International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.3, No.1, pp 149-154, Jan-Mar 2011

Study on the Analysis of Human Gallstones using Fourier Transform Infrared Spectroscopic Technique

S.Sikkandar[1]*, S.Jayakumar[2], S.Gunasekaran[3], T.S.Renugadevi[4] and B.Alwar[5]

[1,2]Department of Physics, R.K.M.Vivekananda College, Chennai-600004, India.
[3]Periyar University , Salem-636011, India.

[4]Department of Physics, Women's Christian College, Chennai-600006, India.

[5]Department of Chemistry , D.G.Vaishnav College, Chennai-600106, India.

Corres.author: sikkandar_khan@yahoo.com, Phone No. 00919840395950

Abstract: The present study is to investigate the composition of gallstones from human population. Gallstone samples removed Laparoscopically were washed, dried and analyzed for composition by Fourier Transform Infrared (FTIR) spectrometry and by Qualitative analysis to identify various bio-chemical components. Results based on FTIR spectrometry and Qualitative analysis suggests that cholesterol and bile pigment either with or without calcium content are the most predominant components in gallstones. Chemical analysis of gallstone using FTIR spectroscopic technique and Qualitative method often clues to the pathogenesis of the gallstone disease. Results obtained using FTIR Spectra is well correlated with that of result obtained by Qualitative analysis.
Keywords: Gallstones, Chemical composition, FTIR, Qualitative analysis, Cholesterol, Bilirubin and Calcium carbonate.

1. INTRODUCTION

The gall bladder is a pear shaped digestive storage organ that is situated under the liver on the upper right side of the human abdomen. Its job is to store and slowly release bile into the digestive system for the digestion of fats. When we eat fat, both the liver and the gall bladder excrete bile to the stomach for digestion. Once digestion stops, and if the gall bladder is not emptied out completely, the bile that remains in the gall bladder can become too concentrated with cholesterol and gallstones are created. Gallstones are one of the most common problem associated with the gall bladder and can become dangerous if left untreated. Gallstone disease remains a serious health concern for human beings, affecting millions of people throughout the world[1, 2]. Removal of the gall bladder by surgical method is the only solution available to the gallstone disease today. The identification of the components of gallstone is essential as it provides information that could be useful for medical practitioner to find out the underlying cause of gallstones and to decide whether to treat gallstone patients surgically or therapeutically [3-8] .Recent years, there have seen an increasing trend in the number of gallstone cases. Absorption measurements based upon Infrared radiation find widespread application for the composition determination of large variety of inorganic and organic species .FTIR spectroscopy has been widely employed for structural analysis on variety of bio molecules. No reagents are required in FTIR based analytical methods, which is an advantage over chemical based analytical methods. FTIR spectrometry provides a simple and rapid means for analyzing many chemical substances of interest in the biosciences. . In the recent past, the use of FTIR method has enhanced our understanding in different branches of medicine. Since the pathogenesis of gallstones is not clearly understood, its analysis using FTIR spectroscopic technique and Qualitative analysis method have provided some clues. The results based on this study will help to improve our understanding on the pathophysiology of gallstone diseases. This study is mainly aimed to define the patterns of gallstone

Composition in different types of gallstone samples using FTIR technique. There are many interesting reports in the literature on the characterization of gallstones[2-5] .Though, the investigation on gallstone samples are done by many, not much work is done by FTIR spectroscopy. The present study aims to quantify the spectral difference between the types of gallstone samples using FTIR spectroscopic technique. Also, in the present study, we present comparative results on the characterization among the various types of gallstones using FTIR technique.

2. COMPOSITION OF GALLSTONES

The gallstones are solid crystalline precipitates in the biliary tract usually formed in the gall bladder. Gallstones may occur as one large stone or hundreds of tiny stones almost in any combination. The two main substances involved in gallstone formation are cholesterol and calcium bilirubinate [9].Gallstones derived from bile consist of mixture of cholesterol, bilirubin with or without calcium. Gallstones found in the gall bladder are classified as cholesterol, pigmented or mixed stones based on their chemical composition. Gallstones can be mostly white, yellow, brown, black and green colored[10]. Approximately 80% of the gallstones are cholesterol gallstones, which chiefly consist of cholesterol plus bile salts. Cholesterol stones are usually green, but are sometimes white or yellow in color. These are divided into two sub type as pure (90-100%cholesterol) or mixed
(50-90% cholesterol).Cholesterol gallstones develop when bile contains too much cholesterol and not enough bile salts. Pure stones often are solitary, whitish, and larger than 2.5 cm in diameter. Mixed stones usually are smaller, multiple in number, and occur in various shapes and colors. The remaining 20% are usually referred to as pigmented gallstone, which chiefly consists of bilirubin (the pigment) and calcium salts such as calcium carbonate. These occur in two sub types brown and black. Brown stones are usually made up of calcium bilirubinate.Black stones typically form in the gall bladder result when excess bilirubin enters the bile and polymerizes into calcium bilirubinate.

