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*Article*

# Analysis of Gallbladder Stones by X-ray Fluorescence

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**Abstract:** Gallstone disease impacts millions globally. Over the decades modern techniques have been applied in laminar. Nevertheless, the fundamental cause of its formation has continued to be a topic of speculation. The current analysis of present sunshine utilizes various methods, including infrared. Through FTIR spectroscopy, we investigated the role of cholesterol, bilirubin, and calcium carbonate in gallstone formation within the gallbladder. Our experimental findings indicated that cholesterol, whether alone or in conjunction with bilirubin and calcium carbonate, is the predominant component of human gallstones and serves as the primary cause of gallstone formation. Gallstone samples were identified using WDXRF. Some of the interiors exhibited tones of pebbles in various colors, while the outer casing was brown and black. FTIR analyses are presented, along with WD-XRF analyses and other findings related to gallstone samples. This clearly demonstrates that calcium and copper constitute the salts of bilirubin's to a greater extent than the brown and black coloration of the central part of gallstones

**Keywords:** Gallstones, Gallbladder, X-Ray Fluorescence (Xrf), Elemental Analysis, Cholesterol, Calcium Carbonate

## 1. Introduction

X-ray Fluorescence (XRF) Spectroscopy entails the measurement of the intensity of X-rays emitted from a specimen based on energy or wavelength. The energies of prominent intensity lines are distinctive to the atoms within the specimen. The intensities of the observed lines for a specific atom fluctuate according to the quantity of that atom present in the specimen. Qualitative analysis focuses on identifying the atoms present in a specimen by correlating the observed characteristic lines with their respective atoms. Conversely, quantitative analysis aims to ascertain the quantity of each atom in the specimen by analyzing the intensity of the measured characteristic X-ray lines. The emission of characteristic atomic X-ray photons occurs when a vacancy is created in an inner electron state, prompting an outer orbit electron to transition into that vacant state. The energy of the emitted photon corresponds to the difference in electron energy levels during the transition. Since the electron energy levels are unique to each atom, the energy of the emitted photon is also characteristic of that atom. Molecular bonds typically form between the outer electrons of a molecule, leaving the inner electron states unaffected. Given that X-ray fluorescence involves transitions to inner electron states, the energy of the characteristic X-ray radiation is generally not influenced by molecular chemistry. This attribute renders XRF a highly effective tool for chemical analysis across various materials. In a liquid medium, fluoresced X-rays are typically minimally influenced by other atoms present, and the line intensities are generally directly proportional to the quantity of that atom in the liquid. In solid materials, the atoms of the specimen both absorb and enhance the characteristic X-ray radiation. These interactions are referred to as 'matrix effects', and a significant portion of quantitative analysis utilizing XRF spectroscopy focuses on compensating for these effects.

Although the underlying principles remain consistent, various types of instrumentation are employed to conduct X-ray fluorescence spectroscopy. There are two primary categories of instruments: Wavelength Dispersive and Energy Dispersive. Wavelength Dispersive spectrometers assess X-ray intensity based on Wavelength, whereas Energy Dispersive spectrometers evaluate X-ray intensity according to energy. A crucial element of X-ray fluorescence spectroscopy is the technique employed to create the inner orbital vacancy. One method involves bombarding the sample with high-energy X-rays. Other techniques include bombarding the sample with high-energy electrons and protons. When a photon beam strikes the specimen, it undergoes a photon absorption interaction, whereas electron and proton beams primarily engage in a Coulomb interaction with the specimen-ray tubes accelerate high-energy electrons towards a target within the tube, which subsequently fluoresces X-rays. The resultant X-ray beam comprises both a continuum and characteristic lines corresponding to the tube target. Additionally, radioactive sources can be utilized to produce beams of X-rays, electrons (beta emitters), and protons (alpha emitters). While X-ray tubes can generate a high-power X-ray beam, the radiation produced is not monochromatic. In contrast, radioactive sources yield monochromatic beams, albeit at a lower power level. Proton-Induced X-ray Emission (PIXE) employs a proton beam. Wavelength Dispersive Spectrometry (WDS) typically utilizes an X-ray tube, similar to Energy Dispersive X-ray Spectrometry (EDX).

Instruments such as the electron microprobe and electron microscope bombard the sample directly with high-energy electrons to eject inner orbital electrons (EDS). It is important to note that the charged particle beam methods necessitate that the specimen be electrically conductive.

