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THERMAL ANALYSIS OF HUMAN GALLSTONE

R. Ganapathi Raman#, R. Selvaraju*

Department of Engg. Physics, Annamalai University, Annamalainagar -608 002.

***Corres.author: *drselvarajufeatau@yahoo.co.in**
#ganapathiraman83@gmail.com

ABSTRACT: Human gallstones were collected from Rajah Muthiah Medical College and Hospital (RMMC&H), Annamalai University and the stones were classified according their visual and chemical composition. Thermal properties of the classified stone samples were investigated using Thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC). From this study, the thermal analysis also used as one of the method for characterizing the human gallstones.

Keywords : human gallstone, Thermal analysis, TGA, DSC.

INTRODUCTION

Gallstones are complex biomineralized deposits formed in the gallbladder[1], is still a major health problem all over the world. 20 - 30% of western and around 10% of non-western population have been affected by gallstones disorders[2,3]. The stones were separated into the following three groups like Brown, Black and Mixed gallstones. Pigment gallstones are further subdivided into laminated brown stones and amorphous black stones[4]. Cholesterol stones contain more than 70% of cholesterol whereas pigment stones contain mainly of various bilirubinate salts with less than 20% cholesterol by weight. Black stones are formed in gallbladder and associated with hemolysis, cirrhosis and old age[5,6]. Brown and black stones are chemically, morphologically and clinically distinct. Brown stones are conveniently associated with biliary infection and are more common in elderly. Brown stones have been reported to contain Ca-bilirubinate in combination with Ca-palmitate and usually with small amounts of cholesterol. Brown stones though to be predominant in Eastern populations, especially in certain section of India[7,8], but presumably uncommon in western populations[6].

We have analyzed the major constituent of gallstones using Fourier transform infrared (FTIR) spectroscopy[9]. FTIR analysis was useful in characterizing gallstone[10]. TGA measure changes in weight that occurs to a sample as a function of temperature (^{o}C) over time. Only the limited study has been carried out for analysis of three gallstone groups by TG-DTA and DSC.

MATERIALS AND METHOD

Human gallstones were collected from adult patients at Rajah Muthiah Medical College and Hospital (RMMC&H), Annamalai University, Annamalainagar, India. All the patients belong to the same socio-economic status. The stones were washed with running deionised water for an hour to remove debris, blood and preserved under sterile conditions at 4 °C. Stones were photographed and classified according to their visual appearances and chemical composition by FTIR. Color was considered

***Corres.author:**
R.Selvaraju
Reader in Physics
Department of Physics
Faculty of Engineering and Technology
Annamalai University
Annamalainagar -608 002.
Tamilnadu, India.
Mobile no: +91 9976715576
Email – ganapathiraman83@gmail.com
drselvarajufeatau@yahoo.co.in

irrespective of stone locations. Thermogravimetric analysis were obtained under nitrogen atmosphere at a heating rate 8 °C min^{-1} in Pt cell and a gas flow of cm^3 min^{-1} on TA Instruments Model SDT Q600 apparatus in the temperature from ambient to 800 °C. TGA can be a useful and versatile instrument in a variety of fields. It has the ability to measure accurately large amount of samples, both in weight and volume.

RESULTS AND DISCUSSION

Randomly selected representative (Brown -5, Black -5, Mixed -5) gallstone samples were subjected to thermal analysis. Stones were classified by using FTIR spectroscopy as described by certain peaks[9]. The larger sample size simply provides the user a more representative sample to analyze. The representative group samples gave the similar spectra in TGA and DSC. This represent our classifications are correct.

The thermograms of representative mixed, brown and black human gallstone samples are shown in Fig 1a-3a and Table 1 shows the thermal analysis of mixed, brown and black human gallstones samples. It shows multi stage weight loss for mixed, brown, black gallstone groups, but the transition temperature varies for each groups. Residues from TGA analysis are mainly composed of carbonaceous material.

