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MINERALOGICAL COMPOSITION ANALYSIS ON CERTAIN HUMAN GALLBLADDER STONES USING X-RAY DIFFRACTION TECHNIQUE

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ABSTRACT

The present investigation aims to determine the mineralogical composition of certain human gallstones using Powder X-Ray Diffraction technique (PXRD). The minerals present in the samples are identified by analysing Bragg's angle (2θ), Lattice spacing (d), Miller Indices (h k l) and full width at half maximum (FWHM) values of the sample with the standard data. Results suggest that calcium phosphate or calcium carbonate with or without magnesium content is the most predominant mineralogical component in gallstones.

KEYWORDS: Gallstones, X-Ray Diffraction, Bragg's Angle and Miller Indices

INTRODUCTION

Gallstone disease is a major worldwide health problem in human life, affecting millions of people throughout the world [1, 2]. Stone formation in the gallbladder causes gallstone disease (Cholelithiasis). Gallstone mostly accumulates in the gallbladder and occasionally in the bile ducts leading from the liver to small intestine.

The gallbladder is found in the upper right corner of the abdomen beneath the liver. Gallbladder contracts and forces the concentrated bile through the bile duct into the duodenum. The result is a mixture of food and bile in which the individual nutrients are more completely dissolved and more easily digested. When bile becomes cholesterol heavy, due to increased hepatic secretion of cholesterol, the cholesterol may crystallize and begin to form a stone. This happens in most of the cases of obese patients. Cholesterol can also crystallize when bile flow is slowed. Less commonly, other elements of bile, such as bilirubin, may precipitate out, causing Bilirubinate stones also called Pigment stones [3, 4]. When the gallbladders can no longer adequately contract, or when the opening of the gallbladder or the bile ducts are partially or completely blocked by gallstones, the painful symptoms begin [5]. The abdominal ultrasonography is the standard diagnostic test for gallstone detection [6].

The identification of the components of gallstone is essential as it provides information that could be useful for medical practitioners to find out the underlying cause of gallstone and to decide whether to treat gallstone patients surgically or therapeutically [7].

According to the National Institutes of Health International Gallstone Workshop [NIH] [8], the gallstones are classified into cholesterol, pigment and mixed stones. Other substances found include calcium salts of phosphate, mucin, glycoprotein, phospholipids and some metals [9]. It has been reported that 14 elements namely, sulphur, chloride, potassium, calcium, titanium, vanadium, chromium, manganese, iron, nickel, copper, zinc, bromide and lead are present in the gallstones and play a significant role in the formation of gallstones [10].

As there are no clear reports on the chemical composition, the stone formation process still remain unclear. There are many interesting reports in literature on the characterization of gallstones [11-14]. Though many studies on gallstone samples were carried out [15-18], so far there are not many investigations done on mineral identification on gallstone using X-Ray diffraction technique. Hence, an attempt has been made to investigate X-Ray Diffraction technique as a tool to identify the mineral composition of the gallstone.

MATERIALS AND METHODS

The gallstone samples are collected from the Department of Digestive diseases in Selvarangam hospital, Anna Nagar, Chennai, Tamilnadu, India. The gallstones are classified depending on their colour as black, dark brown, brown, Whitish yellow (Mixed type) and whitish brown (Mixed type).The gallstones are washed with distilled water to remove the loose debris such as bile, mucous and then air dried. For analysis of constituents, gallstones are broken into halves and then crushed and dry grounded with a pestle and porcelain mortar. The fine powder obtained from all the layers are intimately mixed in the mortar. This powdered stone sample is used for the X-Ray diffraction analysis.

The X-Ray diffraction patterns of selected gallstones from each type are recorded at room temperature and data is taken in the diffraction angle range of $1^{\circ} \leq 2\theta \leq 70^{\circ}$ in a step of 0.04° with monochromatic CuKα radiation (λ =1.540598A$^{\circ}$) source using SEIFERT X-ray diffractometer from the Department of Nuclear Physics , University of Madras, Guindy campus , Chennai. For each measurement 2 - 4 mg of finely powdered gallstone sample is used.