## Background

### 1.1 X-Ray Fluorescence (XRF)

The X-ray fluorescence (XRF) technique is a straightforward tool. The ionizing electromagnetic radiation, known as X-rays, is utilized to displace electrons from their orbital energy levels, resulting in ionization. In simpler terms, X-ray photons can eject an electron from its atomic orbit, which is referred to as ionizing radiation, thereby initiating ionization—the process of removing one or more electrons from atoms due to the incident radiation, which leaves behind electrically charged particles, specifically an electron and a positively charged ion. Both X-rays and gamma rays have the capability to expel an electron, forcing it to exit its inner orbital; however, this action leads to the formation of an unstable atom. Consequently, an electron from the outer shell will compensate for the missing electron in the inner shell. As a result, energy is released due to the differing affinities of electrons to their respective shells (the internal electron has a greater affinity for the shell than the external electron). Therefore, the emitted energy will vary from one item to another. The XRF technique is applicable for multi-elemental analyses. The XRF method has been employed to ascertain trace element concentrations across a diverse array of samples, including biochemical samples, chemical samples, and archaeological samples. Nevertheless, the portable XRF system often lacks the capability to analyze large samples. Thus, larger samples must be processed into a homogeneous powder. Furthermore, the XRF system typically struggles to detect elements with atomic numbers exceeding 92.

## 1.2 Electromagnetic Radiation, Quanta

X-rays are a form of electromagnetic radiation. They constitute a highly energetic segment of the electromagnetic spectrum in Table 1 and possess short wavelengths ranging from approximately 0.1 to 100 angstroms (Å). These rays are flanked by ultraviolet light on the longer wavelength side and gamma rays on the shorter wavelength side. X-rays that fall within the 50 to 100 Å range are classified as soft X-rays due to their lower energy levels and higher absorption rates.

**Table 1.** Energy and names of various wavelength range.

| Energy range (eV) | Wavelength range | Name |
|---|---|---|
| $< 10^{-7}$ | cm to km | Radio Waves (short, medium, long waves) |
| $< 10^{-3}$ | mm to cm | Micro Wave |
| $< 10^{-3}$ | mm to mm | Infra Red |
| 0.0017 – 0.0033 | 380 to 750 nm | Visible Light |
| 0.033 – 0.1 | 10 to 380 nm | Ultra Violet |
| 0.11 – 100 | 0.01 to 12 nm | X-Rays |
| 10 - 5000 | 0.0002 to 0.12 nm | Gamma Radiation |

The range of interest for X-ray is roughly between 0.1 and 100 Å. Although angstroms are utilized throughout these notes, they are not recognized as an SI unit.

Wavelengths ought to be represented in nanometers (nm), which correspond to 10-9 meters (1 Å = 10-10 m); however, the majority of texts and articles concerning microprobe analysis continue to employ angstroms. Another frequently used unit is the micron, which should more accurately be referred to as a micrometer (µm); a micrometer equals 10^4 Å. The connection between the wavelength of electromagnetic radiation and its corpuscular energy (E) is established as follows. For all forms of electromagnetic radiation:

$$E = h\,\nu \;\ldots\ldots\ldots\; (1\text{-}1)$$

where:

h is the Planck constant (6.62* 10-34 J.s); ν is the frequency expressed in Hertz.

For all wavelengths,

$\nu = c / \lambda \;\ldots\ldots\ldots(1\text{-}2)$

where:

c = speed of light (3* 108 m/s);

$\lambda$= wavelength (Å).

## 1.3 Properties of X-Rays

A general summary of the properties of X-rays is presented below:

a. Invisible
b. Propagate with velocity of light (3.108 m/s)
c. Unaffected by electrical and magnetic fields
d. Differentially absorbed in passing through matter of varying composition, density and thickness
e. Reflected, diffracted, refracted and polarized
f. Capable of ionizing gases
g. Capable of affecting electrical properties of solids and liquids
h. Capable of blackening a photographic plate
i. Able to liberate photoelectron. And recoils electrons
j. Emitted in a continuous spectrum
k. Emitted also with a line spectrum characteristic of the chemical element

l. Found to have absorption spectra characteristic of the chemical element

### 1.4 X-Ray Interactions with Matter

When X-rays are directed towards an object, some photons interact with the particles of the matter, leading to their energy being either absorbed or scattered. This process of absorption and scattering is referred to as attenuation. Conversely, other photons may pass entirely through the object without interacting with any of the material's particles. The quantity of photons that successfully transmit through a material is influenced by the material's thickness, density, atomic number, and the energy of the individual photons. Even if photons possess the same energy, they can travel varying distances within a material, which is determined by the likelihood of their interaction with one or more particles of the matter and the nature of the interactions that occur. As the probability of an encounter rises with the distance traveled, the number of photons arriving at a specific point within the matter diminishes exponentially with the distance traversed, see Figure 1.

