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Novel Role of Fourier Transform Infrared and Chemical Analysis in Differential between Gallstone Diseases

ANTESAR RHEEM OBEAD[1], MOHEND A.N. AL-SHALAH[2], MAHA F. SMAISM[3*]

[1]*Clinical Biochemistry Dept., College of Medicine, University of Babylon, Iraq.*

[2]*Surgery Dept., College of Medicine, University of Babylon, Iraq.*

[3*]*Clinical Biochemistry Dept., College of Medicine, University of Babylon, Iraq.*

[3*]*mahasmaism@gmail.com*

Abstract.

Background: The aim of the present study was to evaluate component of variety color of gallstone samples using Fourier Transform Infrared Spectroscopic Technique (FTIR), also study the cost and simple method of Fourier Transform Infrared Spectroscopic Technique (FTIR) compare with result of quantitative chemical analysis like total cholesterol, calcium, total bilirubin, total protein.

Material and method: The material in the study were gallstones removed from 140 patient (94 female, 46 male) after surgical. All samples were stored in sterile dried counter and used for FTIR spectral analysis and quantitative chemical analysis.

Result: The results of FTIR were suggested that cholesterol and mixed gallstones content were a major component, cholesterol gallstones were characterized by the band 2949,1456,1053 cm^{-1}, while in mixed stones the band of cholesterol between 2800-3000 cm^{-1} due to asymmetrical stretch vibration of CH_2 and CH_3group, and quantitative chemical analysis were explained the cholesterol stones and mixed stones were higher cholesterol compared to pigmented stones which were richer in total bilirubin and total protein.

Conclusion: The investigation were suggested thatFourier Transform Infrared Spectroscopic Technique (FTIR) is the less cost and simple method to find component of differential gallstone samples and this method conformed the cholesterol and mixed with calcium carbonate, bilirubin and total protein is the major component of human gallstones from Babylon, Iraq.

Keywords. Gallstone, FTIR, Qualitative Analysis, Total Cholesterol, Total Bilirubin, Calcium, Total Protein.

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INTRODUCTION

The most prevalent gastrointestinal diseases is Gallstone disease (GD), which is characterized by the formation of gallstones in the hepatic bile duct, or bile duct, or (1). Gallstones (GS) were formed due to a wide range of disorders(2) and impaired metabolism of cholesterol, Bilirubin and bile acids which is characterized by the formation of stones(3,4).The composition of gallstone is identification to provide information about cause of gallstone and to decide treatment gallstone patients by surgical or therapeutically (5-8) . FTIR spectroscopy is utilized for basic investigation to determine inorganic and organic compound (9). No reagents were required in FTIR based analytical strategies(10,11) .The present study is aim to evaluate component variety type of gallstone samples utilizing FTIR spectroscopic method , and study the novel characterization of FTIR spectroscopic compare with result of quantitative chemical analysis for total cholesterol, total bilirubin, calcium and total protein .

MATERIAL AND METHOD

In this study, the material utilized were gallstones after removal gallbladder from patient by surgically (Iraq, Babylon Hospital). The gallstones were placed in sterile contour and exposure to air dry and washing by deionized water to remove bile and debris. Gallstone samples were classified into cholesterol, black or brown pigment and were process to produce fine homogeneous powder of gallstone samples. A 10mg stone powder was placed in 3 ml chloroform in a test tube to determine total cholesterol and total bilirubin. the cylinder was kept in bubbling water shower for 2 min.

A10 mg stone powder was disintegrated in 3 ml IN HCl in proceeded10 ml cylinder and its last volume was made up to 10 ml with refined water (12)., the cylinder was kept in bubbling water shower for one hour to determine calcium, solvent protein,. total cholesterol were measured by enzyme colorimetric technique for CHOD PAP strategy, France (13), bilirubin were measured by colorimetric technique for Accurex Biomedical Pvt. Ltd (14), solvent protein were measured by colorimetric technique for Biuret, Biolabo Reagent, France (15) calcium were measured by OCPC unit strategy for Miles India Ltd.(16), the stones were put away at 2-8°C, when not being used, and another stone examples were put away in clean dried condition and later utilized for FTIR Spectroscopic investigation and for identification of different component of gallstone samples. The potassium bromide (KBr) material was transparent IR without the example was checked as for each sample and were added at spectra for resolution 2 cm^{-1}.

