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TG and DSC investigation of gallstone samples collected from patients submitted to cholecystectomy

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Abstract

In the present work are performed the TG and DSC study of gallstone samples collected from patients submitted to cholecystectomy at the Hospital Universitário Onofre Lopes of Universidade Federal do Rio Grande do Norte, in Natal, Rio Grande do Norte State, Brazil. The gallstone samples were characterized by elemental analysis and infrared spectroscopy. Standard cholesterol was used for all analysis as comparison with the gallstones. Is verified that cholesterol is the main chemical component of all studied gallstone samples. The thermoanalytical study was performed on air and nitrogen atmospheres. The presence of cholesterol as the main chemical component of all gallstone samples is confirmed by TG and DSC results.

Keywords: Gallstones; Cholesterol; Cholecystectomy; Thermogravimetry

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1. Introduction

A considerable amount of the world population is affected by the occurrence of gallstones. Such fact justify the study of the chemical composition of gallstones as a way to understand its formation mechanism, which will, in a broad sense, allows the proposition of medical procedures in order to avoid its formation and damages to the patients.

In the present work, the is performed the thermoanalytical (TG and DSC) study of gallstone samples collected from patients submitted to cholecystectomy at the Hospital Universitário Onofre Lopes of Universidade Federal do Rio Grande do Norte, in Natal, Rio Grande do Norte State, Brazil. Is verified that TG and DSC can be successfully employed for the investigation of such biological samples.

2. Experimental

The gallstone samples were collected from patients submitted to cholecystectomy at the Hospital Universitário Onofre Lopes of Universidade Federal do Rio Grande do Norte, in Natal, Rio Grande do Norte State, Brazil. The gallstones and bile samples were collected by the chirurgical center team of Hospital Onofre Lopes, between October 21, 1999 and April 25, 2000. A total of 34 bile samples (after pH measuring, the bile samples were discarded) and 34 gallstone samples were collected. The collected samples were handled at thermal containers and stored at $-10\,^{\circ}\mathrm{C}$ before analysis. For comparison with gallstone samples, standard cholesterol and bilirubin samples from Sigma were employed.

After washing with deionized water to remove the bile residues, the gallstones were dried at room temperature under vacuum for 24 h. Hence, the samples were turned into powders by it in a mortar. The obtained powders were then dried under vacuum at room temperature for 12 h.

The FT infrared spectra were obtained in KBr discs in the 4000–400 cm^{-1} range by using a Perkin Elmer FTIR-16

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Table 1
Age and sex of the patients, and pH values of the bile for the eight considered gallstone samples

Sample	Age	Sex	pH (bile)
1	49	M	6.9
2	39	F	7.5
3	32	F	7.4
4	50	F	7.7
5	45	F	6.7
6	52	F	6.8
7	44	F	7.9
8	74	F	7.6

PC apparatus. The carbon, hydrogen and nitrogen elemental analysis were performed in a Perkin Elmer micro analyzer model 2400. The atomic absorption analysis were performed in a Varian Spectra A-110 equipment. Thermogravimetric (TG) curves were obtained under air and nitrogen atmospheres at a heating rate of 10 °C min^{-1} and a gas flow of 50 cm^3 min^{-1} on a Shimadzu TGA-50H apparatus.

3. Results and discussion

From the 34 gallstone collected samples, 80% were form female and 20% from male patients. Such fact evidences the higher occurrence of gallstones on the female population. Since the obtained results are very similar for the 34 studied samples, only the results obtained for five representative samples will be presented. The age and sex of the patients, as well as the pH value of the bile for the eight considered gallstone samples are summarized in Table 1. As can be observed from Table 1 data, the bile exhibits pH values around 7.0, that is, a neutral pH.