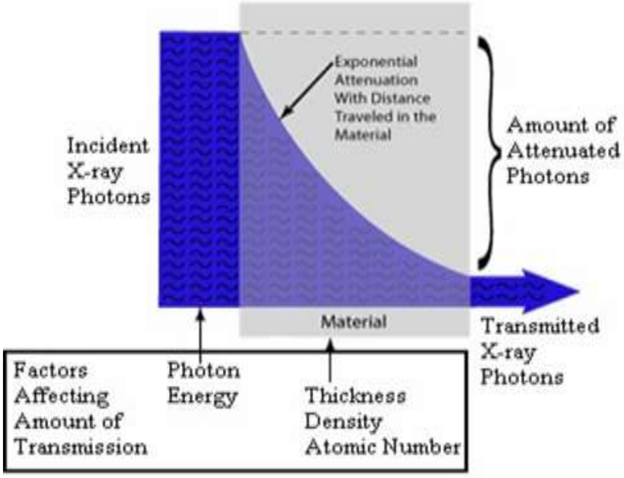

**Figure 1.** Exponential attenuation of photon energy with distance travelled in the material.

### 1.5 Gallbladder stone

Urinary stone disease, also referred to as renal calculi or urolithiasis, results in significant pain and can occasionally lead to renal failure. This condition has impacted humanity since 4800 BC, yet the mechanisms behind the disease remain incompletely understood to this day. Urinary stones are hard, solid formations that can be located in three areas of the urinary tract: the kidney, ureter, and bladder. Finlayson conducted a review of various global geographic surveys and identified regions with high and low incidence rates. Numerous researchers have focused on specific areas over designated time frames to comprehend the particular characteristics of urinary stones in those regions. Additionally, Daudonetial has identified the sex and age-related composition of urinary stones. The etiological factors believed to contribute to stone formation include metabolic disorders, ion transport within the intestinal tract and kidneys, as well as dietary influences such as fluid intake. External factors that lead to urinary stones encompass geographical location, water quality, hot climates, occupation, stress, and factors induced by drugs and bacteria. Significant research is ongoing to elucidate the three stages of stone formation: heterogeneous nucleation, crystal growth, and crystal aggregation, along with the roles of modifiers (inhibitors, promoters, and complexors) in the stone formation process. To grasp the impact of environmental and etiological factors on stone growth, it is crucial to understand the constituents of the stones. The composition of stones can differ from one patient to another and may also change over time within the same individual. The Saurashtra region of Gujarat state in Western India, characterized by a hot climate, low rainfall, and increased groundwater salinity, is particularly susceptible to urinary stone disease; however, no comprehensive studies on urinary stone analysis in this area have been conducted. The advent of analytical tools has enabled the examination and identification of urinary stone constituents through various techniques.

## 2. Materials and Methods

### 2.1. Sample Collection

All gallbladder stone samples were obtained from the urologist in Karbala city following the patient's surgery or after the spontaneous expulsion of the stones. The specifics of the samples collected from different patients are illustrated, see Figure 2.

All stones were dried for a week and subsequently ground into a powder prior to measurement.Multiple stones from individual patients were ground separately, after which analytical measurements were conducted.The elemental concentrations of these samples were analyzed using X-Ray fluorescence.The samples were prepared by grinding the teeth samples with a mechanical mortar, followed by pressing 5 grams of the powder into a disk with a diameter of 1 cm under a pressure of 3.5 tons. Figure 3 depicts the teeth samples post-grinding and pressing.

**Figure 2.** Number of gallbladder stone samples, (F and M) mark to female and male gender.

**Figure 3.** Some of samples after grinding and pressing.

The X-ray fluorescence spectrometer utilized is the Model 2010 from Spectro Analytical Instruments, located in Kleve, Germany. It operates with an X-ray tube at a voltage of 44.69 kV and a current of 0.55 mA, employing a palladium target. Figure 4 illustrates a typical X-ray fluorescence spectroscopy setup. Specialized software was employed to analyze the secondary X-rays emitted from the samples, allowing for the identification of their elemental composition.

