Preliminary Kinetic Study for Heterogenous Degradation of Cholesterol - Containing Human Biliary Stones

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In this paper, we present a continuation of our dedicated study regarding the analysis by instrumental techniques of different types of human stones, with different etiopathology. We focused in a preliminary kinetic study for heterogeneous degradation of cholesterol-containing human biliary stones. A number of eight samples of gallstones collected from human subjects were prepared and the kinetic parameters were determined using the Coats-Redfern method.

Keywords gallstone, cholesterol, human biliary stone, kinetic analysis; Coats-Redfern

Cholesterol ((3β)-cholest-5-en-3-ol, abbreviated CH, Figure 1) is a sterol-type compound which is endogenous biosynthesized at cellular level, but also widely available for human intake from dietary sources, such as animal fats [1]. The biochemical importance of CH resides in the fact that is a main structural component of cell membranes of animals, and also play important role in maintaining their structural integrity. CH also plays an important role in the *in-vivo* synthesis of hormones (like aldosterone, cortisol, progesterone, testosterone and estrogens), vitamin D and bile acids *via* multistep cytochrome P450-mediated oxidation process [2-3].

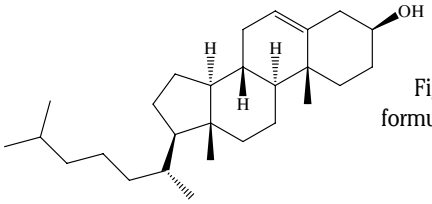

Fig. 1. The structural formula of cholesterol (CH)

The formation of gallstones and/or (accompanied by) pseudoliths (sludge) was studied, as well the influence of bile acids and cholesterol concentration in microcrystal formation [4]. As treatment schemes, oral formulations containing as active pharmaceutical ingredinets (APIs) chenodeoxycholic acid and/or ursodeoxycholic acid are used to dissolve cholesterol gallstones [5]. Since it's a global concern, the *in vivo* levels of CH n humans is monitored and maintained under control by different therapeutic schemes, including statins [6-7].

Since the incidence of cholelithiasis is worldwide high, and the composition of stones differs from one patient to another, the instrumental analysis of intraoperative collected stones [8], offer a perspective corroboration of the type of stone with the treating scheme.

As mentioned in a previous published paper [8], the composition of human gallstones is rich in CH, with a medium value over 85%. There were also identified black stones – containing less than 20% CH, traces of calcium phosphate and the colour is due to presence of bilirubin-based pigments but as well mixed stones, with a composition consisting in CH between the above-mentioned limits, along with biliary pigments and calcium salts (palmitate, stearate, bilirubinate, carbonate, phosphate). [9]

Kinetic analysis is nowadays a currently-employed method for establishing the mechanism of decomposition, stability and thermal behaviour of chemical compounds. The kinetic study regarding the heterogeneous decomposition of a solid can be carried out by two major ways of processing the data – model-fitting models and model-free models, by isothermal or non-isothermal experiments. Even if the superiority of using model-free methods was proven in numerous studies [10-14], a preliminary study using model-fitting methods can suggest a global view of the decomposition process [15].

Previously, we have reported the importance of thermal analysis in characterization of human stones [8,16], but as well bioactive compounds and newly synthesised derivatives [17-19], and the coreelation if obtained data with the ones from other instrumental techniques.

This paper is a continuation of our study regarding the characterization of the eight human gallstones samples (TG/DTG/HF, FTIR and PXRD data) [8] with a preliminary kinetic analysis regarding the heterogeneous decomposition of cholesterol-containing human biliary stones. According to our knowledge, kinetic study for the decomposition of gallstones was not previously reported in literature. Following this, a number of eight samples of gallstones (GS1 to GS8) collected from human subjects were prepared and the kinetic parameters (E_a-activation energy and A- pre-exponential factor) were determined using the Coats-Redfern method.