Bilirubin stones are formed from cholesterol and bilirubin. They tend to develop in people who have cirrhosis, biliary tract infections and hereditary blood disorders such as sickle cell anemia[11].Composite (Mixed) stones also occur in the gall bladder. ie. Those consisting of a mixture of cholesterol, bilirubin and calcium.Diets high in cholesterol and fat, increase the chance of developing stones. Gallstones have high prevalence among elderly adults. Unfortunately gallstone composition is heterogeneous and varies with and amongst the populations around the world [3-8].

3. MATERIALS AND METHODS

The materials utilised for this study were gallstones removed from patients by expert surgeons using Laparoscopic cholecystectomy surgery. The extracted gallstones were placed on sterile gauze to air dry, and then washed carefully with doubly distilled de-ionized water (to remove bile and debris) and dried over silica gel transferred into a paper envelope bearing the name, age and sex of the patient as well as the date. Stone specimens were first examined for shape, size and color. They were classified as cholesterol, black or brown pigmented stones. After noting the morphological features, single gallstone from each patient (heaviest ones in case of multiples) is taken for stone analysis. This process produces a fine homogeneous powder stored in a sample tube, kept over silica gel in dark cabinet until analyzed for composition[12, 13]. All stone samples were stored in sterile dried condition and later used for FTIR Spectroscopic analysis and Quantitative analysis for the identification of various bio-chemical components. IR transparent KBr material without the sample was scanned as background for each spectrum and 10 scans were added at spectral resolution of 2 cm^{-1}. The FTIR measurements were performed and spectra were analyzed at Sophisticated Analytical Instrumentation Facility (SAIF), IIT-Madras using Perkin-Elmer Spectrum-one FTIR Spectrometer in the frequency range of 400 – 4000 cm^{-1} at 2 cm^{-1} resolution .For each measurement 4-8mg of finely powdered stone sample was used to make KBr discs. The collected signal was transferred to the personal computer .The data were processed by windows based data program spectrum software. The evaluate menu provided in the FTIR spectrometer software performed the entire process automatically. The spectra were baseline corrected and they were normalized to acquire identical area under the curve and the minimum absorption values of the characteristic bands were noted. The gallstones were analyzed for the identification of bio-chemical components by Qualitative analysis method in the clinical laboratory at Hi-tech diagnostic centre, Chennai.

Table 1 : Identification of gallstones by Qualitative analysis method

Label of the sample	Color of Gallstones	Cholesterol + Bile Pigments	Calcium	Carbonate
A	White	Present	Absent	Absent
B	Brown	Present	Absent	Absent
C	Whitish Brown	Present	Absent	Absent
D	Dark Brown	Present	Present	Absent
E	Black	Present	Present	Absent
F	Greenish Black	Present	Present	Present

Table 2 : Infrared vibrations band frequency assignment of gallstones by FTIR analysis

Vibrational frequency in cm^{-1}	FTIR Band Assignments	Literature values [13,14,16] in cm^{-1}
Bands due to Cholesterol		
3392	CH asymmetric stretching of CH_2	3410
2936	CH asymmetric stretching of CH_3	2725
2899	CH symmetric stretching of CH_3	2860
2866	CH symmetric stretching of CH_2	2860
1464	CH bending of CH_2	1460
1367	CH bending of CH_3	1380
1052	C-C stretching	1050
Bands due to bilirubin		
1660	OC=O stretching	1670
1627	C=O carbonyl stretching	1626
1574	C=C stretching	1575
1250	C-O-C stretching	1244
1171	C-H in plane bending	1167
1023	C-C-H plane bending	1021
936	C-C ring stretching	923
877	C-H out of plane bending	879
838	C-H out of plane bending	832
Bands due to calcium and carbonate		
1498	C-O stretching$CaCO_3$	1481
1435	C-O stretching $CaCO_3$	1445
1196	C-C stretching	1166
1135	C-O stretching of $CaCO_3$	1143
1052	C-C stretching	1050
882	C-O bending of $CaCO_3$	875
854	C-O bending of $CaCO_3$	855
837	C-O bending of $CaCO_3$	819

4. RESULTS

The composition of gallstones identified by Qualitative analysis method is presented in Table 1.The Infrared vibration bands frequency assignment of gallstones from the present study along with literature values are characteristically and systematically assigned in Table 2.

