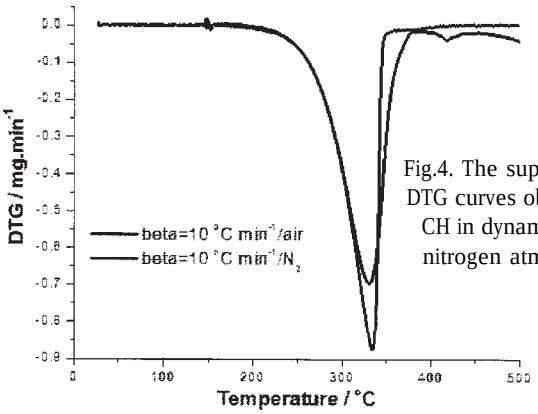

Fig.4. The superimposed DTG curves obtained for CH in dynamic air *vs.* nitrogen atmosphere

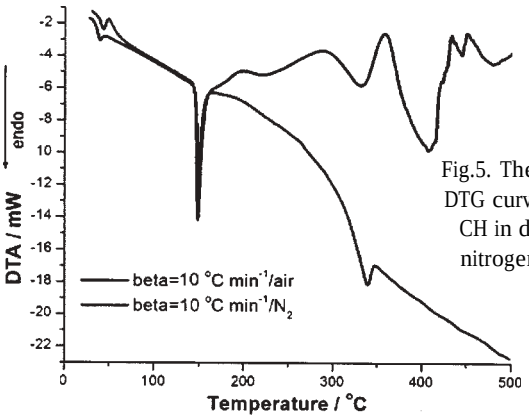

Fig.5. The superimposed DTG curves obtained for CH in dynamic air *vs.* nitrogen atmosphere

too, the DTA_{max} at 42 °C. However, this polymorphic transition is not influencing the FTIR spectra of the sample. The DTA curve in air shows the melting of CH at 148°C, which is in good agreement with the data suggested by the MSDS of the supplier. The melting is independent of the surrounding atmosphere. The DTA curve obtained in oxidative atmosphere is more complex that the one recorded in nitrogen, due to multiple oxidations and skeleton breakdowns. The DTA pattern is complex, showing overlapped processes, with maximums at 288 and 357°C. An endothermic event is observed, with a maximum at 407°C, which cannot be associated with a clear physical process. Even if the MSDS of the supplier suggest that the boiling of CH occurs at 360°C, our study proves that isothermal heating for 15 min at 300°C determines a considerable breakdown of the molecule, and this process can be associated with evaporation of smaller moieties and not of the entire CH structure.

However, the DTA curve recorded in nitrogen atmosphere suggest that the thermodegradative processes do not occur, the pattern being more simplistic, suggesting solely the melting (as previously mentioned) and the boiling at 340 °C, other thermal events weren't noticed.

Conclusions

In this study, we set our goal in investigating the thermal behavior of cholesterol in both dynamic oxidative atmosphere (air) and inert one (nitrogen), in order to determine the stability. It was shown that CH is thermally stable up to considerable high temperatures in both surrounding atmospheres, but the mass loss occurs through different mechanisms: in air, the main processes are a polymorphic transitions around 40 °C, followed by phase transition (solid to liquid), and decomposition, while in nitrogen, solely physical processes are involved in the mass loss: melting around 148 °C, followed by evaporation and boiling at 340 °C.

Also, FTIR spectroscopy was used as complementary instrumental technique in the analysis of CH samples treated in isothermally conditions at 50, 100, 150, 200, 250 and 300 °C. It was shown that polymorphic transition that occurs at 39 °C does not influence the aspect of the FTIR spectra. Only the isothermal treatment of CH at 300 °C for 15' determines a considerable modification of composition, as suggested by the spectroscopic investigation.

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