In brown and black stone groups, there is meager amount of mass loss before 295 °C, so we can conclude there is feeble amount of cholesterol or crystallized water present in the samples. In mixed stone groups the fast mass loss (~89%) between the temperature 25 and 315 °C because cholesterol is the main constituent in mixed stone so it is surely represent the loss of cholesterol, it supports the endothermic peak at 145.20 °C, this is due to melting of cholesterol. This region is related to devolatilization, during which carbon, hydrogen, and oxygen compounds are released[10]. The observed weight loss in this region may be suggested that this residue is composed mainly of carbonaceous material formed only in the presence of oxygen. After weight loss of 89% it was observed that there was no more thermal degradation, which may be ascribed due to stability of chemical substances like calcium bilirubinate, calcium phosphate or hydroxapatite present in the sample in the range of temperature. Another mass loss (9%) at mixed stone group started around ~340 °C and ended with 573 °C, this is due to some other organic matter present in the sample.

In brown stone samples, transition temperatures are in between 25-68 °C, 68-298 °C, 298-566 °C and 506-727 °C respectively, for black stones at 25-93°C, 93-292 °C and 292-665 °C respectively. Mass loss in the temperature between 25 and 95 °C may due to loss of water of hydration. The slow mass loss between 140 and 315 °C are due to cholesterol melting. In pigment gallstone cholesterol is minor quantity so mass loss is very slow. Above the temperature 400 °C, inorganic (calcium palmitate, calcium oxalate, calcium bilirubinate and some salts of calcium) constituents are retained in the sample, this was confirmed by Rautray *et al*[10]. This are also supports by DTA spectrum of endothermic peak of 145.20 and 303.22 °C in mixed gallstones group, and exothermic peak around 341, 439 and 513 °C in brown gallstones group and around 357 and 517 °C for black gallstone groups.

Residue in the gallstone samples, for mixed 2.156±0.635%, for brown 5.011±2.732%, for black 32.588±2.716%. The minimum residue percentage for mixed stone is because of it contains more than 80% cholesterol by weight. In brown and black stone contain bilirubin complexes, calcium phosphate or hydroxyapatite; these are thermally stable even after 800 °C. Gallstones are due to biomineralization, contains complex of molecules, so it could not say losses due to certainty of composition.

The phase transitions of gallstones were studied using DSC. The DSC thermogram of representative mixed, brown, and black human gallstone samples are shown in Fig 1b-3b. The calorimeter was computer controlled to allow automated DSC control data acquisition, and analysis. Few milligrams of sample sealed in one pan, and similar empty pan was used as the reference. The first endothermic peak on the DSC curve are associated with the melting of cholesterol, and second one with its vaporization and thermal degradation. Cholesterol monohydrate loses its water of hydratetion at 86 °C, shows a transition to smectic liquid crystalline phase at 124 °C, and finally melts at 156 °C[11]. For all mixed gallstone samples, the melting temperatures are very close to those observed for standard cholesterol in previous study[12,13] so it showing that DSC is also a reliable tool to identify the presence of cholesterol on gallstone samples, i.e., for a qualitative identification[14].

However, apparently there is no correlation between the cholesterol content of the gallstone sample and the ΔH values.

CONCLUSION

From the present investigation, Cholesterol is the main constituent of gallstone. Thermal analysis also the tool to analyze gallstone samples qualitatively and quantitatively. The melting range of cholesterol is varies from 147-150 °C, the phase transition of cholesterol also identified in the temperature range. Similar appearing stones might contain different amounts of various constituents. There is no drastic change between brown and black pigmented gallstones in the temperature between 25-500 °C, later more residues for black and less for brown stone found. Thermal analysis also used as tool for classification of human gallstones.