4.1 FTIR SPECTROSCOPY OF BROWN AND BLACK GALLSTONES FROM ADULTS

IR Spectra of black stones showed characteristic bands of bilirubinate salts in the region between 1500-1700 cm^{-1}.The stretching vibrations of C=C(at1574 cm^{-1}),C=O carbonyl group(at 1627 cm^{-1}),OC=O (at1646 and1660 cm^{-1}) arising from bilirubinate salts[14]. Also, the presence of triplet (1574, 1627, 1660cm^{-1}) with two additional bands observed between 1500-1700 cm^{-1}, confirms the presence of bilirubinate . The strong bands around 1052 cm^{-1} from the brown type stones as well as black stone shown in the Fig.1 confirm the existence of cholesterol .Cholesterol in the brown and black stones was also characterized by the bands between 2800-3000cm^{-1} due to C-H stretching vibrations of CH2 and CH3 group[15]. From the absorbance of black and brown stones in the region between 1500 -1800 cm^{-1}, it can be deduced that in adults, the bile pigment content of black stones was much higher than that of brown stones. In addition, brown stones had a higher cholesterol / bile pigment ratio compared to the black stones. The FTIR spectral analysis of black stones from adults showed that bile pigments were dominant in black stones and cholesterol in mixed stone. The FTIR overlaid spectra of brown and black gallstones are shown in Fig.1.

4.2 FTIR SPECTROSCOPY OF BLACK AND GREENISH BLACK GALLSTONES FROM ADULTS

For the first time, a greenish color black gallstone from an adult patient was identified. It was green in color all over the surface and black in color inside the gallstone. The spectrum for the greenish black stone in the region between 1400 -1800 cm^{-1} was entirely different from the black stone. This is due to the varying cholesterol content of these stones. Black stones contained low amounts of cholesterol and higher quantities of bile pigments. In contrast, cholesterol was a major component in the greenish black stone, with much less content of bilirubin. This is evident in the spectra in the region between 2800-3000 cm^{-1} as shown in Fig.2.Results show that black stones from adults are rich in bilirubin. Green stones removed from an adult which is very rare, were found to be composed mainly of cholesterol. Surprisingly, the composition of white stone is similar to that of the greenish black stone having a very high content of cholesterol. In addition, dominant bands at 1052 and 854 cm^{-1} indicated that the greenish black stone had a high content of cholesterol and calcium carbonate.$CaCO_3$ present in green stones give rise to two characteristic IR bands at 1498$cm^{-1}$and 854 cm^{-1}due to C-O stretching and bending vibrations respectively[13,19]. The FTIR overlaid spectra of black and greenish black gallstones are shown in Fig.2.