Table 2
Main infrared bands for cholesterol and bilirubin standard samples

Band (cm^{-1})	Attribution
Cholesterol	
3400	ν(OH)
2930	ν(CH)
1630	ν(C=C)
1466/1376	δ(CH$_2$)/(CH$_3$) and δ(OH)
1056	ν(C–O)
Bilirubin	
3425	ν(OH)
1670	ν(C=O)
1610	δ(NH)
1475	δ(OH)
1250	ν(C–O)
1020	ν(C–N)

The main infrared bands observed in the infrared spectra obtained for cholesterol and bilirubin are summarized in Table 2. The infrared spectra for gallstone (sample 1) is shown in Fig. 1. The band at 3400 cm^{-1} attributed to ν(OH) is typical of anhydrous cholesterol and different from those observed for cholesterol monohydrate [1], for which a broad “two peak” band is observed in the same region. So, despite the importance of cholesterol monohydrate on the human mixed gallstone samples [1] the obtained infrared data are consistent with anhydrous cholesterol samples.

In the infrared spectra obtained for gallstone (sample 1) can be observed bands at 3450, 2934, 1475, 1400 and 1075 cm^{-1}, attributed to cholesterol, as well as bands at 1650 cm^{-1} due to the C=O stretching mode of calcium bilirubinate [2], and another band at 1586 cm^{-1} associated to the ν(COO^{-}Me^{+}) vibration mode of calcium bilirubinate. For sample 2 an infrared band at 1580 cm^{-1} associated to

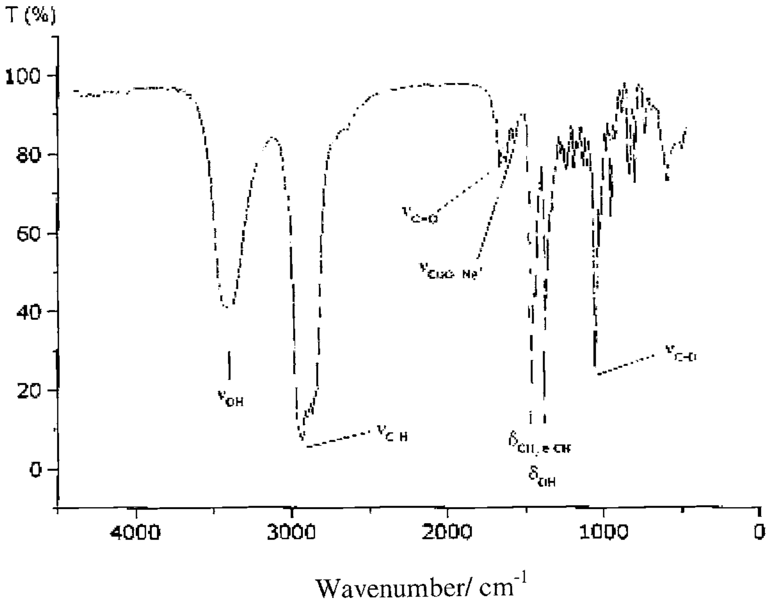

Fig. 1. Infrared spectra for a gallstone (sample 1).

