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Identification of Gallstones using Spectroscopic technique

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Abstract: : Gallstone formation is the primary underlying disease that results in gall bladder illness. Gallstone formation in the gall bladder is a common disease and constitutes a major health problem world wide. There are two major types of gallstones - cholesterol stones and pigment stones. Knowing the composition of gallstones, crystallized samples are prepared and the results are compared. . The aim of the study is to assess the constituents and their composition of gallstones using infrared spectroscopy.
Keywords : Gallstone, Gall bladder, Spectroscopic technique.

1. Introduction

Gallstones are clumps of solid material that are formed in the bile stored in the gall bladder. The main function of the gall bladder is to concentrate bile by the absorption of water and sodium. Cholesterol stones are composed primarily of cholesterol. Pigment stones are composed of bilirubin and other substances such as calcium, which are found in the bile. The occurrence of gallstone disease is 2-3 times more common in women than in men[1]. The disease is especially common in certain sections of India and China, areas of high incidence are found in southern United States and western coast of South America. The causes for this striking variation in geographic incidence is obscure, through differences in diet and climate are frequently mentioned as possible factors[2].

Infrared spectroscopy is one of the most powerful analytical techniques, which offer the possibility of chemical identification. One of the most important advantages of infrared spectroscopy over the other usual methods of structural analysis is that it provides useful information about the structure of molecules quickly without tiresome evaluation methods[3,4]. The infrared region of the spectrum encompasses radiation with wave numbers ranging from about 12,800 to 10cm^{-1}. The large majority of analytical applications of infrared region are confined to the limited portion between 4000-400 cm$^{-1.}$ For a molecule to absorb IR the vibrations or rotation with a molecule must cause a net change in the dipole moment of the molecule. IR was employed for the first time by Beischer in the year 1955 for investigating urinary stones[5]. With the advent of computer technology and the introduction of Fourier Transform Infrared (FTIR) to the clinical laboratory, sensitivity of the measurements has been greatly improved and the analytical time shortened.

2. Materials and methods

The domains of biochemical science may be broadly viewed to encompass a number of specialties, which differ greatly in the types of problems that are encountered and the means employed towards their solution. Human gallstones were collected from adult patients and were preserved under sterile conditions. The stones were cleaned carefully to remove all foreign materials and ground to a fine powder by an agate mortar. The main compositions of the gallstones were found to be cholesterol, bilirubin, calcium carbonate and calcium oxalate[6]. By knowing the composition of gall bladder stones obtained from the patients, stones were crystallized in the chemical laboratory and used for FTIR spectral studies. FTIR spectra were taken using the powder of the gallstones obtained from patients and crystallized samples. The infrared spectra of the samples were recorded at CLRI, Chennai, India using Perkin-Elmer Spectrum-one FTIR spectrometer under identical conditions.

3. Results and Discussion

The representative FTIR absorption spectra of the black, brown, yellow and mixed gallstones and crystallized samples are presented in Fig.1-5 respectively. The chemical components and its corresponding IR absorption bands of gallstones are characteristically and systematically assigned in Table 1 and 2.

3.1 FTIR Spectroscopy of black and brown stones:

Cholesterol in the black and brown stones was characterized by the bands between 2800-3000cm^{-1} due to asymmetric and symmetric stretching of $-CH_2$ and $-CH_3$ vibration as presented in Table 1. Black stones show characteristic bands in the region between 1500 - 1700cm^{-1} due to the stretching vibrations of C=C, CO and C-N at 1572cm^{-1}, 1626cm^{-1}, 1661cm^{-1} respectively arising from bilirubinate salts[7,8]. Also the triplet between 1500-1700cm^{-1} explicitly indicates the presence of bilirubinate in the metal complexed form whereas a doublet characterizes the unconjugated bilirubin.

3.2 FTIR spectroscopy of brown and yellow stones:

The FTIR spectrum for the yellow stones shows peaks, which are in the region around 1500-1700cm^{-1}. The triplet in that region which is an indication of bilirubinate in the metal complexed form is missing in the spectrum of yellow stone. It indicates that composition of cholesterol present in component is varying. Brown stones are composed of varying amounts of cholesterol and bilirubin and the corresponding peaks show varying intensity, which agrees with our experimental results. In contrast, the cholesterol was the major component of the yellow stone with less content of bilirubin.

3.3 FTIR Spectroscopy of black and mixed stones:

The spectra of black stones showed characteristic IR bands for bilirubin in the region between 1500-1700cm^{-1} as reported in earlier results[9,10]. The peak intensity around 1600cm^{-1} regions is stronger in black stone compared to mixed stone. This shows that black stones are rich in bilirubin. In contrast, the spectra of the mixed stones showed the presence of higher cholesterol content, which is evident by the higher absorbance in the region between 2800-3000cm^{-1} and lower absorbance in the region 1500-1700cm^{-1} for bilirubin.