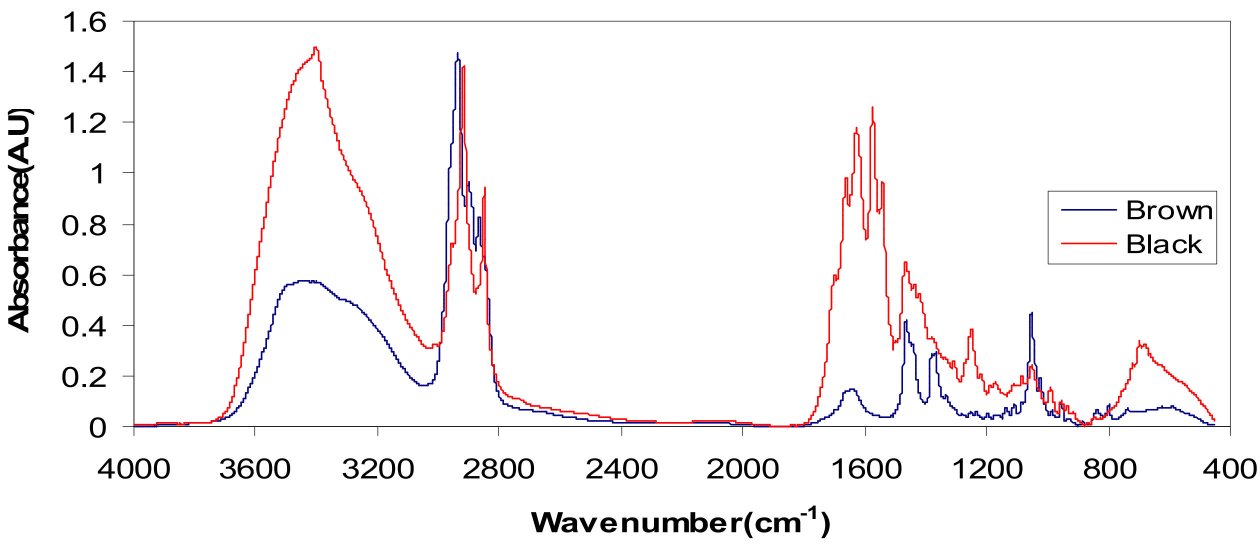

Figure 1 : Overlaid FTIR Spectra of brown and black gallstone

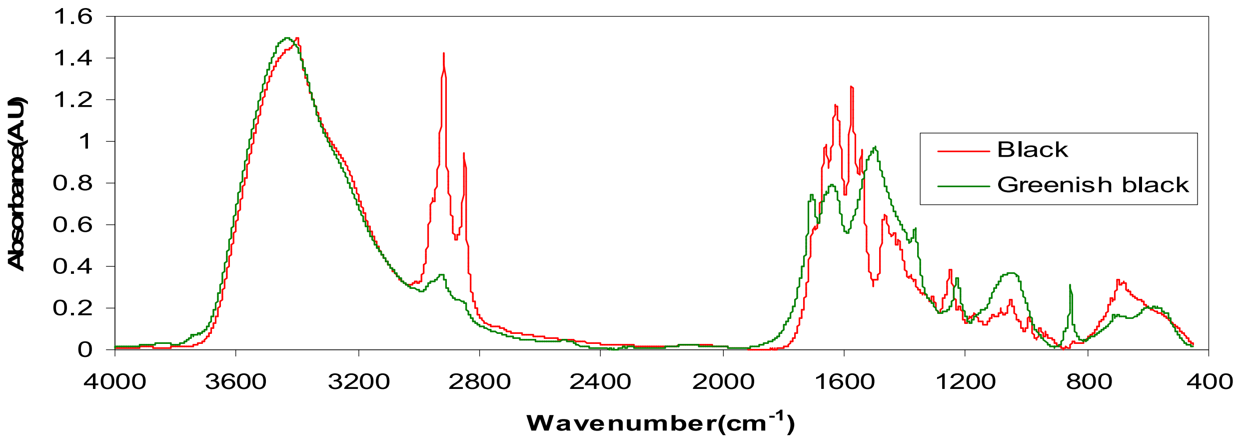

Figure 2 : Overlaid FTIR Spectra of black and greenish black gallstone

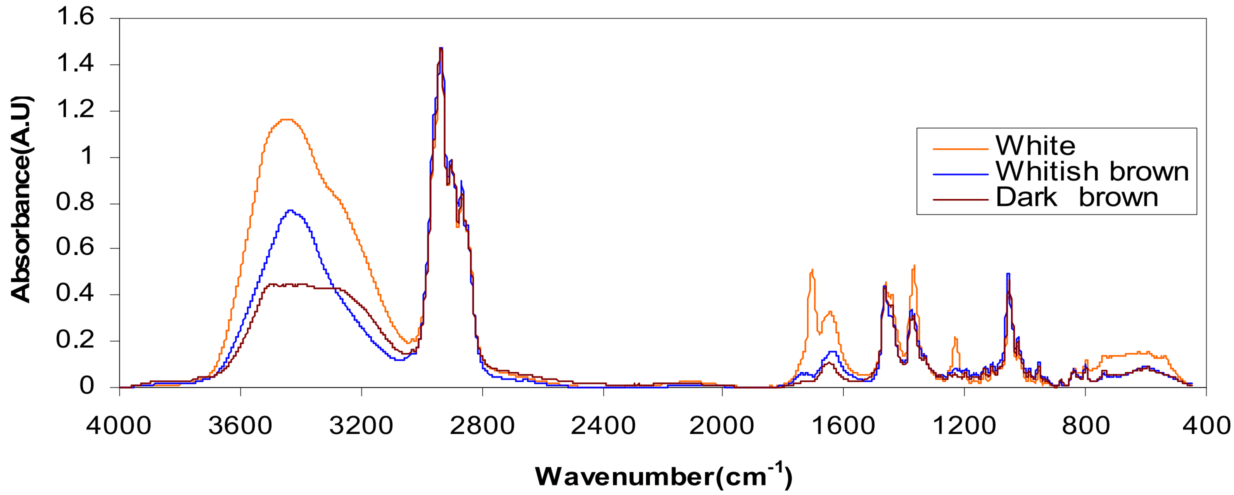

Figure 3 : Overlaid FTIR Spectra of White, Whitish brown and dark brown gallstone

4.3 FTIR SPECTROSCOPY OF WHITE, WHITISH BROWN AND DARK BROWN GALLSTONES

The gallstones which contained more than 80% cholesterol were classified as pure cholesterol gallstones [14].Pure cholesterol gallstones were characterized by the bands around 2936, 1464 and 1052 cm^{-1}. The spectra of the mixed composition stones showed the presence of higher cholesterol content (whitish brown and dark brown gallstones), which is evident by the higher absorbance was characterized in the region between 2800 –3000 cm^{-1} due to C-H stretching vibrations of CH2 and CH3 groups[16] and lower absorbance in the regions 1500-1700 $cm^{-1}$for bilirubin .The FTIR overlaid spectra of white, whitish brown and dark brown gallstones are shown in Fig.3.