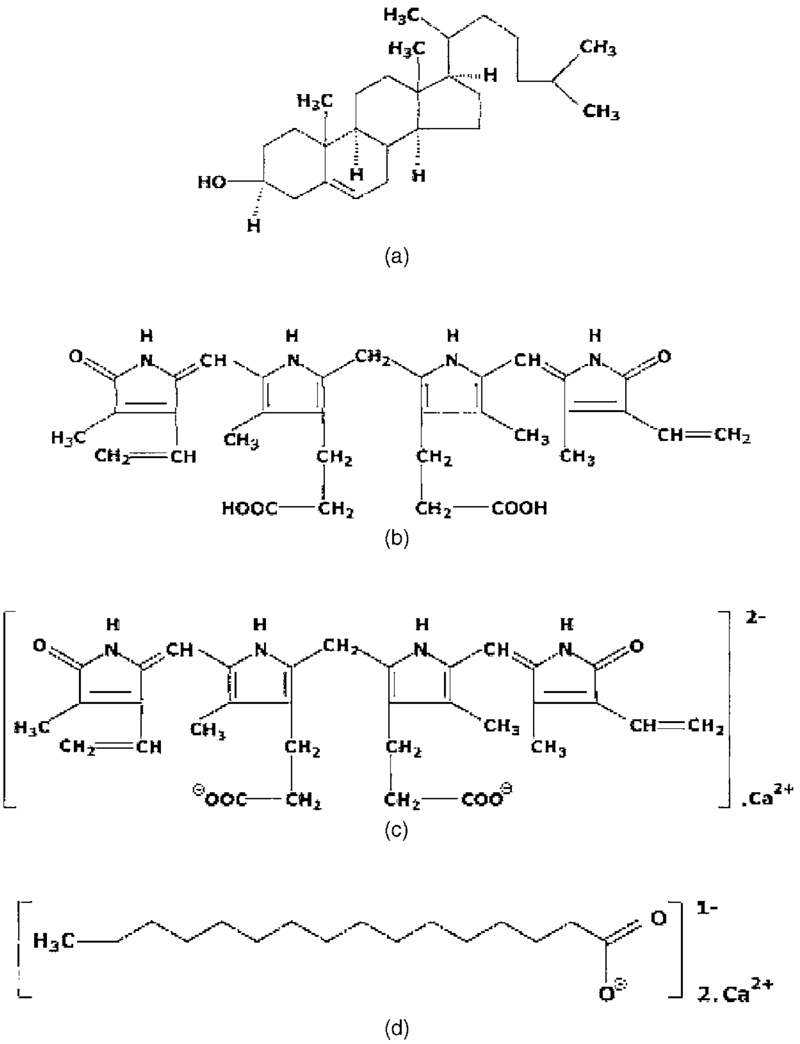

Fig. 2. Structural formulae for cholesterol (a), bilirubin (b), calcium bilirubinate (c) and calcium palmitate (d).

calcium bilirubinate and calcium palmitate is observed. For the other 33 gallstone samples, very similar spectra were obtained, and so, could be concluded that the main chemical species present in the gallstone sample are cholesterol, bilirubin, calcium bilirubinate and calcium palmitate, whose structural formulae are shown in Fig. 2. These results are also in agreement with a pervious study [3] that point out cholesterol as the main chemical species on gallstone samples from western countries.

The CHN elemental analysis for the studied samples are summarized in Table 3. The metal analysis results are summarized in Table 4. Based on the obtained infrared, atomic absorption spectroscopy and X-ray diffraction data, it is proposed that cholesterol, bilirubin, calcium bilirubinate, calcium palmitate and calcium carbonate are the main components of the studied gallstones. Hence, based on Tables 3 and 4 data, as well as on the chemical formulae (calculated chemical composition) for cholesterol, bilirubin, calcium bilirubinate, calcium palmitate and calcium carbonate, the percentages of these main chemical substances on the gallstone samples can be calculated, and such results are summarized in Table 5. As can be verified, cholesterol is the main chemical component for all gallstone samples.

The TG and DTG curves obtained for a cholesterol standard sample under air and nitrogen atmospheres are shown in Fig. 3. As can be observed, under air atmosphere there is a larger residue in the range 350–550 °C not observed for

Table 3
Carbon, hydrogen and nitrogen elemental analysis results for the eight considered gallstone samples

Sample	C (%)	H (%)	N (%)
1	82.4	11.4	0.4
2	82.9	11.7	0.1
3	82.0	11.6	–
4	80.9	11.4	0.1
5	82.0	11.6	0.1
6	77.1	10.7	1.7
7	79.4	11.0	0.2
8	81.3	11.4	0.8

Table 4
Metal contents (mass%) of the considered gallstone samples as obtained by atomic absorption spectroscopy

Metal	Sample							
	1	2	3	4	5	6	7	8
Ca	0.088	0.144	0.625	1.260	0.026	1.219	1.125	0.26
Na	0.028	0.038	0.017	0.043	0.072	0.120	0.065	0.050
Mg	0.015	0.007	0.008	0.031	0.007	0.018	0.030	0.015
K	0.003	0.005	0.004	0.003	–	0.005	0.013	–
Cu	–	–	–	–	–	0.003	–	0.016
Fe	–	–	–	–	0.005	–	–	0.009
Zn	–	–	–	0.001	–	0.001	0.001	0.002

the TG curve obtained under nitrogen atmosphere. So, could be concluded that this residue is composed mainly of a carbonaceous material formed only in the presence of oxygen. For the gallstone samples, very similar TG curves were obtained, and for comparison the TG and DTG curves for the a gallstone sample (sample 1) are shown in Fig. 4. The observed mass loss steps for the cholesterol standard under air and nitrogen atmospheres are summarized in Table 6. The