3.4 FTIR Spectroscopy of black stones and crystallized samples:

The peak intensity (0.1534a.u) around 1600cm^{-1} region is higher in black stone compared to the absorbance found in the crystallized sample spectrum. In contrast, the spectra of crystallized samples show a higher intensity peak around 2800-3000 cm^{-1} which shows the presence of cholesterol[11]. The band at 2933 cm^{-1} and 2899 cm^{-1} due to $-CH_2$ asymmetric and $-CH_3$ symmetric stretching IR bands pertaining to cholesterol indicate that crystallized samples are rich in cholesterol and show the presence of small quantity of bilirubin.

4. Conclusion

FTIR spectral studies on gallstones characterize the composition present in different types of gallstones. The major challenge is medical prevention of gallstone formation in susceptible individuals, although cholecystectomy reminds the cornerstone of treatment. Hence studying the gallstone disease will certainly contribute to understand its pathogenesis and hence prevention.

TABLE 1 : Constituents of the Crystallised sample

Label of the sample	cholesterol %	calcium carbonate %	calcium oxalate %	Bilirubin %
A	90	5	-	5
B	90	-	5	5
C	80	15	-	5
D	80	-	15	5
E	80	10	-	10
F	80	-	10	10
G	75	20	-	5
H	70	20	-	10
I	60	30	-	10
J	50	40	-	10

TABLE 2 : Frequency assignments to gall stones

Frequency In cm^{-1}	FTIR Band Assignments
Bands due to Cholesterol	
3396	CH_2 asymmetric stretching
2866	CH_2 symmetric stretching
2933	CH_3 asymmetric stretching
2899	CH_3 symmetric stretching
1463	CH_2 bending
1371	CH_3 bending
1052	C-C stretching
Bands due to bilirubin	
3396	CH_2 asymmetric stretching
2866	CH_2 symmetric stretching
2933	CH_3 asymmetric stretching
2899	CH_3 symmetric stretching
1463	CH_2 bending
1371	CH_3 bending
1052	C-C stretching
1626	C=O carboxylate group
1570	C=C stretching
1244	C-O-C stretching
1167	C-H in plane bending
1021	C-C –H plane bending
923	C-C ring stretching
879	C-H out of plane bending
832	C-H out of plane bending
465	C-C-C bending
Bands due to oxalate, phosphate and carbonate	
1443	C-O stretching of $CaCO_3$
1331	C-O stretch of calcium oxalate
1228	PO_2^{--} asymmetric stretching
1166	C-C stretching
1143	C-O stretching of $CaCO_3$
1052	C-C stretching
994	PO_4^{--} absorption band
882	C-O bending of $CaCO_3$
879	C-O bending of $CaCO_3$
819	C-O bending of $CaCO_3$