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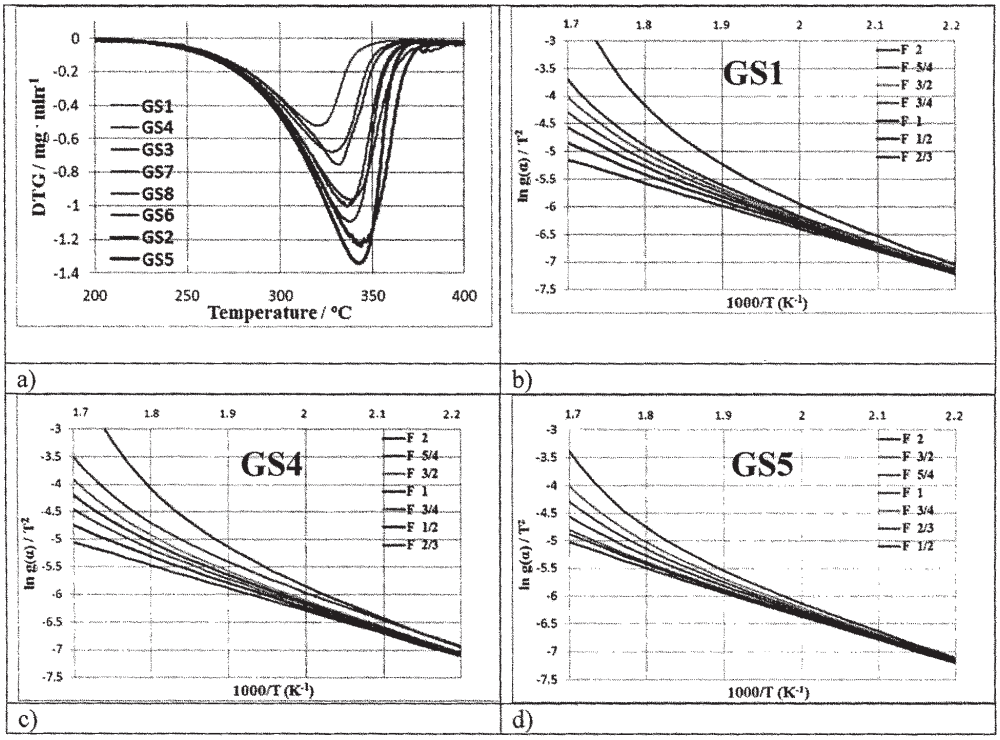

Fig. 2. a) The superimposed DTG curves for analysed process for GS1-GS8; b)-d) graphic representation for Coats–Redfern method (β=10 °C·min^{-1})

Experimental part

Materials and methods

Gallstones (GS) were collected intraoperative from six patients of different ages hospitalized at the Department of Surgery II, First Surgical Clinic (Timiş). The instrumental analysis of the stones was carried out with patients agreement.

The labelling of the samples, the number of stones extracted from each patient, macroscopic aspect and sample preparation for carrying instrumental analysis was elsewhere reported [8]. The sample numbering was maintained as in our previously published paper (GS1 to GS8), as well the preliminary selection and characterization of samples [8].

Thermoanalytical data (TG/DTG/HF) was recorded using a Perkin-Elmer DIAMOND device. Samples with mass around 6mg were heated in temperature range 25 - 550 °C in open aluminium crucibles, in non-isothermal condition, with a heating rate β=10 °C·min^{-1}, under a dynamic flow of synthetic air (100 mL·min^{-1}).

Results and discussions

Previously [8], by corroboration of UATR-FTIR, PXRD study and thermal analysis, we proved that the analysed samples GS1-GS8 consist mainly in CH, along with other organic traces, including bile pigments and long-chained esters. In none of the cases, traces of inorganic compounds, such as apatite, aragonite, calcite or phosphates were observed. Following this observation, we analysed the kinetic decomposition of the main compound (CH), in all samples, in order to determine if the mechanism of decomposition is influenced by the presence of other compounds.

The decomposition processes analysed for each sample was chosen by the analysis of the DTG curve (fig. 2a). The temperature range data is presented in table 1.

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Sample	Temperatures on DTG (K)		
	Onset	Endset	Max
GS1	463	640	590
GS2	484	653	617
GS3	489	648	600
GS4	479	637	596
GS5	480	650	612
GS6	483	644	606
GS7	479	658	610
GS8	481	649	605

Table 1
TEMPERATURE RANGE DATA FOR DECOMPOSITION OF SAMPLES (DTG CURVE)

The Coats-Redfern method is a model-fitting one, with $f(\alpha)=(1-\alpha)^n$ and described by the following equations:

$$\lg\left[\frac{-\ln(1-\alpha)}{T^2}\right] = \lg\frac{A\cdot R}{\beta\cdot E_a} - \frac{E_a}{2{,}303\cdot R\cdot T}, \text{ when } n=1 \quad (1)$$

$$\lg\left[\frac{1-(1-\alpha)^{1-n}}{T^2\cdot(1-n)}\right] = \lg\frac{A\cdot R}{\beta\cdot E_a} - \frac{E_a}{2{,}303\cdot R\cdot T}, \text{ when } n\neq 1; \quad (2)$$

where: α-conversion degree, n- reaction order, T-temperature (K), A- preexponential factor (min^{-1}), R-universal gas constant (8.314 J·mol^{-1}·K^{-1}), β- heating rate (K·min^{-1}), Ea– activation energy (kJ·mol^{-1}), f(α)-conversion degree function.