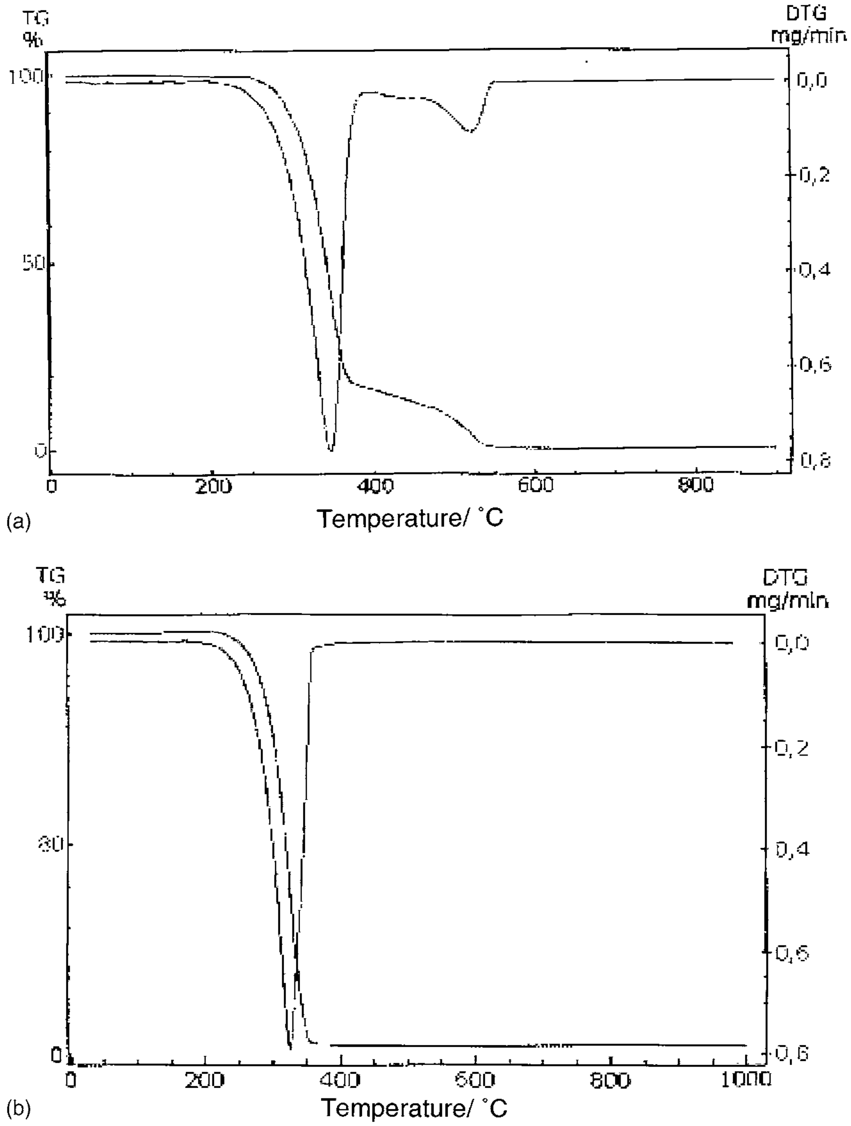

Fig. 3. TG and DTG curves for a cholesterol standard sample obtained under (a) air and (b) nitrogen atmospheres.