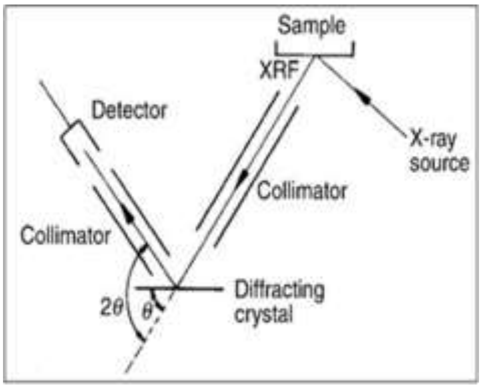

**Figure 4.** The experimental setup for X-ray fluorescence spectroscopy (wavelength-dispersive).

## 3. Results

### 3.1 X- Ray Fluorescence ( XRF) for males

The results of the correlation between the concentration of the elements and the age are shown in Figure 5. Mgo, P2O5, Cl, MNO, ZNO decreased with age and increased in SiO2, CaO.

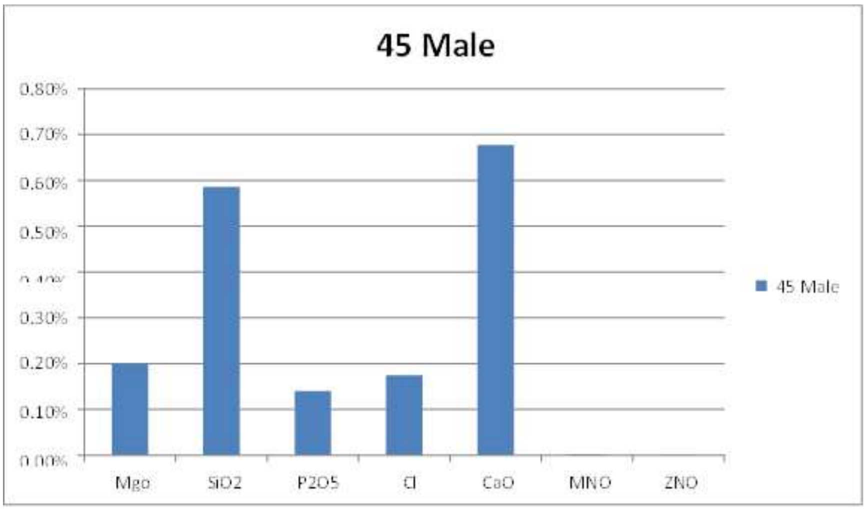

**Figure 5.** X - Ra y Fluores cence ( X RF) for mal e' s different age gro up.

### 3.2 X- Ray Fluorescence ( XRF) for women's

X-ray fluorescence (XRF) was used to analyze elemental contents in the gallbladder. Figure 6 shows the elements identified in women by XRF. Many elements have been identified such as MgO, SiO2, P2O5, Cl, CaO, MNO, ZNO. The proportion of these elements varies with age, with the highest concentration of toxic elements appearing SiO2, P2O5, CaO in the oldest. Compared to the younger ones. While the concentration of trace elements MgO, Cl, MNO, ZNO decreases with age.

