Elemental Analysis as a Strong Tool for the Determination of Gallstones Composition

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Abstract. Elemental analysis of C, H, N, S and Ca was used for the classification of gallstones from 94 patients from the Western Region of Saudi Arabia. Fourier transform infrared spectroscopy (FT-IR) was used to provide rapid qualitative information about the gallstones composition. The density, color and surface of the stones were found to give a good indication of stones composition. From the studies made, it was found that 64.9% of the stones analyzed were cholesterol stones, 25.5% were mixed stones, which contain cholesterol and some calcium salt. The rest of the stones were rare stones which contain either calcium carbonate or a mixture of calcium carbonate and ammonium and magnesium salt.

Introduction

Gallstones are formed in the biliary tree, usually in the gall bladder. They occur when certain biliary solutes such as cholesterol or calcium salt precipitate as solid crystals that subsequently grow and aggregate within the mucine layer lining the gall bladder.

There are two major categories of gallstones; cholesterol stones and calcium stones. Cholesterol stones consist of crystalline cholesterol ($C_{27} H_{45}$ OH-M.W. 386.67) with variable amounts of other components including mucine glycoprotein, and calcium bilirubin. The predominant components of calcium stones are calcium salts of organic and inorganic anions, especially bilirubin ($C_{33} H_{36} N_4 O_6$-M.W. 584.67). Black gallstones are mostly hard, dense and brittle. They are composed of calcium bilirubinate with inorganic calcium salt of carbonate and phosphate. Some of these stones contain substantial proportion of calcium soaps of fatty acids.

Cholesterol stones represent about 80% of all gallstones in United States[1], 46% in Southeast Asia[2], 100% in Himalayas (Sikkim and north Bengal)[3], less than 2% in Coimbatore District of Tamil Nadu State[4], 34% in Ghana[5], 56% in Sanata Cruz, Bolivia[6], 94% in Northern India[7]. In Siena, Italy[8], out of 1226 gall bladder stones 94 were black pigment gallstones Out of these, 13 patients were found to have black pigment gallstones and cholesterol gallstones in their gall bladders.

The knowledge of the chemical composition of gallstones is very important from the viewpoint of litho-genesis and possible medical therapy of stone dissolution.

Different techniques have been used for the determination of the components of the gallstones. Thermogravimetry has been used for the determination of cholesterol, calcium carbonate and calcium oxalate[9]. Enzymes method was used for the determination of total cholesterol in gallstones[10]. FT-IR was used as the main analytical technique for the determination of human gallstone structural composition[11]. Computed tomography (CT) was used for the analysis of gallstone composition *in vivo*[12]. Correlation between chemical analysis and *in vitro* CT scan was investigated[13]. The analysis of minor and trace elements in the gallstones was made using neutron activation analysis (NAA), proton induced X-ray emission (PIXE) and X-ray fluorescence spectroscopy (XRF)[14]. Also inductive-coupled-plasma atomic emission spectroscopy (ICP-AES) and atomic absorption spectroscopy (AAS) were used for the determination of Co, Cr, Cu, Fe, Mn, Ni and Zn in gallstones after digestion with HNO_3 and H_2O_2[15].

Experimental

Gallstones were received from the Faculty of Medicine at King Abdulaziz University Hospital, Jeddah, Saudi Arabia. Any possible superficial contamination of the stones was removed by washing with distilled water after which the stones were dried in oven at 50ºC for 24 h. The physical properties were recorded including the weight, number, color, shape and surface. Photographs of the stones were taken to document any special feature. In all cases only one stone was taken for analysis. Half of the dried stone was ground carefully to a fine powder in porcelain mortar. The resulting powder was analyzed by FT-IR and elemental analyzer. The density of each stone was measured on the other half of the stone using density bottle.

Physical Measurements

1 – *FT-IR Analysis:* About 5 mg of the dry stone powder was ground with 25 mg of pure KBr (Analytical grade) in an agate mortar. The resulting powder was put in the sample cavity of Perkin Elmer Spectrum RXI-FT-IR System. Background correction was made with pure KBr.