Table 5
Percentage composition for the main chemical substances found in the considered gallstone samples

Sample	Cholesterol	Bilirubin	Calcium bilirubinate	Calcium palmitate	Calcium carbonate
1	96	3	1	0	0
2	98	0	1	1	0
3	98	0	0	0	2
4	96	0	1	0	3
5	98	1	1	0	0
6	77	7	13	1	2
7	94	0	2	2	2
8	91	5	4	0	0

Table 6
Thermogravimetric data for the thermal degradation of a cholesterol standard sample under air and nitrogen atmospheres[a]

Atmosphere	T_i (°C)	T_f (°C)	Δm (%)	T_i (°C)	T_f (°C)	Δm (%)
Air	281	363	84.3	432	539	15.7
N_2	269	347	100.0	–	–	–

[a] T_i and T_f are the initial and final temperatures for a given thermal process.

TG data obtained for the eight considered gallstone samples are summarized in Tables 7 and 8, respectively. As can be observed on Table 7 data, for samples 3, 4, 6 and 7, a third mass loss step, not observed for the cholesterol standard sample is verified. As a general trend, the obtained results are in good agreement with some previously studied gallstone samples [4,5].

The observed mass on the TG curves obtained under nitrogen atmosphere (Table 8) are plotted as function of the cholesterol percentages, as calculated by elemental analysis results (Table 5) as shown in Fig. 5. The linear correlation observed (linear coefficient = 0.997) is a “graphical proof” that thermogravimetry can be successfully employed to determine the total amount of cholesterol on gallstone samples.

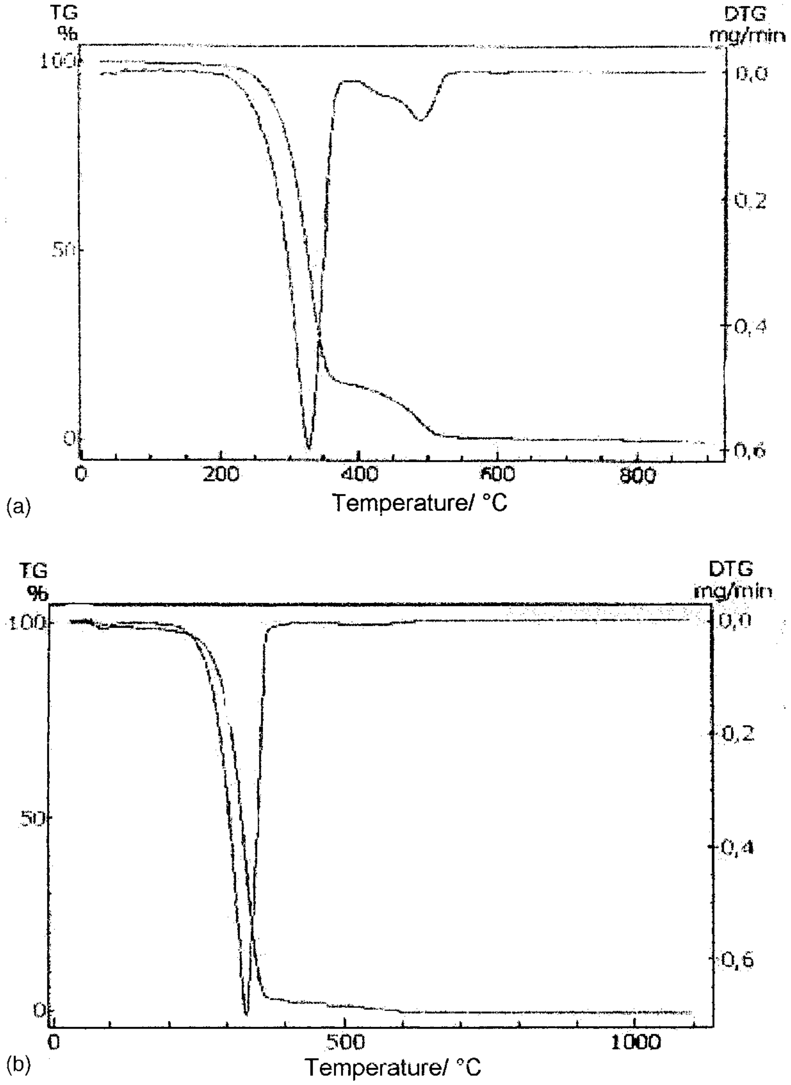

Fig. 4. TG and DTG curves for a gallstone sample (sample 1) obtained under (a) air and (b) nitrogen atmospheres.