FIGURE 1: Representative FTIR spectrum of black gallstone

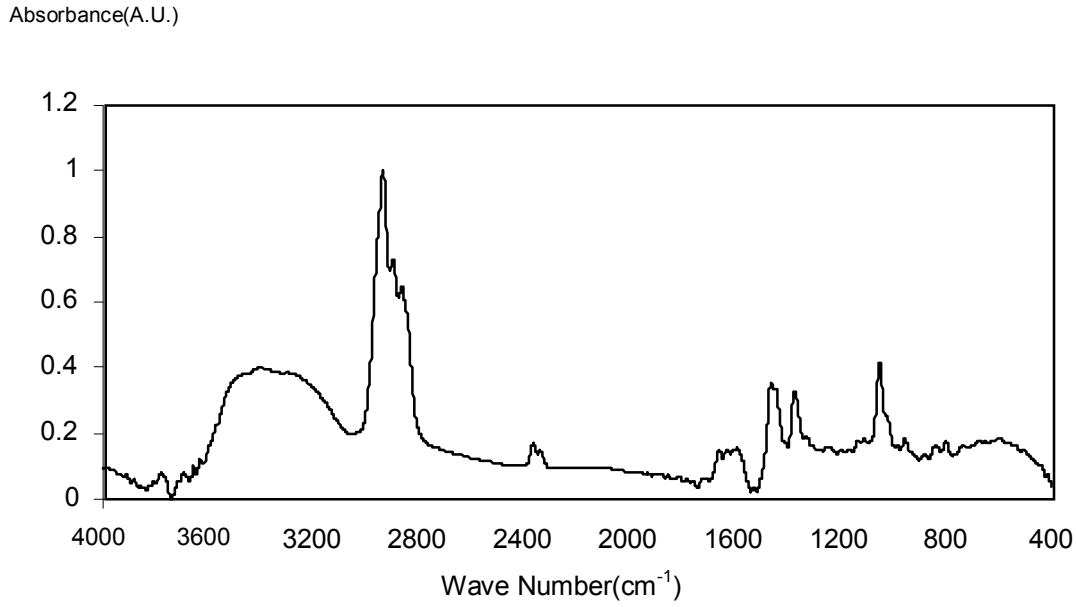


FIGURE 2: Representative FTIR spectrum of brown gallstone

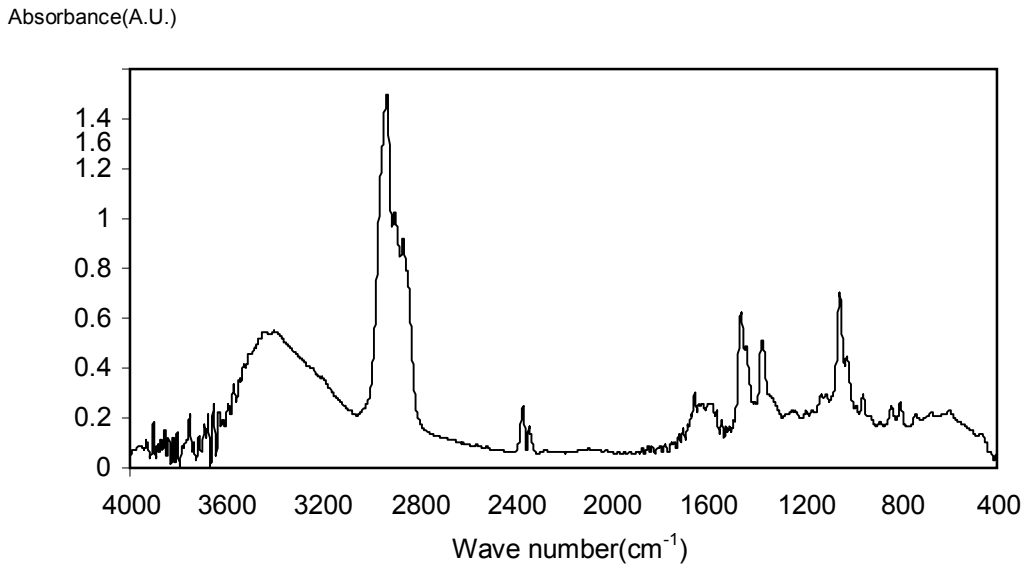


FIGURE 3: Representative FTIR spectrum of yellow gallstone