RESULTS AND DISCUSSIONS

X-ray powdered diffraction analysis shows that spectrum consisting of well resolved sharp peaks indicating the crystalline nature. The peak positions of the observed pattern match those reported on the JCPDS file no. as mentioned in the Table 1 to 5. The principles of reflections occurring at the d-spacing confirm the presence of a major mineral phase in the sample. The atomic spacing‘d’ values and intensities from the X-Ray diffraction data are compared with the standard data from powder diffraction file. By comparing both these data, the constituents present in the respective samples are identified and percentage analyses of the constituents are made by measuring the relative maximum peak intensities of different compounds. The Relative percentage of selected gallstones are tabulated in Table 1 to 5 and their X-Ray diffraction patterns are displayed in Figure 1 to 5.

Black Colour Gallstone

The X-Ray diffractogram (Figure1) of Black colour gallstone sample exhibits the presence of Dolomite (Calcium Magnesium Phosphate) indicated by the occurence of two peaks in the spectrum with the 2θ values 11.84° and 17.64° is observed in large amount due to the presence of reflections with d spacing values of 7.468Å and 5.023Å. Dolomite (Calcium Magnesium Carbonate) is also noticed in this sample as indicated by the presence of peak with the 2θ value of 23.24° is observed in lesser amount (Minor constituent) due to the presence of reflections with d spacing value of 3.824 Å.

From Table 1, it is clear that only traces of Glauberite (Calcium Sodium Sulphate Hydrate), Pyrite (Iron sulphide) and Apatite Hydroxy (Calcium phosphate) are found (Minor constituent). Hence, the sample contains minerals namely Dolomite, Glauberite, Pyrite and Apatite hydroxy in Tetramineralic form. The FWHM values observed in this sample indicate that calcium magnesium phosphate is present in major phase and Calcium Sodium Sulphate Hydrate, Calcium Magnesium Carbonate, Iron sulphide & Calcium phosphate are present in minor phase. The variation observed in the FWHM values suggests that the composition of Dolomite (Calcium Magnesium Phosphate / Carbonate), Apatite Hydroxy (Calcium phosphate), Glauberite (Calcium Sodium Sulphate Hydrate) and Pyrite (Iron sulphide) phases vary from one sample to another. Among 6 peaks, 100 intensity peak has occurred at 012 plane, the most reliable and useful diagnostic peak for Calcium magnesium carbonate may be considered as most reliable and useful diagnostic peak and is used to identify Calcium magnesium carbonate in the sample by the occurence of peak in the spectrum with the 2θ value of 23.24° and corresponding d spacing value of 3.824Å.

Table 1: Comparison between Observed and Standard Values of 'd', '2θ ' and 'I/Io' of a Black Colour Gallstone Sample

Sample Type / Colour	Observed Value			Standard Value			hkl	FWHM	JCPDS FILE#	Mineral Names	Chemical Names
	d(Å)	2θ (deg)	I/I_o	d(Å)	2θ (deg)	I/I_o					
Black Colour gallstone	7.468	11.84	33	8.0623	10.965	12	012	0.16	87-1582	Dolomite	Calcium Magnesium Phosphate
	5.868	15.12	41	5.9945	14.766	78	110	0.16	89-8618	Glauberite	Calcium Sodium Sulphate Hydrate
	5.549	15.96	65	5.6995	15.535	1	002	0.12	89-6928	Pyrite	Iron sulphide
	5.023	17.64	56	5.1646	17.155	38	110	0.16	87-1582	Dolomite	Calcium Magnesium Phosphate
	4.573	19.40	59	4.4161	20.091	2	100	0.08	86-1585	Apatite Hydroxy	Calcium phosphate
	3.824	23.24	100	3.8204	23.264	5	012	0.24	43-0697	Dolomite	Calcium Magnesium Carbonate