Table 7
Thermogravimetric data for the thermal degradation of eight considered gallstone samples under air atmosphere[a]

Sample	T_i (°C)	T_f (°C)	Δm (%)	T_i (°C)	T_f (°C)	Δm (%)	B (%)	T_i (°C)	T_f (°C)	Δm (%)
1	264	354	84.4	428	513	14.4	1.4	–	–	–
2	274	362	86.2	450	521	13.8	0.8	–	–	–
3	263	357	85.0	437	516	13.1	1.9	596	655	1.1
4	268	357	83.5	447	505	13.7	2.8	633	689	1.4
5	265	357	82.1	442	515	15.7	2.2	–	–	–
6	257	351	69.8	490	561	25.8	3.2	607	689	2.7
7	262	347	79.6	451	525	15.3	5.1	660	851	1.8
8	265	354	77.3	452	517	21.5	1.1	–	–	

[a] B is the residue at 550 °C. T_i and T_f are the initial and final temperatures for a given thermal process.

Table 8
Thermogravimetric data for the thermal degradation of eight considered gallstone samples under nitrogen atmosphere[a]

Sample	T_i (°C)	T_f (°C)	Δm (%)	B (%)
1	268	363	97.2	2.2
2	270	362	98.6	1.4
3	277	359	97.6	2.4
4	286	381	96.2	3.8
5	275	362	97.4	2.6
6	251	366	74.2	25.8
7	264	360	94.8	5.8
8	260	353	90.1	9.9

[a] B is the residue at 400 °C. T_i and T_f are the initial and final temperatures for a given thermal process.

The DSC curves obtained for a standard cholesterol sample and a gallstone (sample 1) sample are shown in Fig. 6. Very similar DSC curves were obtained for all other gallstone samples. The obtained DSC data for all considered sample are summarized in Table 9. The first endothermic peak on the DSC curves are associated with the melting of cholesterol, and the second one with its vaporization and thermal degradation. As can be observed, for all gallstone sample the melting temperatures are very close to those observed for standard cholesterol, and the obtained DSC curves exhibit a very similar profile to those of the cholesterol standard sample, showing that DSC is also a reliable tool to identify the presence of cholesterol on gallstone samples, that is, for a qualitative identification. However, apparently there is not a correlation between the cholesterol content of the gallstone sample and the ΔH values.

By comparison of Figs. 4b and 6b, it can be seen that two endothermic peaks in the DSC curve are associated with only one thermal degradation process in the TG curve for a gallstone sample (sample 1). So, is supposed that the two endothermic peaks are due to vaporization and thermal degradation of cholesterol in the gallstone sample, despite the fact that in the TG curve these two processes are visualized as a single one. The presence of another compounds (4%) other than cholesterol, could be considered as responsible differences observed in the DSC curves obtained for a cholesterol standard sample and sample 1.