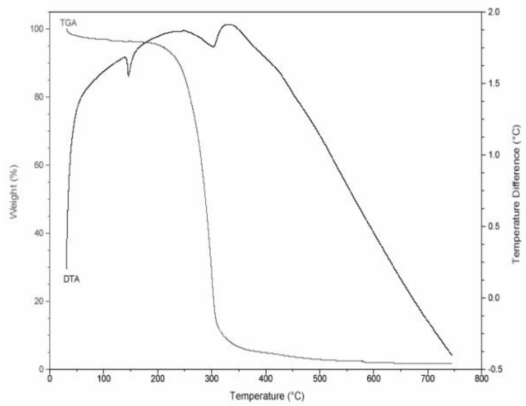

Fig 1a

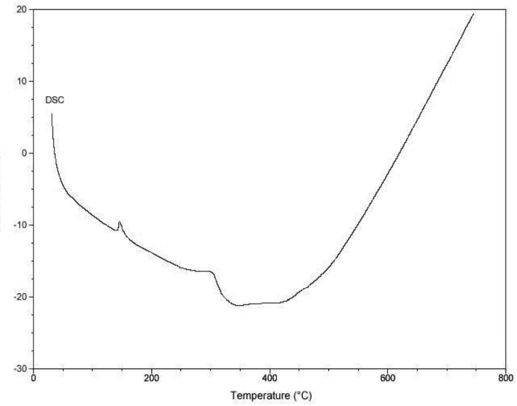

Fig 1b

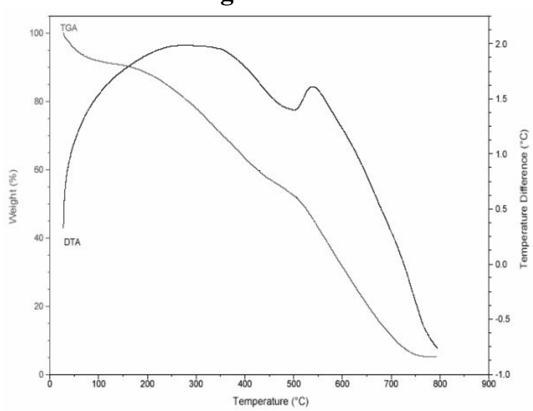

Fig 2a

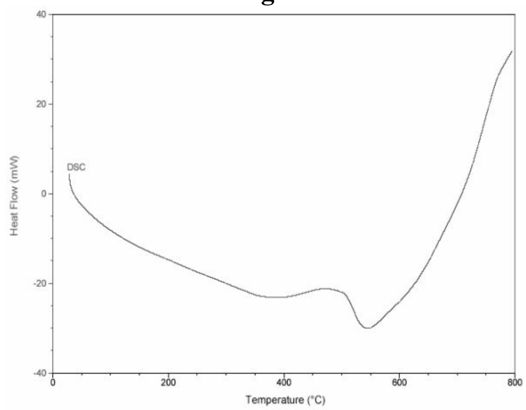

Fig 2b

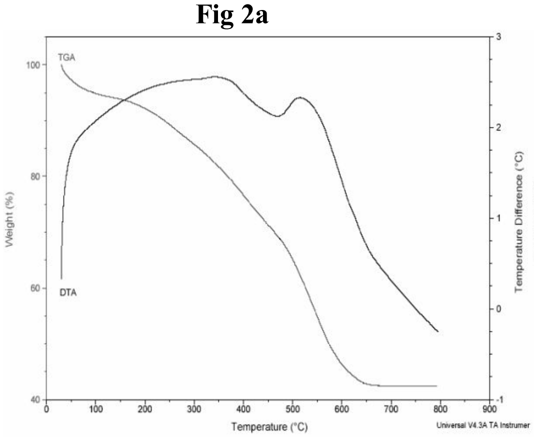

Fig 3a

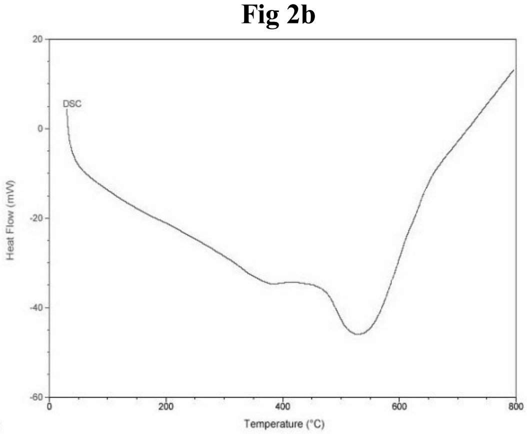

Fig 3b

Fig 1a-3a Thermogravimetry analysis and 1b-3b Differential Scanning Calorimetry analysis of representative mixed, brown and black gallstone group