The FTIR estimations were performed and spectra were analyzed at SHIMADZU, in the frequency range 400 – 4000 cm^{-1} at 2 cm^{-1}resolution.

RESULT

The identification of composition of gallstone samples were presented by qualitative analysis method in table (1) and infrared vibration band frequency assignment of gallstone by FTIR analysis in table (2).

Table 1. Identification of gallstones by quality analysis method

Label of the sample	Color of Gallstone	Type of stone	Number of gallstones
1	**Whitish Brown**	**Cholesterol**	**41**
2	**Black**	**pigment**	**16**
3	**Brown**	**pigment**	**20**
4	**White**	**Mixed**	**13**
5	**Yellow**	**Mixed**	**33**
6	**Greenish black**	**Mixed**	**20**

Table 2. Infrared vibration band frequency assignment of gallstone by FTIR analysis;;

Wave number(cm^{-1})	FTIR Band Assignments
Bands due to cholesterol in cholesterol, pigment and mixed group	
3450	**Free O-H Stretch**
3412	**CH Asymmetrical stretch of CH_2**
2949	**CH Asymmetrical stretching of CH_3**
2883	**CH Asymmetrical stretch of CH_3**
1631	**CH Asymmetric stretching of CH_2**
1456	**CH Bend of CH_2**
1371	**CH Bend of CH_3**
1053	**C-C Stretch**
Bands due to bilirubin in pigment group	
1624	**OC=O Stretch**
1448	**C=O Carbonyl stretch**
1384	**C=C Stretch**
1049	**C-H in plane bend**
860	**C-C Ring stretch**
694	**C-H out of plane bending**
599	**C-H out of plan bending**
Bands due to calcium and carbonate in mixed group	
1629	**C-O Stretch of $CaCO_3$**
1462	**C-O Stretch $CaCO_3$**
1373	**C-C Stretch**
1055	**C-O Stretch $CaCO_3$**
954	**C-C Stretch**
837	**C-O bend $CaCO_3$**
800	**C-O bend $CaCO_3$**
599	**C-O bend $CaCO_3$**
Band due to protein	
1247	**Strong amide III**

FTIR Spectroscopy for brown and black stones

The bands of bilirubin were showed in region between 1500-1700cm-1 in black stones and extending of vibrations in stretch C=C(at1373cm^{-1}),C=O carbonyl group(at 1462 cm^{-1}),OC=O (at1624 cm^{-1}) arising from bilirubin (17).Also two bands between 1500-1700 and the strong groups around 1055 cm^{-1}were confirm the presence of bilirubin and in black stones were appeared the Fig.1.Cholesterol in the brown and dark stones were additionally described by the bands between 2800-3000cm^{-1} because of C-H symmetrical vibrations of CH_2 and CH_3 group (18)