2 – *C,H,N and S Analysis:* was made using Perkin Elmer Elemental Analyzer (model 2400).

3 – *Al, Ba, Ca, Cu, K, Mg, Mn, Na, Sr and Zn Analysis:* Exact weight of the stone powder was heated in a porcelain crucible at 700ºC using muffle furnace. The residue was digested with 5 ml concentrated HNO_3 (Analytical grade) for one hour on a water bath, then evaporated to dryness and dissolved in dilute HNO_3 (0.1 M). The solution was transferred to a 25 ml volumetric flask and the volume was completed to the mark using 0.1 M HNO_3. The blank was prepared in the same way. Standard solutions of Al, Ba, Ca, Cu, K, Mg, Mn, Na, Sr and Zn were prepared using the nitrate salt of these metals (Analytical grade). Na and K were determined using a Perkin Elmer, model 5000, atomic absorption spectrometer. The rest of metals were determined using a Perkin Elmer inductively coupled plasma spectrometer, model Optima 4100-DV.

Results and Discussion

The stones from 94 patients from the Western Region of Saudi Arabia have been studied. 86.17% of the patients were women and 13.83% were men. 40.43% of the cases have single stone only, 8.51% of the cases have two stones, 25.53% of the cases have between 3-10 stones and more than 10 stones in the rest of the cases. In one case 40 stones were found.

Different shapes of the stones were observed. 44% of the stones were spherical, 18% egg shape, 11% almost cubic, 11% pyramidal shape, 8% different shapes and the rest were crushed pieces.

The color of the stones was observed too. 67% of the stones were brown, 13% were black and the rest of the stones were either white or light gray.

The surface of the stones was smooth in 80% of the cases, rough in 17% of the cases and needle like in three stones.

The photographs of some of these stones are shown in Fig. (1). The figure shows the different shapes and different colors of some studied stones.

FT-IR Spectra

FT-IR spectra provide a rapid qualitative information about the chemical composition of gallstone[11]. Figure (2) is the spectrum of stone No. 44 which shows bands at 3372, 2936, 1465, 1377 and 1057 cm^{-1} is almost similar to the spectra of pure cholesterol. Figure (3) is the spectrum of stone No. 15 which exhibits bands at 3286, 2929, 2517, 1793, 1487, 1082, 876 and 713 cm^{-1} is similar to the spectrum of pure $CaCO_3$. Fig. (4) is the spectrum of stone No. 70 which gives bands at 3282, 1665, 1250, 1036 and 564 cm^{-1}, is an indicative of the presence of N-H group.