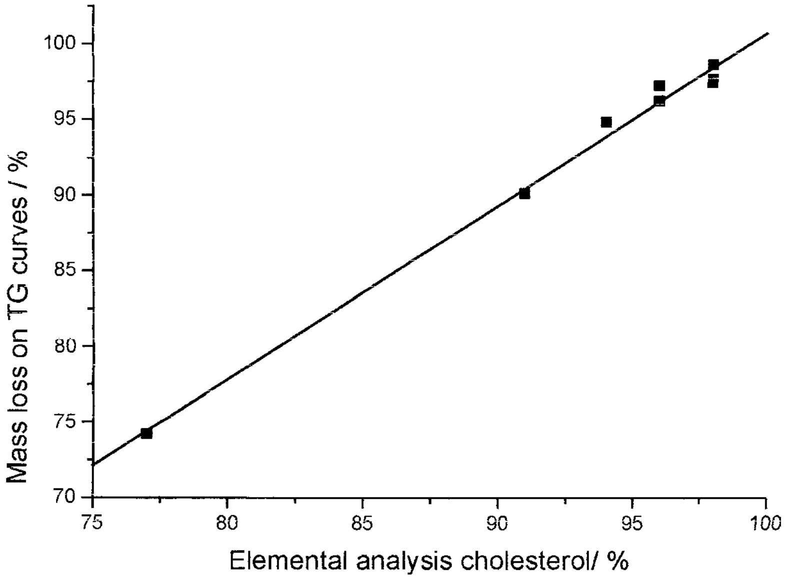

Fig. 5. Mass loss percentage on TG curves obtained under nitrogen atmospheres, as the cholesterol percentage as calculated by elemental analysis results for the eight considered gallstone samples.

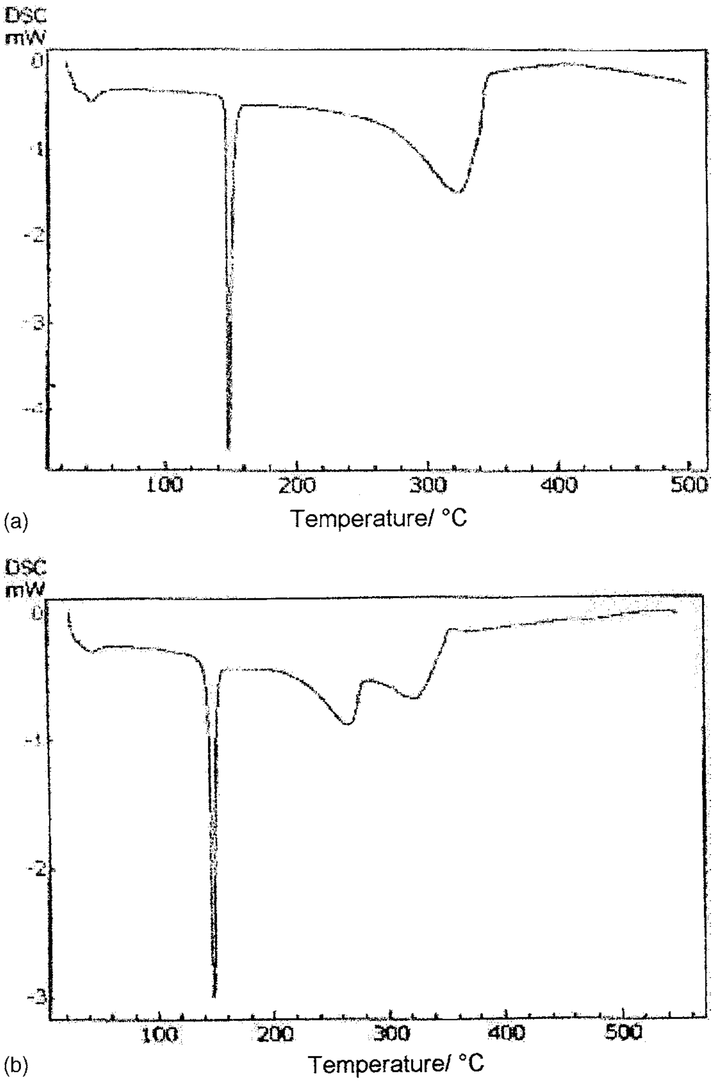

Fig. 6. DSC curves for (a) a cholesterol standard sample and (b) a gallstone sample (sample 1).

Table 9
DSC data summary for cholesterol standard sample and the eight considered gallstone samples[a]

Sample	T_i (°C)	T_f (°C)	ΔH (J g^{-1})	T_i (°C)	T_f (°C)	ΔH (J g^{-1})
Cholesterol	145	154	80	257	341	214
1	140	155	67	213	354	243
2	142	156	69	211	350	284
3	145	156	70	224	345	26
4	141	155	67	212	359	289
5	139	156	75	224	340	186
6	138	155	41	211	389	166
7	141	155	61	197	343	184
8	143	156	69	219	344	166

[a] T_i and T_f are the initial and final temperatures for a given thermal process.

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