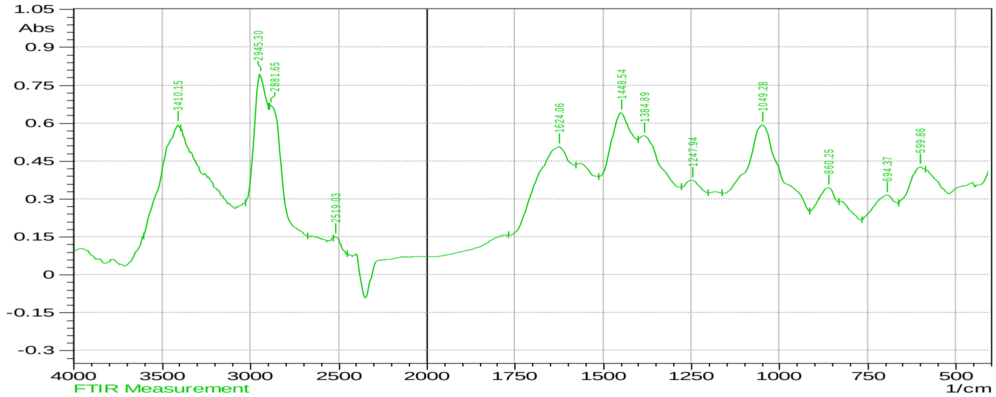

Figure 1. FTIR spectra of black gallstone

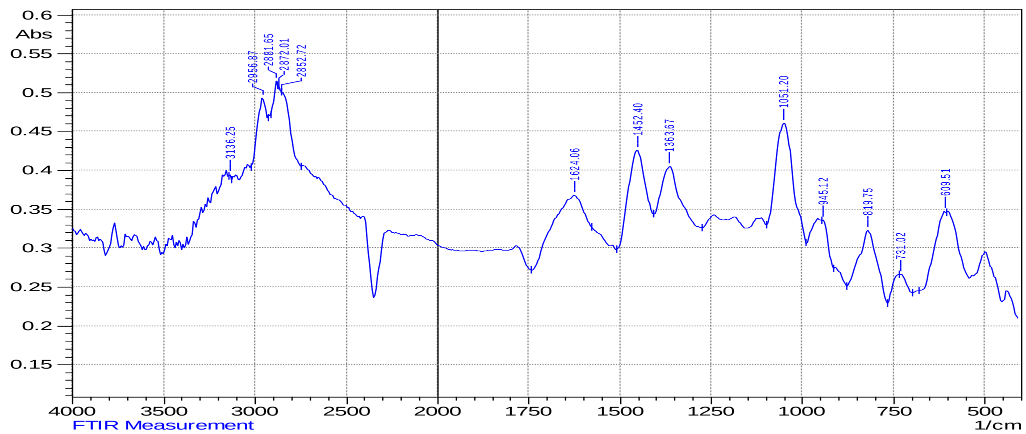

Figure 2. FTIR spectra of brown gallstone

FTIR Spectroscopy for greenish black stones

The bands of cholesterol in greenish black stones and yellow were appeared in region 2500-3500 cm-1 due to the different cholesterol content of these stones significantly. Also The component of white stones were like that of the greenish dark stones had been high amount of cholesterol. The stretch of$CaCO_3$ waspresentin green stones and yellow at 1629cm^{-1}and 599 cm-1due to C-O extending and bending vibrations (19,20). The FTIR spectra of greenish dark stones and yellow stones were performed in Fig.3.,fig4 respectively.