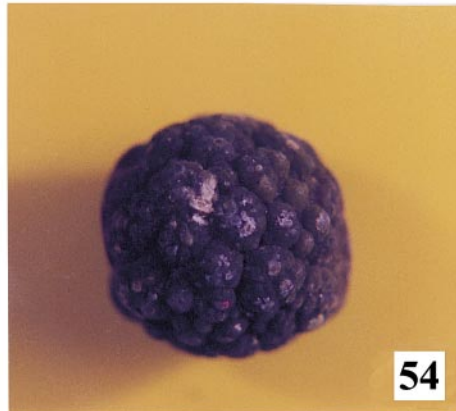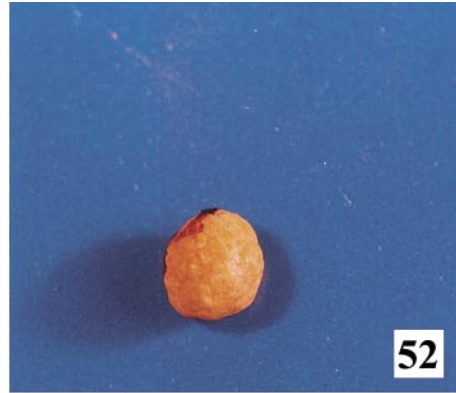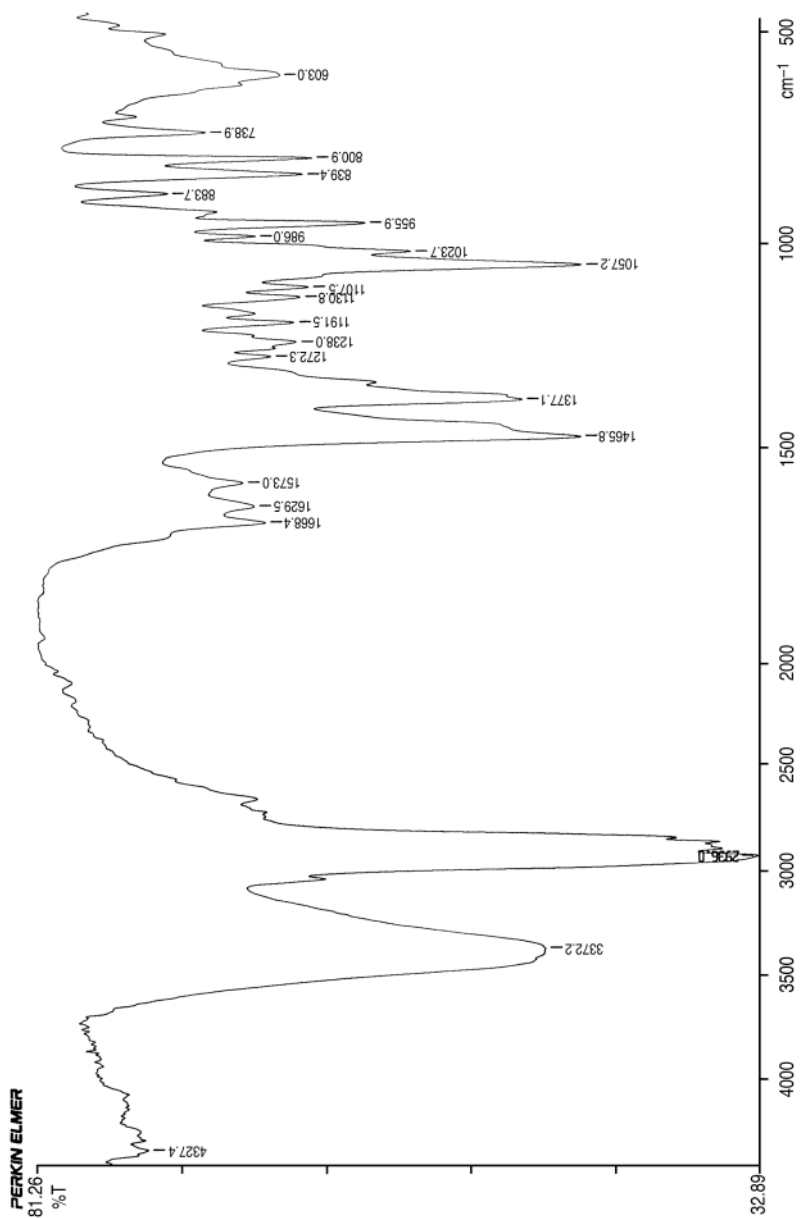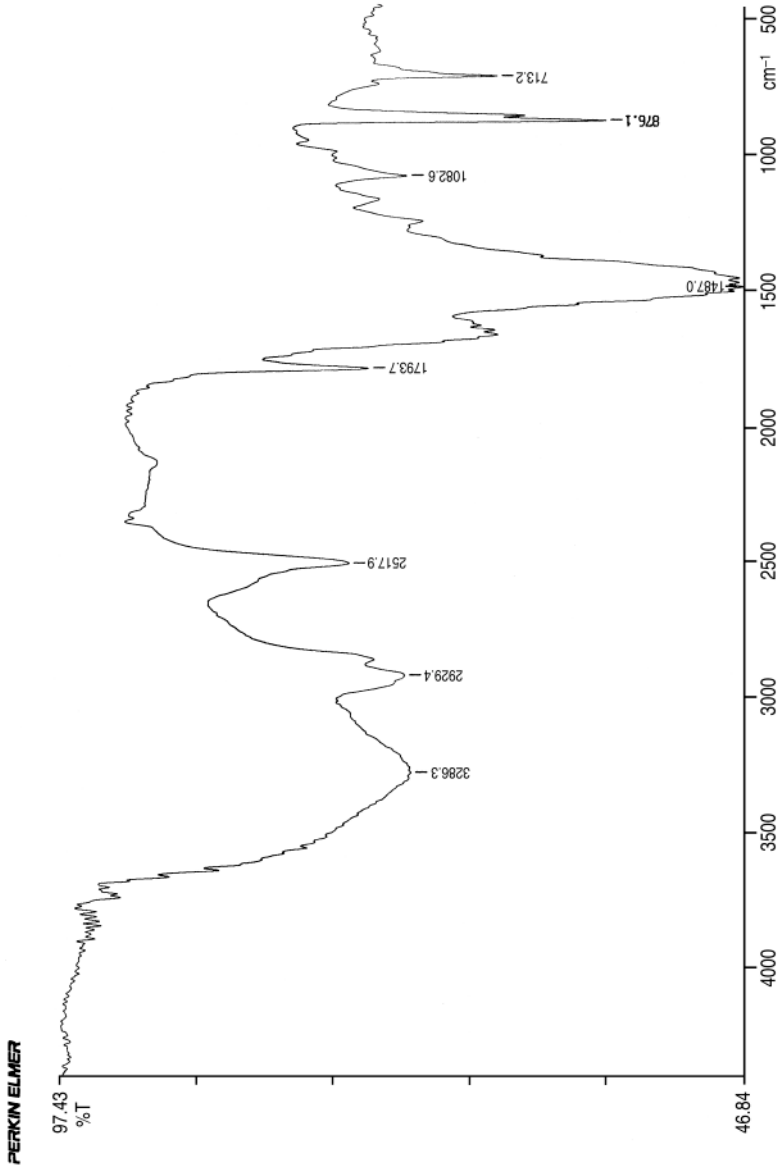**Fig. 1. Photographs of some gallstones and their corresponding numbers.**