Dark Brown Colour Gallstone

The X-Ray diffractogram (Figure 2) of Dark Brown colour gallstone sample exhibits the presence of Dolomite (Calcium Magnesium Phosphate) indicated by the occurence of two peaks in the spectrum with the 2θ values 11.075° and 26.578° is observed in this sample in large amount due to the presence of reflections with d spacing values of 7.982Å and 3.351Å. Dolomite (Calcium Magnesium Carbonate) is also noticed in this sample as indicated by the presence of peak with the 2θ value of 23.263°is observed in lesser amount (Minor constituent) due to the presence of reflections with d spacing value of 3.821Å. Table 2 shows that only traces of Pyrite (Iron Sulphide) are found (Minor constituent). Hence, the sample contains the minerals namely Dolomite and Pyrite in Bimineralic form. The FWHM values observed in this sample indicate that calcium magnesium phosphate is present in major phase; Calcium magnesium carbonate and Iron sulphide are present in minor phase. The variation observed in the FWHM values suggests that the composition of Dolomite (Calcium Magnesium Phosphate/Carbonate) and Pyrite (Iron sulphide) phases vary from one sample to another. Among the 4 peaks, intense reflection is caused by the reflection of 012 plane which is the most reliable and useful diagnostic peak for Calcium

magnesium phosphate and is used to identify Calcium magnesium phosphate in the sample by the occurence of peak in the spectrum with the 2θ value of 11.075° and corresponding d spacing value of 7.982 Å.

Table 2: Comparison between Observed and Standard Values of 'd', '2θ ' and 'I/Io' of a Dark Brown Colour Gallstone Sample

Sample Type / Colour	Observed Value			Standard Value			hkl	FWHM	JCPDS FILE#	Mineral Names	Chemical Names
	d(Å)	2θ(deg)	I/I_o	d(Å)	2θ(deg)	I/I_o					
Dark Brown colour gallstone	7.982	11.075	100	8.062	10.965	12	012	0.08	87-1582	Dolomite	Calcium Magnesium Phosphate
	4.713	18.813	57	4.704	18.847	1	101	0.12	89-6928	Pyrite	Iron sulphide
	3.821	23.263	51	3.82	23.264	5	012	0.04	43-0697	Dolomite	Calcium Magnesium Carbonate
	3.351	26.578	41	3.327	26.778	9	122	0.16	87-1582	Dolomite	Calcium Magnesium Phosphate

Brown Colour Gallstone

The X-Ray diffractogram (Figure 3) of Brown colour gallstone sample exhibits the presence of Dolomite (Calcium Magnesium Phosphate) indicated by the occurence of three peaks in the spectrum with the 2θ values 11.921°, 17.359° and 22.447° is observed in this sample in large amount due to the presence of reflections with d spacing values of 7.418Å, 5.104Å and 3.957Å. From Table 3, it is evident that only traces of Pyrite (Iron Sulphide) are present (Minor constituent). Hence, the sample contains minerals namely Dolomite and Pyrite in Bimineralic form. The FWHM values observed in this sample indicate that calcium magnesium phosphate is present in major phase and Iron sulphide is present in minor phase. The variation observed in the FWHM values suggests that the composition of Dolomite (Calcium Magnesium Phosphate) and Pyrite (Iron sulphide) phases vary from one sample to another. Out of 4 peaks, the particular peak with highest intensity 100 caused by the reflection of 012 plane may be considered as most reliable and useful diagnostic peak and is used to identify Calcium magnesium phosphate in the sample by the occurence of peak in the spectrum with the 2θ value of 11.921° and corresponding d spacing value of 7.418 Å.

Table 3: Comparison between Observed and Standard Values of 'd', '2θ ' and 'I/Io' of a Brown Colour Gallstone Sample

Sample Type / Colour	Observed Value			Standard Value			hkl	FWHM	JCPDS FILE#	Mineral Names	Chemical Names
	d(Å)	2θ (deg)	I/I_o	d(Å)	2θ (deg)	I/I_o					
Brown colour gallstone	7.418	11.921	100	8.062	10.965	12	012	0.16	87-1582	Dolomite	Calcium Magnesium Phosphate
	5.104	17.359	61	5.165	17.155	38	110	0.08	87-1582	Dolomite	Calcium Magnesium Phosphate
	4.613	19.226	57	4.704	18.847	1	101	0.08	89-6928	Pyrite	Iron sulphide
	3.957	22.447	53	3.969	22.382	1	116	0.08	87-1582	Dolomite	Calcium Magnesium Phosphate