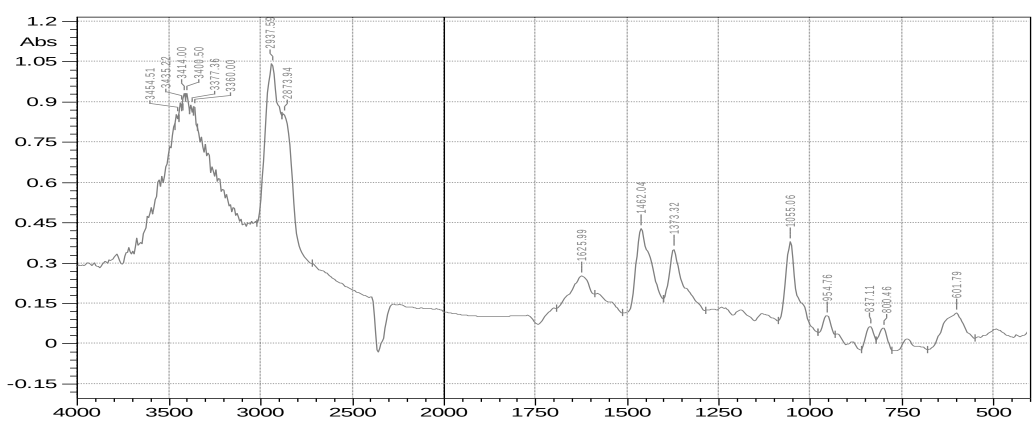

Figure 3. FTIR spectra of Greenish black gallstone

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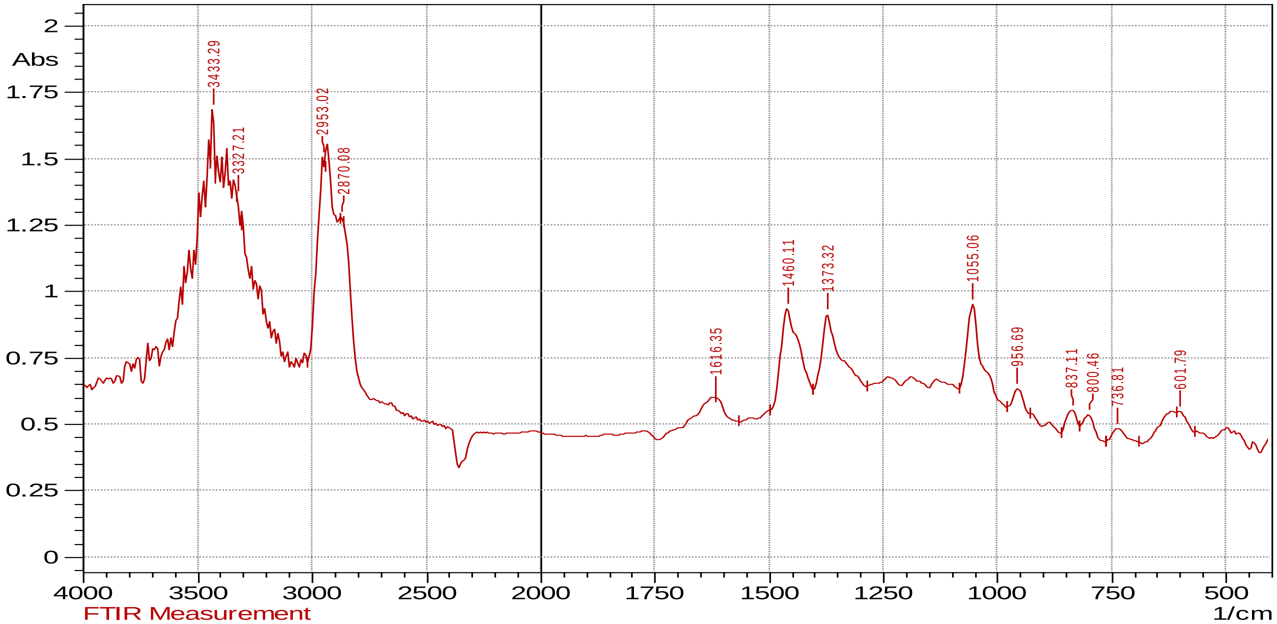

Figure 4. FTIR spectra of yellow gallstone

FTIR Spectroscopy for white and whitish brown stones

The stones were contained over 80% cholesterol were called cholesterol stones. The bands around 2949, 1456 and 1053 cm^{-1}were described pure cholesterol stones. A spectra for blended stones were demonstrated higher a cholesterol component (whitish black, yellow and greenish stones),which were apparent by the more high absorbance and designated in a region between 2800 – 3000 cm^{-1} because of the C-H extending vibrations for CH_2 and CH_3 groups(17).The FTIR overlaid spectra of whitish brown were appeared in Fig.5.