Fig. 1. Contd.

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Fig. 2. The FT-IR spectrum of stone No. 44.

Y: 32 scans, 4.0cm−1, apod weak, smooth
Stone No. 15

Fig. 3. The FT-IR spectrum of stone No. 15.

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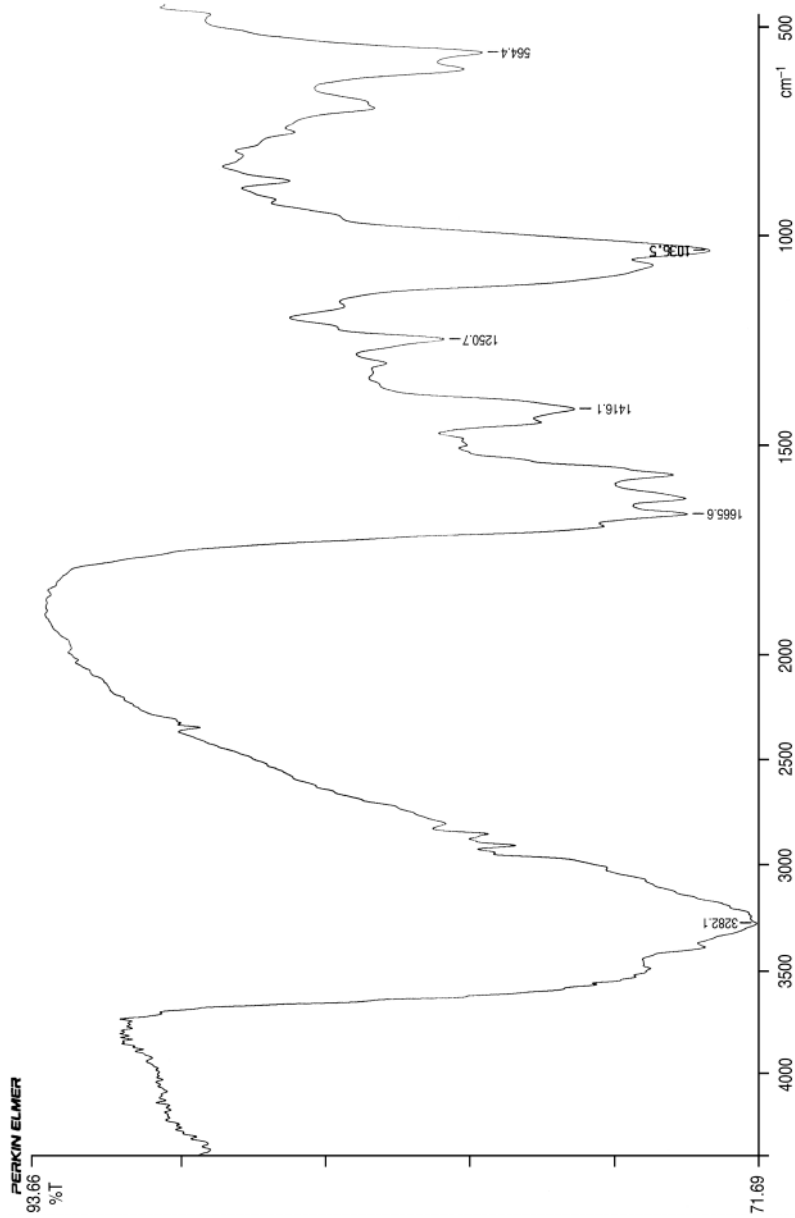
X: 40 scans, 4.0cm–1, flat, smooth
Stone No. 70