Whitish Yellow Colour (Mixed Type) Gallstone

The X-Ray diffractogram (Figure 4) of whitish yellow colour (Mixed type) gallstone sample exhibits the presence

of Dolomite (Calcium Magnesium Phosphate) indicated by the occurence of five peaks in the spectrum with the 2θ values 11.523°, 12.296°, 16.467°, 17.639° and 18.693° is observed in this sample in higher amount (Major constituent) due to the presence of reflections with d spacing values of 7.673Å, 7.192Å, 5.379Å, 5.024 Å and 4.743Å. Dolomite (Calcium Magnesium Carbonate) is also noticed in this sample as indicated by the presence of peak with the 2θ value of 23.24°is observed in lesser amount (Minor constituent) due to the presence of reflections with d spacing value of 3.824Å. From Table 4, it is concluded that only traces of Pyrite (Iron Sulphide), Glauberite (Calcium Sodium Sulphate Hydrate) and Apatite hydroxy (Calcium Phosphate) are found (Minor constituent). Hence, it is concluded that the sample contains minerals namely Dolomite, Glauberite, Pyrite and Apatite hydroxy in Tetramineralic form. The FWHM values observed in this sample indicate that calcium magnesium phosphate is present in major phase and Calcium Sodium Sulphate Hydrate, Iron sulphide, Calcium phosphate are present in minor phase. The variation observed in the FWHM values suggests that the composition of Dolomite (Calcium Magnesium Phosphate /Carbonate), Apatite hydroxy (Calcium Phosphate), Pyrite (Iron Sulphide) and Glauberite (Calcium Sodium Sulphate Hydrate) phases vary from one sample to another. Out of 9 peaks, the particular peak with highest intensity 100 caused by 110 plane may be considered as most reliable and useful diagnostic peak and is used to identify Calcium sodium sulphate hydrate in the sample by the occurence of peak in the spectrum with the 2θ value of 15.079° and corresponding d spacing value of 5.871 Å.

Table 4:.Comparison between Observed and Standard Values of 'D', '2θ' and 'I/Io' of a Whitish Yellow Colour (Mixed Type) Gallstone Sample

Sample Type/ Colour	Observed Value			Standard Value			hkl	FWHM	JCPDS FILE#	Mineral Names	Chemical Names
	d(Å)	2θ (deg)	I/I_o	d(Å)	2θ (deg)	I/I_o					
Whitish yellow colour (Mixed type) gallstone	7.673	11.523	46	8.0623	10.965	12	012	0.08	87-1582	Dolomite	Calcium Magnesium Phosphate
	7.192	12.296	39	6.4486	13.721	22	104	0.24	87-1582	Dolomite	Calcium Magnesium Phosphate
	5.871	15.079	100	5.9945	14.766	78	110	0.16	89-8618	Glauberite	Calcium Sodium Sulphate Hydrate
	5.555	15.942	46	5.6995	15.535	1	002	0.12	89-6928	Pyrite	Iron sulphide
	5.379	16.467	41	5.1646	17.155	38	110	0.16	89-1582	Dolomite	Calcium Magnesium Phosphate
	5.024	17.639	42	5.1646	17.155	38	110	0.16	87-1582	Dolomite	Calcium Magnesium Phosphate
	4.743	18.693	37	4.768	18.594	2	113	0.08	87-1582	Dolomite	Calcium Magnesium Phosphate
	4.574	19.39	38	4.4161	20.091	2	100	0.08	86-1585	Apatite Hydroxy	Calcium phosphate
	3.824	23.24	54	3.8204	23.264	5	012	0.24	43-0697	Dolomite	Calcium Magnesium Carbonate