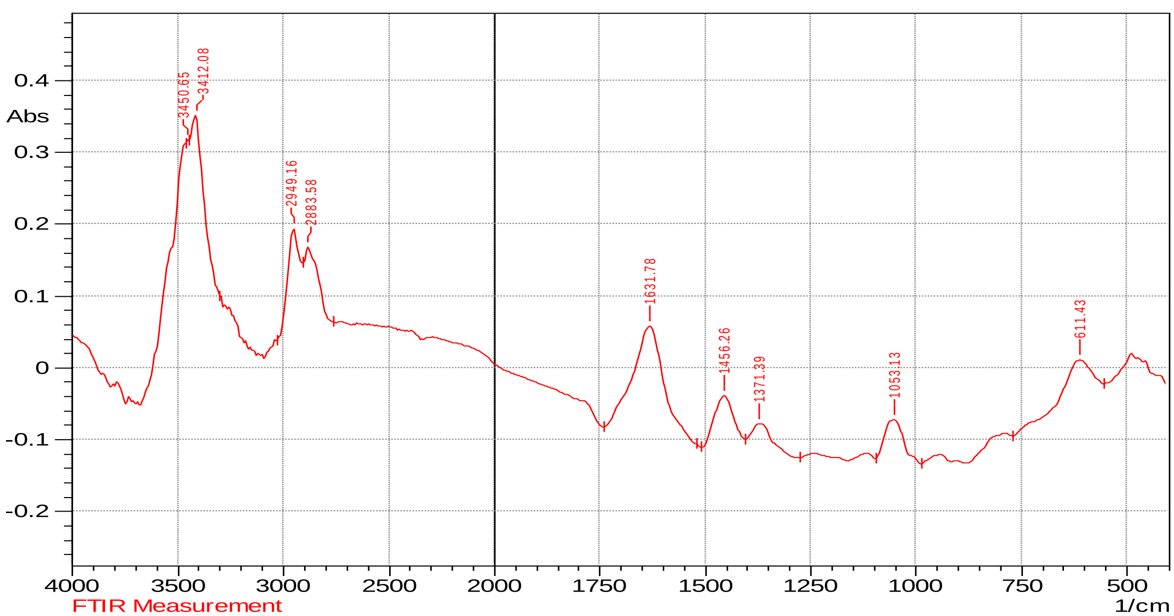

Figure 5. FTIR spectra of Whitish Brown gallstone

Quantitative chemical analysis in table (3) was showed significantly of the total cholesterol more high in cholesterol stones when total cholesterol compared to pigment stones (p<0.0001) and in mixed stones as compared to pigment stones significantly (p<0.002).

Table 3. Quantitative analysis of metabolites in dry stone powder (mg/gm)

Parameter mean± SD	Type of stones			p-value
	cholesterol	mixes	pigment	
NO.	41	63	36	
Cholesterol(mg/dl)	522.8±21.2	442±34.4	263.3±46.3	0.002* 0.0001** 0.064***
Calcium (mg/dl)	8.7±1.7	16.9±2.8	10.9±1.3	0.003* 0.244** 0.0001***
Bilirubin (mg%)	2.1±0.5	1.4±0.22	4.2±0.98	0.0001* 0.010** 0.0001***

* Comparison between cholesterol and mixed group.

**Comparison between cholesterol and pigment group.

***Comparison between mixed and pigment group. P-value > 0.0001

DISCUSSION

In the present investigation for the components of gallstones which of the characteristic band features and key band locations were agreement with the Sikkandar(7), Young (17) and Kleiner (20).A brown or black or greenish black stones were mainly composed of cholesterol and additional bilirubin or calcium. A results of black stones were suggest which the composition of bilirubin and cholesterol were differs noticeably .

In the pigment stones were most high of the total bilirubin concentration and lowermost in mixed stones and were agreement with Channa (21). The total bilirubin In pigment stones compared to mixed stones and cholesterol stones were significantly higher (p<0.0001) and the total cholesterol was significantly higher in cholesterol stones as compared with pigment stones (p<0.001).The interpretations were agreement with Varanasi (22), Kanpur (23), Delhi (14) and Haryana (21).the soluble protein content in pigment stones were high and low in mixed stone. The protein content were significantly more high in pigment stones (p<0.0001) were compared to mixed stones and cholesterol stones were compared to mixed stones (p<0.01) . Binette *et al* (24)to facilitate the formation of stones were proposed that the protein were be candidates in mixed gallstones, the mean calcium content were higher and when compared pigment stones and mixed with cholesterol stones were significantly higher (p<0.003) . In different gallstones, were the calcium content for mixed stones more than pigment stones and more than Cholesterol stones.

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