Fig. 4. The FT-IR spectrum of stone No. 70.

Percentage of C, H and Ca in Gallstones

The percentage of C, H and Ca is used in this study to classify the gallstones into three categories:

The first category: the cholesterol stones, which represent 64.9% of the total number of the stones. The percentage of carbon in these stones is more than 80% and in some stones reached 83.9%. These stones contain 10.2 to 13.98% hydrogen and small amount of calcium ranged from 0.01 to 2.15%.

The second category: the mixed stones, which represent 25.53% of the total number of the stones, studied. They contain between 70-80% carbon and between 8.86 to 13.23% hydrogen. The amount of calcium in the stones of this category ranged from 0.63 to 6.82%.

The third category: the rare stones, which represent 9.57% of the total number of the stones and contain less than 70% carbon. The percentage of C, H, N, S and Ca in these stones is shown in Table (1).

Percentage of N and S in Gallstones

Some of the stones of first category contain little amount of N and S. The percentage of N in these stones ranged from zero to 2.73% and the percentage of S ranged from zero to 1.6%.

Some of the second category stones contain nitrogen in the range from zero to 3.82%, where all of them contain S in the range from 1.19 to 1.41%

In the third category, the percentage of N and S in the rare stones is recorded in Table (1).

Table 1. Analytical data (%) composition of C, H, N, S and Ca in the rare gallstones.

Stone no.	% C	% H	% N	% S	% Ca
15	18.04	0.66	3.05	0.15	41.10
16	53.32	6.27	3.04	0.85	0.84
20	14.77	0.82	0.94	0.08	34.78
28	62.01	8.57	0.36	1.01	7.50
50	40.05	4.96	5.16	–	10.38
67	69.50	10.94	–	1.23	6.98
70	32.25	4.56	4.04	–	22.3
77	53.84	7.03	–	–	8.52
88	46.05	5.60	6.43	–	0.44

Trace Elements in the Gallstones

Al, Cu, Sr and Zn were not present in any of the stones, which have been analyzed by the Perkin Elmer inductively coupled plasma spectrometer, model Optima 4100-DV., having detection limit of 0.1 µg/g for Cu, Sr and Zn and 0.5 µg/g for Al.

Ba was present in three stones of the first category in the range from 175-693 µg/g.