Table 1 Thermo gravimetry analysis of Human gallstone samples

Sample id	T_i (°C)	T_f (°C)	Mass (%)	T_i (°C)	T_f (°C)	Mass (%)	T_i (°C)	T_f (°C)	Mass (%)	T_i (°C)	T_f (°C)	Mass (%)	T_i (°C)	T_f (°C)	B (%)
Mixed-1	25.00	122.85	94.95	122.85	260.12	91.43	260.12	341.25	10.50	341.25	520.86	4.95	520.86	800.00	1.65
Mixed-2	25.00	118.65	96.25	118.65	258.54	90.58	258.54	343.58	13.58	343.58	518.69	6.43	518.69	800.00	3.13
Mixed-3	25.00	118.59	96.81	118.59	255.84	93.16	255.84	349.24	9.25	349.24	515.73	2.33	515.73	800.00	2.46
Mixed-4	25.00	120.68	95.62	120.68	263.78	92.91	263.78	339.63	11.58	339.63	521.49	2.98	513.45	800.00	1.85
Mixed-5	25.00	119.89	96.48	119.89	257.23	92.57	257.23	342.75	12.69	342.75	518.65	3.84	518.65	800.00	1.69
Brown-1	25.00	68.54	93.55	68.54	295.70	78.65	295.70	505.28	51.76	505.28	727.30	7.53			5.05
Brown-2	25.00	66.41	95.65	66.41	292.60	80.52	292.60	503.50	55.65	503.50	716.90	12.56			9.51
Brown-3	25.00	67.55	93.95	67.55	299.82	82.63	299.82	498.60	57.32	498.60	721.50	8.29			2.24
Brown-4	25.00	65.86	95.62	65.86	298.60	79.67	298.60	501.69	54.58	501.69	715.69	11.58			4.57
Brown-5	25.00	63.84	96.51	63.84	293.70	76.85	293.70	506.80	52.65	506.80	712.35	9.65			3.69
Black-1	25.00	90.16	92.47	90.16	286.70	86.37	286.70	665.17	32.07						31.34
Black-2	25.00	90.16	95.15	90.16	291.60	84.65	291.60	643.24	42.98						33.33
Black-3	25.00	93.54	97.84	93.54	292.40	82.95	292.40	654.98	34.80						31.77
Black-4	25.00	91.88	90.86	91.88	287.20	79.86	287.20	664.60	31.85						29.66
Black-5	25.00	94.20	95.48	94.20	288.30	80.54	288.30	661.50	39.60						36.85

T_i & T_f - Initial and final temperatures for a given thermal process
B – Residual mass
Mass (%) - % of mass retained in the specific temperature

Table 2 Differential Scanning calorimetry analysis of Human gallstone samples

Sample	T_i (°C)	T_f (°C)	T_{max} (°C)	□□ (Jg^{-1})	T_i (°C)	T_f (°C)	T_{max} (°C)	□□ (Jg^{-1})	T_i (°C)	T_f (°C)	T_{max} (°C)	□□ (Jg^{-1})
Mixed-1	124.68	168.54	145.73	55.52	254.20	340.88	303.22	303.22	398.54	480.62	428.40	174.90
Mixed-2	126.58	167.18	146.01	48.05	264.65	334.51	297.16	143.80	456.30	582.46	524.21	1401.00
Mixed-3	128.65	170.54	146.23	146.23	260.54	350.48	308.40	110.10	514.89	576.54	540.42	168.30
Mixed-4	129.84	169.65	148.84	34.63	270.59	358.78	340.20	252.80	434.65	590.40	502.30	815.10
Mixed-5	226.42	170.58	147.65	62.58	266.92	354.22	318.54	180.64	468.21	586.40	538.41	486.40
Brown-1	310.58	427.54	346.24	327.00	498.64	626.34	542.11	1187.00				
Brown-2	306.51	430.48	349.96	567.80	488.54	628.54	536.07	1723.00				
Brown-3	308.46	434.15	346.58	234.63	477.64	625.56	538.45	252.80				
Brown-4	304.25	429.10	342.50	186.40	486.10	632.70	539.24	1564.00				
Brown-5	301.48	436.40	343.89	215.05	483.54	629.10	540.58	1486.00				
Black-1	235.45	425.35	350.41	495.60	438.48	539.68	285.01	206.40				
Black-2	305.74	430.23	375.16	420.20	467.54	597.12	532.05	2899.00				
Black-3	301.45	384.80	344.50	253.00	420.46	489.52	441.13	702.00				
Black-4	308.58	429.67	365.80	264.10	425.80	523.86	465.80	584.00				
Black-5	306.48	431.59	367.54	158.40	436.58	542.18	458.65	284.00				

T_i & T_f - Initial and final temperatures for a given thermal process

□□ enthalpy

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