Whitish Brown Colour (Mixed Type) Gallstone

The X-Ray diffractogram (Figure 5) of Whitish Brown colour (Mixed type) gallstone sample exhibits the presence

of Dolomite (Calcium Magnesium Phosphate) indicated by the occurence of two peaks in the spectrum with the 2θ values 16.505 ° and 17.994° is observed in this sample in large amount (Major constituent) due to the presence of reflections with d spacing values of 5.367Å and 4.926Å. Dolomite (Calcium Magnesium Carbonate) is also noticed in this sample as indicated by the presence of peak with the 2θ value of 23.345 °is observed in lesser amount (Minor constituent) due to the presence of reflections with d spacing value of 3.807Å. From Table 5, it is reported that only traces of Glauberite (Calcium Sodium Sulphate Hydrate) and Calcite (Calcium Carbonate) are present (Minor constituent). Hence, the sample contains minerals namely Dolomite, Glauberite and Calcite in trimineralic form. The FWHM values observed in this sample indicate that calcium magnesium phosphate is present in major phase and Calcium Sodium Sulphate Hydrate, Calcium Magnesium carbonate, Calcium carbonate are present in minor phase. The variation observed in the FWHM values suggests that the composition of Dolomite (Calcium Magnesium Phosphate/Carbonate), Glauberite (Calcium Sodium Sulphate Hydrate), Calcite (Calcium carbonate) phases vary from one sample to another. Among 5 peaks, the particular peak with highest intensity 100 caused by the reflection of 110 plane may be considered as most reliable and useful diagnostic peak and is used to identify Calcium sodium sulphate Hydrate in the sample by the occurence of peak in the spectrum with the 2θ value of 15.025° and corresponding d spacing value of 5.892Å.

Table 5: Comparison between Observed and Standard Values of 'D', '2θ ' and 'I/Io' of a Whitish Brown Colour (Mixed Type)

Sample Type/ Colour	Observed Value			Standard Value			hkl	FWHM	JCPDS FILE#	Mineral Names	Chemical Names
	d(Å)	2θ (deg)	I/I_o	d(Å)	2θ (deg)	I/I_o					
Whitish Brown colour (Mixed type) gallstone	5.892	15.025	100	5.994	14.766	78	110	0.24	89-8618	Glauberite	Calcium Sodium Sulphate Hydrate
	5.367	16.505	65	5.165	17.155	38	110	0.12	87-1582	Dolomite	Calcium Magnesium Phosphate
	4.926	17.994	59	4.768	18.594	2	113	0.08	87-1582	Dolomite	Calcium Magnesium Phosphate
	3.885	22.871	39	3.847	23.101	8	012	0.12	85-1108	Calcite	Calcium Carbonate
	3.807	23.345	59	3.82	23.264	5	012	0.16	43-0697	Dolomite	Calcium Magnesium Carbonate

The minerals content in the gallstone samples are summarized in Table 6.

Table 6: The Minerals Content in the Different Types of Gallstone Sample

Sample Type / Colour	Mineral Name	Chemical Name
Black colour gallstone	Dolomite Glauberite Pyrite Apatite hydroxy	Calcium Magnesium Phosphate/Carbonate Calcium Sodium Sulphate Hydrate Iron Sulphide Calcium Phosphate
Dark Brown colour gallstone	Dolomite Pyrite	Calcium Magnesium Phosphate/Carbonate Iron Sulphide
Brown colour gallstone	Dolomite Pyrite	Calcium Magnesium Phosphate Iron Sulphide
Whitish yellow colour (Mixed type) gallstone	Dolomite Glauberite Pyrite Apatite hydroxy	Calcium Magnesium Phosphate/Carbonate, Calcium Sodium Sulphate Hydrate, Iron Sulphide Calcium Phosphate
Whitish Brown colour (Mixed type) gallstone	Dolomite Glauberite Calcite	Calcium Magnesium Phosphate/Carbonate, Calcium Sodium Sulphate Hydrate Calcium Carbonate

CONCLUSIONS

As X-Ray diffraction analysis is confined to crystalline components, the presence of minerals like Dolomite, Glauberite, Pyrite, Calcite and Apatite hydroxy in the Bimineralic, Trimineralic and Tetramineralic forms are observed. By X-Ray Diffraction technique, not only the mineral presents are identified, but also the levels of their presence - viz major, minor or in trace are understood. Calcium content is highest in Black and Dark brown stones which play a very important role and it might be responsible for the higher hardness of the Black and Dark brown stones and Magnesium, Sodium and Iron are also present in almost all the gallstones as trace elements. This is in conformity with the observations made by different authors [19]. An increase in the prevalence of Calcium Phosphate gallstones and the recurrence rate of calcium phosphate has been increased over the past two decades has been reported by different authors [20,21].This is in conformity with the observations made in this study. The intensity of XRD peaks of the sample reflects that the formed gallstones particles are mostly amorphous and partly crystalline and diffraction peaks indicates the composition of gallstones are heterogeneous and differ by patient to patient. X-Ray Diffraction technique is well supported in this study and provides evidence to confirm and carry out the investigation successfully to identify the mineralogical composition of gallstone.

