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Analysis of mixed gallstone by PIXE and FTIR Spectroscopy

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Abstract

The Fourier-transform infrared spectroscopy was carried out to determine the gallstone type. It confirmed that the sample is a mixed gallstone. A mixed gallstone was analyzed by the proton-induced X-ray emission to study the elemental profile. Among the elements detected, Ca and P show the highest concentrations. A small amount of S, Cl, K, Mn, Fe, Cu, and Zn were also found in the sample.

Keywords: Mixed gallstone; PIXE; FTIR; trace element

Introduction

Cholecystolithiasis is a common and the most painful disease throughout the world[1]. The chemical constituents of bile produced by the liver released during digestion in the duodenum are responsible for the formation of the stones in the gallbladder. Accordingly, identifying the components of gallstones is essential to determine the risk factors of the formation of gallstones. The gallstones are traditionally classified into three cholesterol content, pigment stone and mixed stone[2]. Qiao et al. classified the gallstones into 8 types[3]. These include cholesterol stones, pigment stones, calcium carbonate stones, phosphate stones, calcium stearate stones, protein stones, cystine stones and mixed stones. They found more than 10 subtypes of mixed stones, included cholesterol- bilirubinate, bilirubinate-calcium carbonate, cholesterol- calcium carbonate, bilirubinate- phosphate, cholesterol- phosphate, bilirubinate- calcium stearate, calcium carbonate-phosphate, cholesterol- bilirubinate -calcium carbonate, bilirubinate -calcium carbonate- phosphate, cholesterol-bilirubinate- phosphate and cholesterol- calcium carbonate- calcium stearate mixed stones.

In this paper, mixed stones from a patient were collected to identify their subtype. To this end, the FTIR analysis was employed to illustrate the type of the stone and the PIXE analysis was used to determine their elemental composition.

Experimental

Preparation of the materials

The specimen was gallstones from a patient. They were washed with deionized water 6 times and heated at 40°C in an oven for 24 h. the dried sample was ground to a fine powder in a ceramic crucible. Then the pellet was prepared with a thin Ag layer deposited on top for PIXE analysis.

The PIXE analysis was performed at the 3MV Van De Graff accelerator at the Nuclear Science and Technology Research Institute. Measurement was carried out using a 2 MeV proton beam with an average current of 1 nA. The chamber pressure was 10^{-5}Torr. The characteristic X-ray induced by the proton beam was detected by Canberra Si(Li) detector positioned at an angle of 135° to the incident beam direction. The energy resolution of the detector was 165 eV at 6.4 keV. The PIXE spectrum analysis was performed using GUPIX software.

For FTIR spectroscopy, the powdered stone was mixed with KBr and prepared into the pellet. The FTIR spectrum was carried out for the sample in the wave number range of 400–4000 cm^{-1} using a Bruker Tensor 27 Spectrophotometer.

Results and discussion

Fig.1 shows the gallstone samples. They are unsymmetrical stones with a brown core surrounded by yellow layers.

Figure 1. Image of gallstones sample

The FTIR spectroscopy of the stones is displayed in Fig.2.

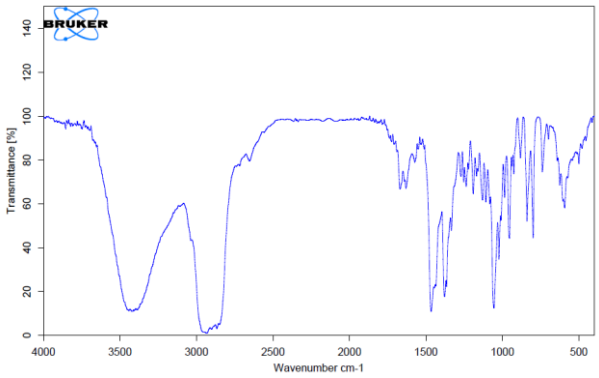

Figure 2. Fourier-transform infrared spectrum of gallstone sample in the spectral range of 4000-400 cm^{-1}

The bands in the FTIR spectrum of the sample reveal that it is a mixed gallstone. The strong bands between 2800 and 3000 cm^{-1} which appear due to asymmetric and symmetric stretching vibrations of CH_2 and CH_3 and the wide band at 3400 cm^{-1} which is due to –OH stretching confirm the presence of cholesterol in the stones. The bands appeared at 1055, 1333, 1378 and 1466 cm^{-1} are also due to the cholesterol contents in the gallstones[4].