5. Discussion

The characteristic band features and key band locations for the components of gallstones in the present investigation were in accordance with the reported in the Literature[14,16, 17]. Pure cholesterol gall stones were not common, only gallstones containing cholesterol, bile pigments mixed with or without calcium were mostly found. Black pigment stones were dark brown or black or greenish black small multiple, and had an irregular surface, they were chiefly composed of calcium bile pigments. The results on black stones suggest that the composition of bilirubin and cholesterol varies considerably. This is clear from Fig.2 where black stone has a higher bile pigment, calcium and a lower cholesterol content. This was observed consistently in other black stones obtained from adult patients. Infra-red Spectroscopy was used to show that black and brown pigment gallstones differ in micro-composition, suggesting that they are formed by different mechanisms. Infra-red Spectroscopy showed that the calcium salts were present in gall stones in compound forms as calcium carbonate and calcium bilirubinate.

6. REFERENCES

1. Harding, AJ., Gallstones :Causes and Treatments. William Heinemann Medical Books ,London, 1964, 42-56.

2 .Kern F, Jr.Epidetmiology and natural history of gallstones. Semin Liver Dis.1983, 3, 87-96(Pub Med)

3 .Kratzer W, Mason RA, and Kachela V. Prevalence of gallstones in Sonographic Surveys world wide. J. Clin. ultrasound 1999,27,1-7.

4 .Walker TM, Hambleton IR, Serjeant GR.Gallstone in sickle cell disease: Observations from the Jamaican cohort studies.

5. Akute OO, Marinho AO, Kalejaiye AO. Prevalence of gallstones in a group of antenatal Women in Ibadan. Nigeria Afr J Med Sci. 1999,3(4),159-61.

6. Kaloo AN, Kanstevoy Sr. Gallstones and biliary disease.Primcare.2001;28:591-606.

7. Stringer MD, Taylor DR, Soloway RD, Gallstone composition: are children different ?. J .Paediatr. 2003 , 142,435-440.

8. Kim MH, Lim BC, Myung SI, Epidemic logical study of Korean gallstone disease .A Nation wide Co-operative study . Dig. Dis. Sci .1999, 44:1674-1683.

9. “Gall bladder Problems” St. Lukes Episcopal Hospital Regi.3, March, 1998.

10. Cholelithiasis article updated August 2, 2006- Author Douglas M. Heuman, M.D, FACP, Director of Hepatology , Virginia Common Wealth University School of Medicine.

11. Management of Gallstones & their Complication by Aijaz Ahmed, M.D.Ramsey.C .Cheung M.D. and Emmet B. Keeffe,M.D. Stanford University school of Medicine, California Published in American Family Physician Clinical Journal, 15, March,2000.

12. The Liver and Biliary System by Antony W.Volwiler, R.A.Wilson, A.M.Larsenand J.D. Ostrow.Cell. Mol. Biol. 1998, 44, 65-73.

13. Kleiner O, Ramesh J, Huleihel M. A Comparative study of gallstones from Children and Adults using FTIR spectroscopy and fluorescence microscopy. BMC gastroenterology 2002,2(3),1-14.

14. Zhou XS, Shen GR, Wu JG. A Spectroscopic study of pigment gallstonesin China. Biospectroscopy 1997, 3,371-80.

15.Wentrup_byrne E , Chana - Annsorn W, St Pierre TG. A Spectroscopic study of thalassemic gallstones. Biospectroscopy 1997, 3, 409-416

16. Identification of gallstones using Spectroscopic technique-S.Kothai, K. Gayathri, V. Kannappan, P.T. Perumal and K. Manimegalai .International Journal of chemTech. Research 2009, 1(3), 430-435

17. Analysis of human gallstones by Fourier Transform Infrared (FTIR)- Naseem A. Channa , Fateh D. Khand,Tayab U Khand, Muhammed H. Leghari, Allah N.Memon. Pak. J. Med. Sci. 2007 , 23(4),546-550.

*****