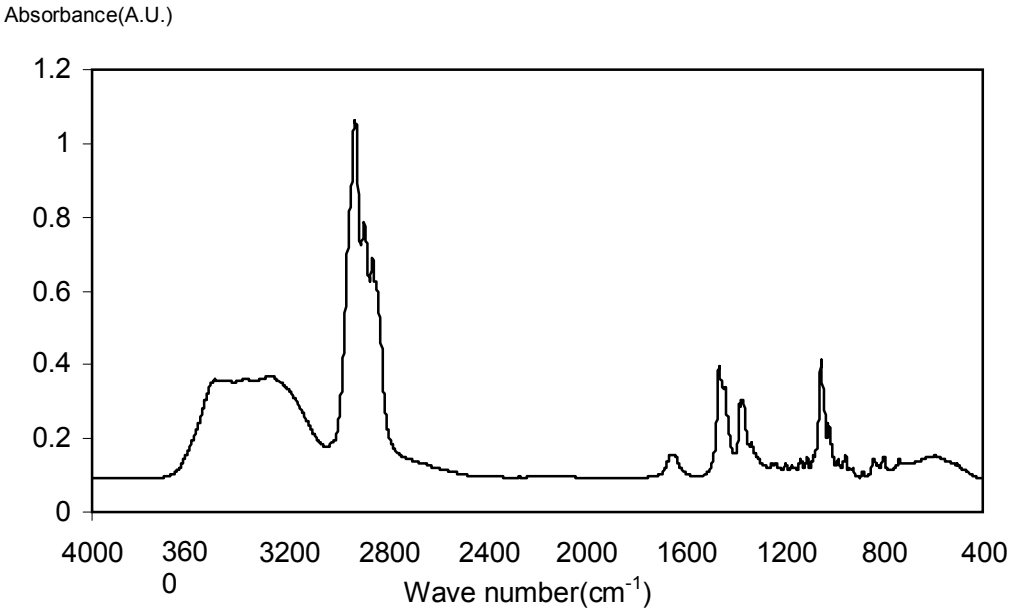


FIGURE 4: Representative FTIR spectrum of mixed gallstone

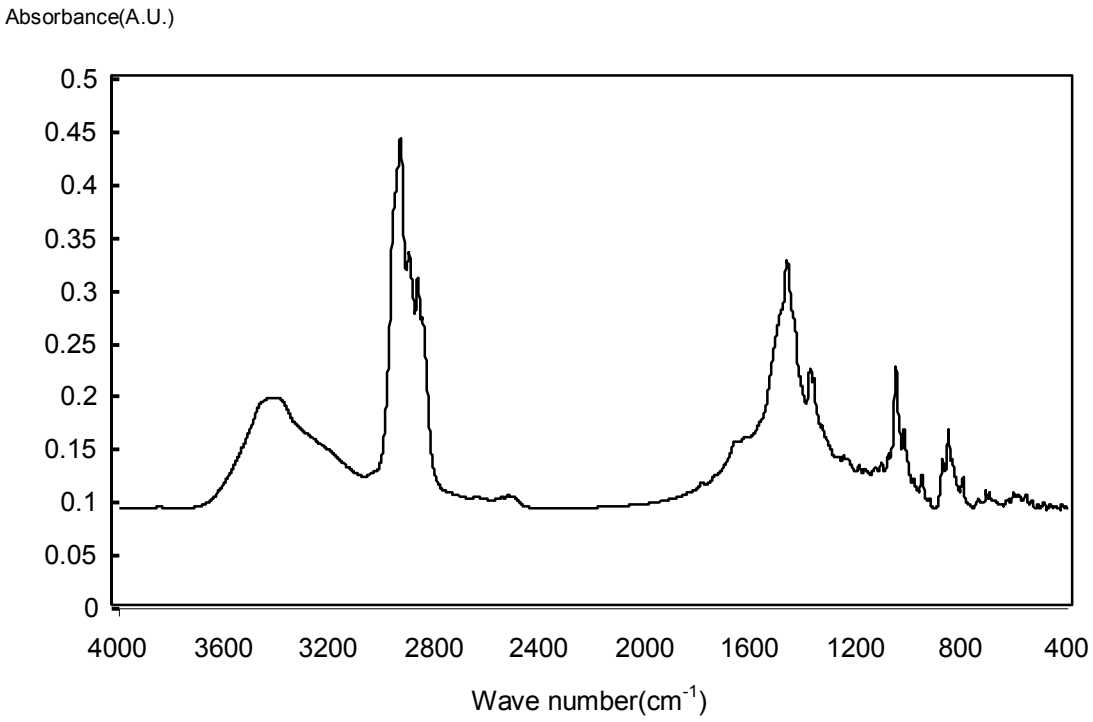
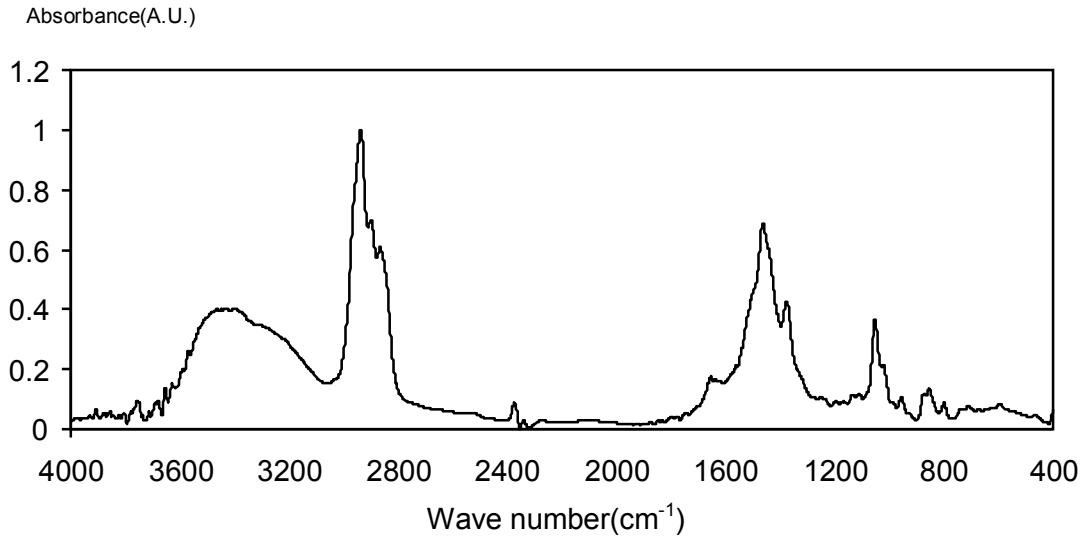


FIGURE 5 : Representative FTIR spectrum of crystallised sample



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