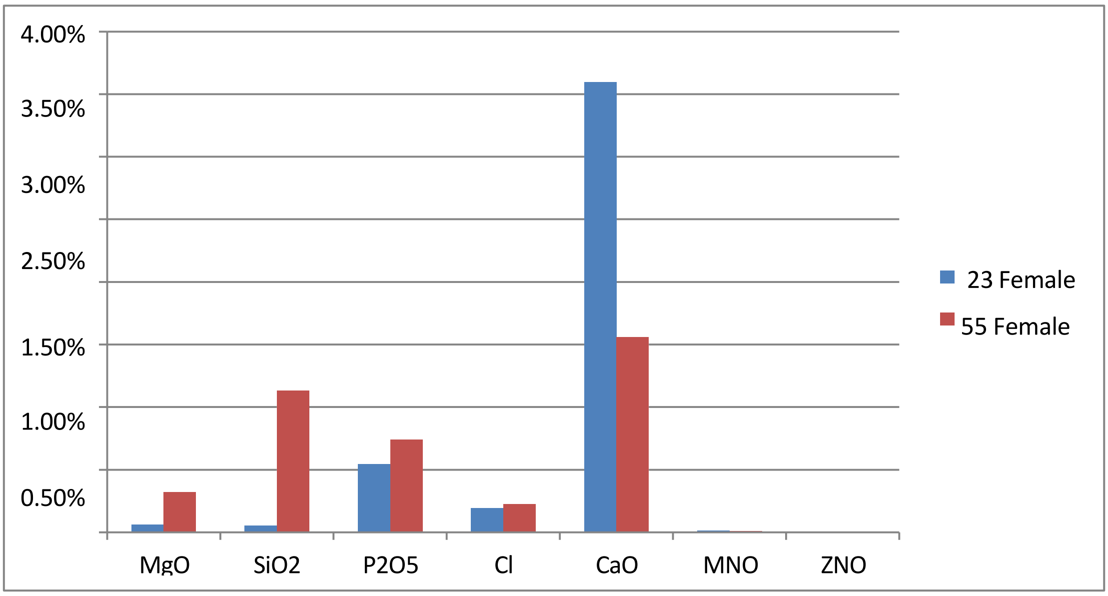

**Figure 6.** X- Ra y Fluorescence ( XRF) for women' s different age group.

## 4. Discussion

The present study highlights the utility of X-ray fluorescence (XRF) spectroscopy as a non-destructive technique for analyzing the elemental composition of gallbladder stones. The results demonstrate that gallstones are composed of varying concentrations of calcium, magnesium, silicon, phosphorus, and trace metals, with noticeable differences between male and female patients and across age groups. These findings are consistent with previous reports that cholesterol, bilirubin salts, and calcium compounds are the major constituents of gallstones, while additional elements such as copper and zinc may play modifying roles in stone coloration and structure.

The observed age-related variation in elemental concentrations is particularly noteworthy. In both male and female groups, a general decline in trace elements such as magnesium and zinc was recorded with increasing age, whereas calcium and silicon compounds appeared to increase. This trend may reflect changes in metabolic activity, dietary patterns, and biliary composition over time, which can alter the physicochemical environment of the gallbladder. Previous studies have also suggested that aging may be associated with altered bile acid metabolism, thereby facilitating the precipitation of calcium salts and cholesterol crystals.

Sex-related differences were also evident. Female samples, particularly from older patients, demonstrated higher concentrations of potentially toxic elements such as silicon and phosphorus oxides compared to males. This may be explained by hormonal influences on bile composition, as estrogens are known to increase cholesterol saturation in bile and promote gallstone formation. The higher prevalence of gallstones among women reported in epidemiological studies aligns with these findings.

The application of XRF provided several advantages over conventional analytical approaches. Its ability to detect multi-elemental profiles without destruction of the specimen allowed for detailed compositional assessment. Moreover, identifying trace metals within gallstones may have clinical implications, as the accumulation of heavy metals such as copper and iron has been proposed as a potential risk factor for gallbladder inflammation and malignancy. Screening for these elements could therefore assist in assessing patient risk for gallbladder cancer or other complications.

Despite these promising results, several limitations should be acknowledged. The sample size was relatively small, and the study was restricted to a single geographic region, which may limit the generalizability of the findings. Environmental and dietary differences are known to influence gallstone composition; therefore, broader multiregional studies are needed to validate these trends. In addition, while XRF identifies elemental concentrations, it does not provide direct information on molecular structures or crystalline phases, which may require complementary techniques such as Fourier Transform Infrared (FTIR) spectroscopy or X-ray diffraction (XRD).

## 5. Conclusion

a. XRF technology can be used for direct chemical analysis of gallbladder samples.
b. A non-destructive analytical technique used to determine the chemical composition of materials
c. The method is characterized by high sensitivity and the elements in the sample are detected.
d. XRF technology makes it easy to quickly identify materials and problems.
e. The presence of many elements such as calcium is detected and decreases with age.
f. We screen for heavy metals found in gallstones to determine their association as potential risk factors for gallbladder cancer.
g. It was possible to differentiate between the male stone and the female stone by exploiting the change in the concentration of the elements in the samples.
h. Identifying the minerals that make up gallstones, and the doctor can warn the patient against eating these foods that increase the concentrations of these stones.
i. In the future, the XRF technology could play a major role in the characterization and characterization of biological samples such as gallstones and kidney stones.

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