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Quantitative analysis and calibration curve of human gallstone using LIBS technology for nanotechnological application

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In this study, quantitative and qualitative analysis of gallstones collected from Iraq patients living in Baghdad, Iraq have been done using LIBS technique. Firstly, to determine the concentration of collected gallstone elements, a calibration curve has been proposed as spectral line intensity against element concentrations. The elements within the stones are divided into two parts. the first one is phosphors (P), sodium (Na), magnesium (Mg), copper (Cu), aluminium (Al) and sulphur (S), while the second one are trace elements such as bismuth, boron, titanium, and antimuon. The results of the ICP-OES calibration demonstrated excellent linearity across all analysed elements, with correlation coefficients (R^2) exceeding 0.98, indicating robust regression models. Magnesium (Mg) and aluminium (Al) exhibited the highest sensitivities due to efficient emission at their selected wavelengths, while sulphur (S) showed lower sensitivity, likely due to weaker spectral lines indicated that it is possible to use this technique to analyse the elements within the gallstone faster and more accurate than any other techniques. clarify that ICP-OES is used as a reference technique to be compared with LIBS.

Keywords: LIBS; Gallbladder stones; ICP-OES.

1. INTRODUCTION

Nanotechnology is a term used in the fields of science and engineering. It is the study of phenomena that occur on a nanoscale. Nanotechnology is considered one of the most widespread technologies in the modern era. Nanotechnology has been used in the modern era in medical fields in general and has been used in particular in the field of disease diagnosis, medical treatments and pain relief. Modern treatments are now manufactured using nanotechnology to provide greater effectiveness and faster treatment than traditional treatments [1]. The human body consists of multiple biological organs, such as the gallbladder, kidneys, stomach, and bladder. Therefore, analysing the elements in these biological components gives us an idea of the health status of the affected parts of the human body [2].

Affected parts show different concentrations of trace elements compared to healthy parts of the body, indicating the presence of an organic disease in one of the body's organs [3]. The human body is composed of the major elements Hydrogen, Oxygen, and Carbon and non-essential elements such as magnesium, zinc, iron, aluminium and calcium. Understanding the basic structure of the biological components of the human body gives us an idea of the nature of the organs, the elements they need, and the nutrients that make up these elements. Changes in the chemical elements in the gallbladder, whether an increase or decrease, can cause gallstones, which are composed of essential elements such as calcium, magnesium, and others. Gallstones are often formed due to an increase in some of the inorganic elements that make them up, such as calcium and magnesium [4].

To diagnose the cause of gallstone growth, it is necessary to study the stones using analytical techniques such as Laser-Induced Breakdown Spectroscopy (LIBS), Energy Dispersive X-ray Spectroscopy (EDX), X-ray Diffraction (XRD), Atomic Absorption Spectroscopy (AAS), and Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES), which provide information about the type of stones and their chemical components. LIBS analysis is a new method and is considered a modern analysis that does not require sample preparation before analysis. It uses a laser to detect the elements and their quantities within the stones to determine the cause of their formation (figure1) [5].

1.2 Laser-Induced Breakdown Spectroscopy (LIBS)

Laser-induced breakdown spectroscopy (LIBS) is a technique developed in the early 1960s, shortly after the invention of the laser [5]. Over time, LIBS systems have undergone considerable miniaturization and performance enhancements, becoming compact, portable, and user-friendly. One of the key advantages of LIBS is its ability to analyse samples without any prior preparation, along with the capability for real-time measurements. The advancement of computer processing power, deep learning algorithms, and reduced equipment costs have made LIBS more accessible to non-experts in the field [6].

In LIBS, a pulsed laser is used to ablate a small portion of the sample, creating a micro plasma. The excited atoms and ions in the plasma emit characteristic spectral lines, which are analysed to determine the sample's composition. That LIBS plasma lifetime is very short (nanoseconds to microseconds), and detection requires time-resolved spectrometers LIBS has proven effective for analysing a wide variety of materials—solids, liquids, gases, slurries, and aerosols. Notable applications include metal analysis (e.g., iron and steel), soil pollution assessment, Martian rock and soil analysis, artwork dating, and even the detection of hazardous substances such as anthrax [5].

The most commonly used laser in LIBS systems is the Nd: YAG (neodymium-doped yttrium aluminium garnet) laser. These lasers are typically pumped by flashlamps or diodes. The pump light excites neodymium ions in the gain medium (the Nd: YAG rod) to higher energy levels. If the pump power is sufficient, population inversion occurs, whereby more ions occupy the upper lasing level than the lower one. Stimulated emission then amplifies photons of the same frequency as the lasing transition [7]. that matrix effects (sample composition influencing plasma properties) can affect LIBS quantification and require calibration or normalization.

When the laser rod is placed inside a resonant cavity formed by two mirrors, and some of the emitted photons are reflected back into the rod, significant optical amplification is achieved. This results in a monochromatic, coherent, and highly collimated laser beam along the optical axis [6-9]. A typical Q-switched laser configuration, used for pulsed LIBS applications, is illustrated in (Figure1). Lasers can employ a range of gain media, including partially transparent crystalline solids, glass, organic dyes in liquid solvents, and various gases or gas mixtures. Among the factors influencing the laser wavelength, the gain medium is the most critical Note that while Nd:YAG (1064 nm) is the most common, other harmonics (532 nm, 355 nm, 266 nm) are also widely used in LIBS to improve sensitivity for certain elements [10,11]. that Q-switching provides short, high-intensity pulses essential for plasma generation, while mode-locking is generally not used in LIBS.