The characteristic absorption peaks appeared at 985, 1169, 1272, 1573, 1630 and 1669 cm^{-1} are for bilirubin[5].

The band at 840 cm^{-1} indicates the presence of calcium carbonate in gallstones. Aragonite type of the $CaCO_3$ is found at the weak intensity of 699 cm^{-1}. Calcite form of the CaCO3 is characterized by bands at 1435 cm^{-1}. It cannot be clearly seen in the spectrum due to the strong band of cholesterol at 1465 cm^{-1}, but the shoulder at this band can be a sign of its existence. The vaterite form of calcium is also observed by the band at 739 cm^{-1}. The band at 881 cm^{-1} shows the presence of calcium oxalate in the sample. The peaks at 604, 593 and 953 cm^{-1} are the characteristic absorption peaks of calcium phosphate. The small peak at 1237 cm^{-1} indicates the presence of apatite, which is also the crystalline form of calcium phosphate[5].

The PIXE spectrum of the sample is shown in Fig. 3.

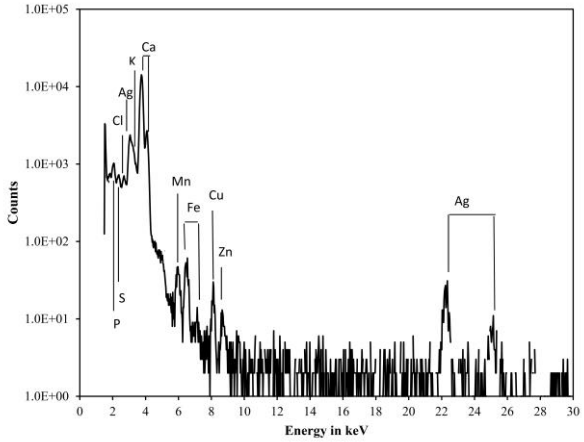

Figure 3. PIXE spectrum of the gallstone sample

The PIXE data analysis showed the presence of P, S, Cl, Ca, Mn, Fe, Cu, Zn. The relative concentration of the detected elements in the sample is shown in Table 1.

Table 1. The concentration of various elements in the gallstone

Elements	Relative concentration in %
P (PO4)	11.3
S	2.3
Cl	2.5
K	1.1
Ca(CaCO3)	82.3
Mn	0.16
Fe	0.19
Cu	0.15
Zn	0.11

Table1 shows that the concentrations of Ca and P are the highest in the sample. As a result, the PIXE analysis suggests this gallstone contains calcium carbonate and phosphate. The small amount presences of S and Zn in the sample indicate that this gallstone can be calcium stearate or pigment types. Since the concentration of S is low, this sample is not the protein and cysteine types.

Conclusions

Analysis of chemical constituents of gallstone using FTIR spectroscopy is a quick, sensitive, and reliable method. The presence of cholesterol, bilirubin, and different salts of calcium such as CaCO3, and calcium phosphate were confirmed by FTIR spectroscopy.

The concentrations of trace elements, in the mixed types of gallstone, were measured by the PIXE analysis. It was found that the concentration of Ca is high in the sample. The trace elements, namely P, S, Cl, K, Mn, Fe, Cu, Zn were also found.

References

[1] L.-Y. Chen, Q.-H. Qiao, S.-C. Zhang, Y.-H. Chen, G.-Q. Chao, L.-Z. Fang, Metabolic syndrome and gallstone disease, World journal of gastroenterology: WJG, 18 (2012) 4215.

[2] L. Ravnborg, D. Teilum, L.R. Pedersen, Gallbladder Stones Classified by Chemical Analysis of Cholesterol Content Frederiksberg, 1987–1988, Scandinavian journal of gastroenterology, 25 (1990) 720-724.

[3] T. Qiao, R.-h. Ma, X.-b. Luo, L.-q. Yang, Z.-l. Luo, P.-m. Zheng, The systematic classification of gallbladder stones, Plos one, 8 (2013) e74887.

[4] B.B.S. Jaswal, P.K. Rai, T. Singh, V. Zorba, V.K. Singh, Detection and quantification of heavy metal elements in gallstones using X-ray fluorescence spectrometry, X-Ray Spectrometry, 48 (2019) 178-187.

[5] R.G. Raman, R. Selvaraju, FTIR spectroscopic analysis of human gallstones, Rom. J. Biophys, 18 (2008) 309-316.