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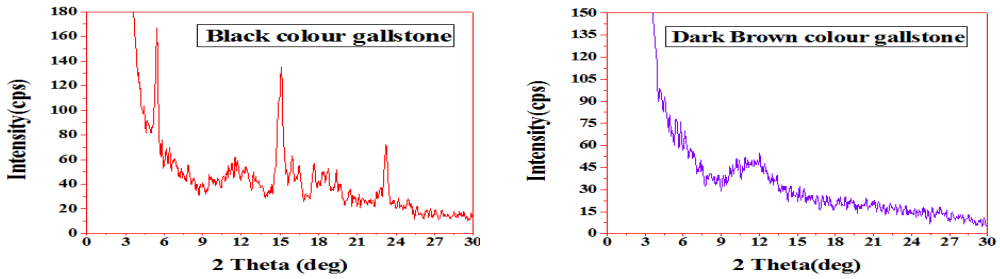


Figure 1 & 2: The X-Ray Diffraction Spectra of Black & Dark Brown Colour Gallstone Samples

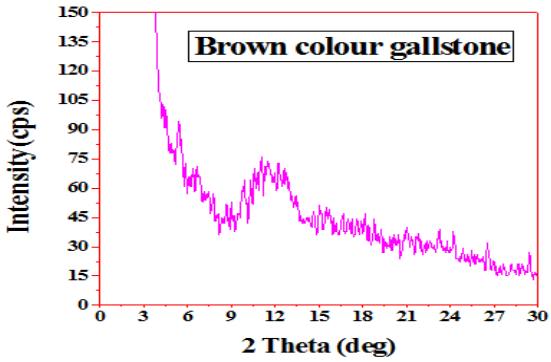
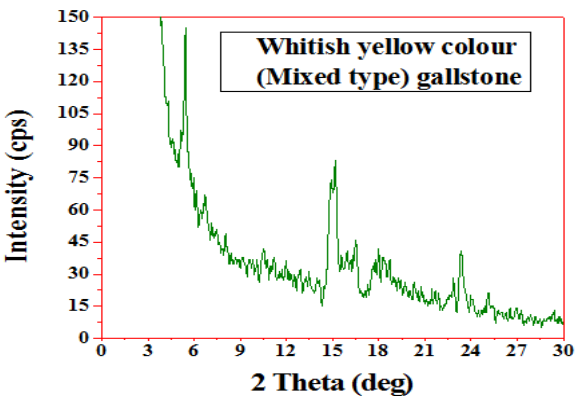


Figure 3: The X-ray Diffraction Spectra of Brown Colour Gallstone Samples



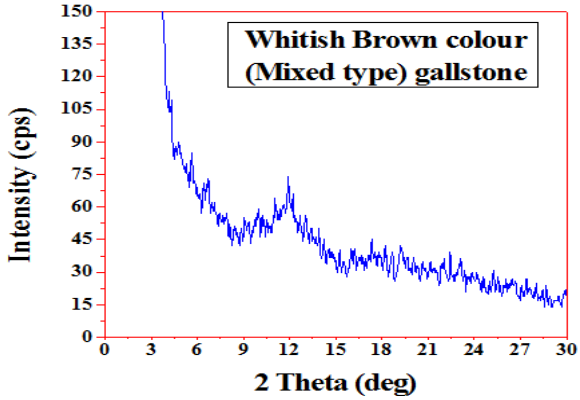


Figure 4 & 5: The X-Ray Diffraction Spectra of Whitish Yellow & Whitish Brown (Mixed) Gallstone Samples

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