By plotting the left side logarithm vs. T^{-1}, linear dependencies are obtained for n=2/3 (for samples GS1, GS3 and GS4) and n=1/2 (for samples GS2, GS5, GS6, GS7 and GS8). The mathematical analysis of these linear dependencies (the slopes and the intercepts) lead to determination of characteristic values for E_a and lnA (in Table 2, the unit for A is min^{-1}).

The results obtained by the Coats-Redfern kinetics, by assuming the dependence using seven models are described in completely for the samples GS1 (fig. 2b, table 2), GS4 (fig. 2c) and GS5 (fig. 2d, table 2). The results obtained after applying Coats-Refdern kinetics and selection of best fitting models for all analyzed samples are presented in table 3.

The preliminary analysis using first-order and second-order kinetics lead to non-linear dependencies, suggesting

Sample	Model	Equation	R^2	Ea (kJ·mol^{-})	ln A
GS1	F2	y = -7902x + 18.36	0.950	151.3	23.2
	F5/4	y = -6512x + 16.23	0.977	124.6	20.8
	F3/2	y = -6102x + 13.95	0.980	116.8	18.5
	F3/4	y = -5531x + 12.08	0.982	105.9	16.5
	F1	y = -5308x + 11.47	0.990	101.6	15.9
	F1/2	y = -4380x + 10.88	0.994	83.8	15.1
	F2/3	y = -4121x + 10.07	0.997	78.9	14.2
GS5	F2	y = -7560x + 16.36	0.956	144.7	21.1
	F3/2	y = -6998x + 13.87	0.983	133.9	18.5
	F5/4	y = -6492x + 12.87	0.988	124.3	17.5
	F1	y = -5198x + 11.87	0.990	99.5	16.2
	F3/4	y = -5102x + 10.58	0.992	97.6	14.9
	F2/3	y = -5091x + 9.997	0.993	97.4	14.4
	F1/2	y = -4860x + 9.08	0.997	93.1	13.4

Table 2
PRELIMINARY ANALYSIS OF COATS-REDFERN KINETICS USING SEVEN MODELS OF DEPENDENCIES FOR GS1 AND GS5

Sample	Model	Equation	R^2	Ea (kJ ·mol^{-1})	ln A
GS1	F2/3	y = -4121x + 10,07	0.997	78.91	14.27
GS2	F1/2	y = -4809x + 9,93	0.998	92.08	14.28
GS3	F2/3	y = -4103x + 9,98	0.997	78.56	14.17
GS4	F2/3	y = -4209x + 10,39	0.998	80.59	14.61
GS5	F1/2	y = -4860x + 9,08	0.997	93.06	13.44
GS6	F1/2	y = -4799x + 10,01	0.998	91.89	14.36
GS7	F1/2	y = -4719x + 9,81	0.997	90.35	14.14
GS8	F1/2	y = -4703x + 9,91	0.996	90.05	14.26

Table 3
RESULTS OBTAINED AFTER APPLYING COATS-REFDERN KINETICS AND SELECTION OF BEST FITTING MODELS FOR ALL ANALYZED SAMPLES

that in all cases, a heterogeneous decomposition with a non-integer value for n occurs.

The best fitting model was selected according to the values of R^2, leading in all eight cases to a grouping of decomposition in two main categories – namely to a n=1/2 model for GS2, GS5, GS6, GS7 and GS8, with values for $R^2>0.996$, and to a n=2/3 model for GS1, GS3 and GS4, with values for $R^2>0.997$.

Even if in our previous study [8], the using of three instrumental techniques suggested some difference between the thermal behaviour of the samples, only a pertinent kinetic analysis can explain the modification of decomposition pattern correlated with the presence of other organic compounds (and traces of other organic compounds, such as bile pigments, esters and/or organic salts, which are thermodegraded in the same temperature range).

Conclusions

A kinetic analysis was used as a preliminary method for describing heterogeneous degradation of cholesterol contained in human biliary stones, extracted from six patients of different ages hospitalized at the Department of Surgery II, First Surgical Clinic (Timiş). The Coats-Redfern kinetic is model-fitting method, which allowed a estimation of parameters from Arrhenius-type dependence, namely the reaction order (n), activation energy (E_a) and pre-exponential factor (A). Coats-Redfern method suggested that the composition of gallstone influence the degradative mechanism (by means of n), allowing a grouping of the eight samples into two main classes: a n=1/2 model for GS2, GS5, GS6, GS7 and GS8, with values for $R^2>0.996$, and to a n=2/3 model for GS1, GS3 and GS4, with values for $R^2>0.997$.

This study is considered a preliminary one, since we will further report a complete kinetic analysis using integral and differential isoconversional methods, as well the non-parametric kinetics (NPK), for a pertinent estimation of parallel steps that are describing the degradative processes of components from human gallstones.

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Manuscript received: 22.12.2014

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