Mn was present in 5 stones of the first category, in 6 stones of the second category and in 4 stones of the third category in the range 23-370 µg/g, 18-290 µg/g and 818-1225 µg/g respectively. In the first category stones, Na was not present in 17 stones, Fe was not present in 7 stones, K was not present in 2 stones and Mg was not present in 20 stones. The rest of the stones in the first category contain the previous four elements in the ranges: 3-1483 µg/g for Na, 3-343 µg/g for Fe, 3-398 µg/g for K and 8-253 µg/g for Mg.

The stones of the second category contain the four previous elements and Mn except one stone, which does not contain Fe, two stones that do not contain Na, two stones, which do not contain Mg, and seven stones, which do not contain Mn. Ba, was present in one stone only. The amounts of Fe, K, Na, Mg and Mn are in the ranges: 13-1280 µg/g for Fe, 8-1278 µg/g for K, 35-3603 for Na, 58-128 µg/g for Mg and 18-290 µg/g for Mn.

Ba was not found in any stone of the third category and the amount of Fe, K, Na, Mn and Mg in µg/g in these stones is recorded in Table (2).

Table 2. Trace elements in rare gallstones in µg/g.

Stone no.	% C	% H	% N	% S	% Ca
15	3288	1225	2798	945	440
20	935	1113	2940	585	530
28	693	–	58	8	75
50	2343	818	5675	1180	848
70	3540	890	5420	1210	920
77	340	–	1120	820	630
88	1703	78	60	33	20

From the results obtained, it was very easy to differentiate between the cholesterol stones and the rare stones from FT-IR spectra. But it seems impossible to differentiate between the cholesterol stones and mixed stones especially when the stones contain small amount of calcium salt. This is due to the bands

of the cholesterol, which are much stronger than the bands of other compounds. Elemental analysis gave quantitative information for the composition of the stones.

The amount of calcium can be used to determine the amount of calcium salt in the stones. The amount of nitrogen can be used to determine the amount of bilirubin salt.

The amount of sulphur helps in determining the amount of mucine in the stones. The mucine is a complex of monosaccharide having sulfonic group with specific proteins.

Conclusion

The gallstones in western region of Saudi Arabia can be classified into cholesterol stones, mixed stones and rare stones. FT-IR analysis is suitable for qualitative differentiation of rare stones from other stones. The elemental analysis is a strong tool to determine quantitatively the different elements in the stones.

From our studies we also conclude that the colour of cholesterol stones is white, light brown or light gray. They have low amount of calcium and other trace elements. Their density is less than one gram per cm^3. The color of mixed stones is either dark brown or black. The density of some of these stones is more than one gram per cm^3. Most of these stones have some amount of mucine and calcium salt of bilirubin. The rare stones are black in color having high density and their surface is either rough or needle like. They are either mainly calcium carbonate or mixed salts of calcium and magnesium carbonate with some magnesium ammonium phosphate. In general, the amount of trace elements in rare stones is higher than the amount of trace elements in cholesterol or mixed stones.

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التحليل العنصري كوسيلة فعَّالة لتعيين مكوِّنات حصوات المرارة

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جـــــدة – المملكة العربية السعودية

المستخلص.   استُخـدم التحليل الدقيق للعناصر Ca, S, N, H, C في تصنيف حصـوات المـرَارة المستـخرجة من أربـع و تسعين مريضًا من المنطقـة الـغربية في المملكة العربية السعودية . يعطي طيف الأشعة تحت الحمراء (FT-IR) معلومات سريعة ، وكيفية عن مكوِّنات الحصوات . كما أنَّ كثافة الحصـوة ، ولونها وطبيعـة سطحـها ، تعطي مؤشِّرًا عـن المكوِّنات الكيميائية للحصوات . وقد وجد من الدراسة أن ٥ , ٦٤٪ من الحصــوات التي حللت عـبارة عــن كـولسـتـرول ، وأن ٨ , ٢٥٪ من الحصوات مكوَّنة من مـزيج من الكولسترول وأمـلاح الكالسيـوم ، أمـَّا بقية الحصوات فهي حصوات نادرة ومكوَّنة من كـربونات الكالسيوم ، أو من مزيج من كربونات الكالسيوم وأملاح المغنيسيوم والأمونيوم.