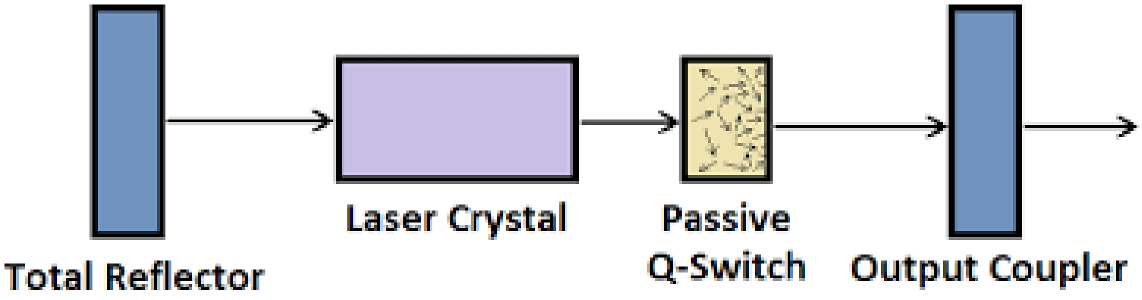


Figure 1 F, flashlamp; LR, laser rod; M, mirror; Q, Q-switch

2. EXPERIMENTAL

2.1 Inductively coupled plasma optical emission spectroscopy (ICP-OES)

To conduct an analysis of the elemental composition of gallstone samples, use the (ICP-OES) Analyzer with the (ICP-OES) (Agilent 7500). The (Agilent 7500) is one of the devices that provides accurate results on the concentration of elements in samples in the (ICP-OES) Analyzer and allows for determining the quantity of these elements in the samples[12].

The samples are subjected to ultrasonic cleaning using deionized water for 10 minutes and then rinsed with boiling deionized water" for scientific clarity to remove sediments such as blood, dirt, etc. They are then dried in oven-dried at 50 °C and then 10 ml of 2% NHO_3 solution is added before conducting (ICP-OES) analysis.

The gallstone samples are then analysed by (ICP-OES) to determine the concentrations of major and minor elements in each sample, such as (Ca, Mg, Na, P, and Al, S). The quantities of these elements determined by (ICP-OES) are then used to create a calibration curve. (Figure 2) shows the cumulative distribution of the analysed elements in the gallstone samples. The (ICP-OES) results are then compared with the equivalent LIBS data , Table 1 summarizes the (ICP-OES) results for all samples tested[13].

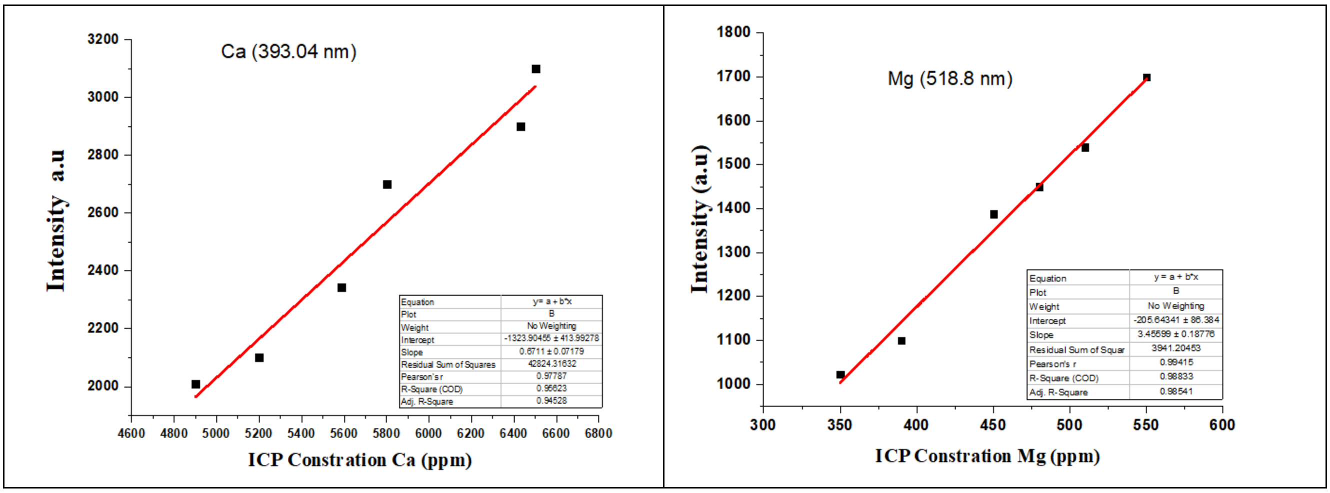
3. RESULT AND DISCUSSION

3.1 Qualitative analysis inductively coupled plasma optical emission spectroscopy (ICP-OES)

Calibration curves (figure 2) obtained by Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES)) are developed around 6 target elements (Ca393.04 nm, Mg 518.8 nm, Na 412.52 nm, P 402.44 nm, Al 526.53 nm, and S 373.03nm). All the elements are calibrated within their working range on the concentration unit, and the emission intensities (a.u.) are measured against the standard concentrations (ppm). Linear Regression analysis is run to obtain the slope, intercept, correlation coefficient and coefficient of determination of each analyte Table 1 summarizes the ICP-OES results for all samples tested. A summary of the calibration results is presented in Table 2.

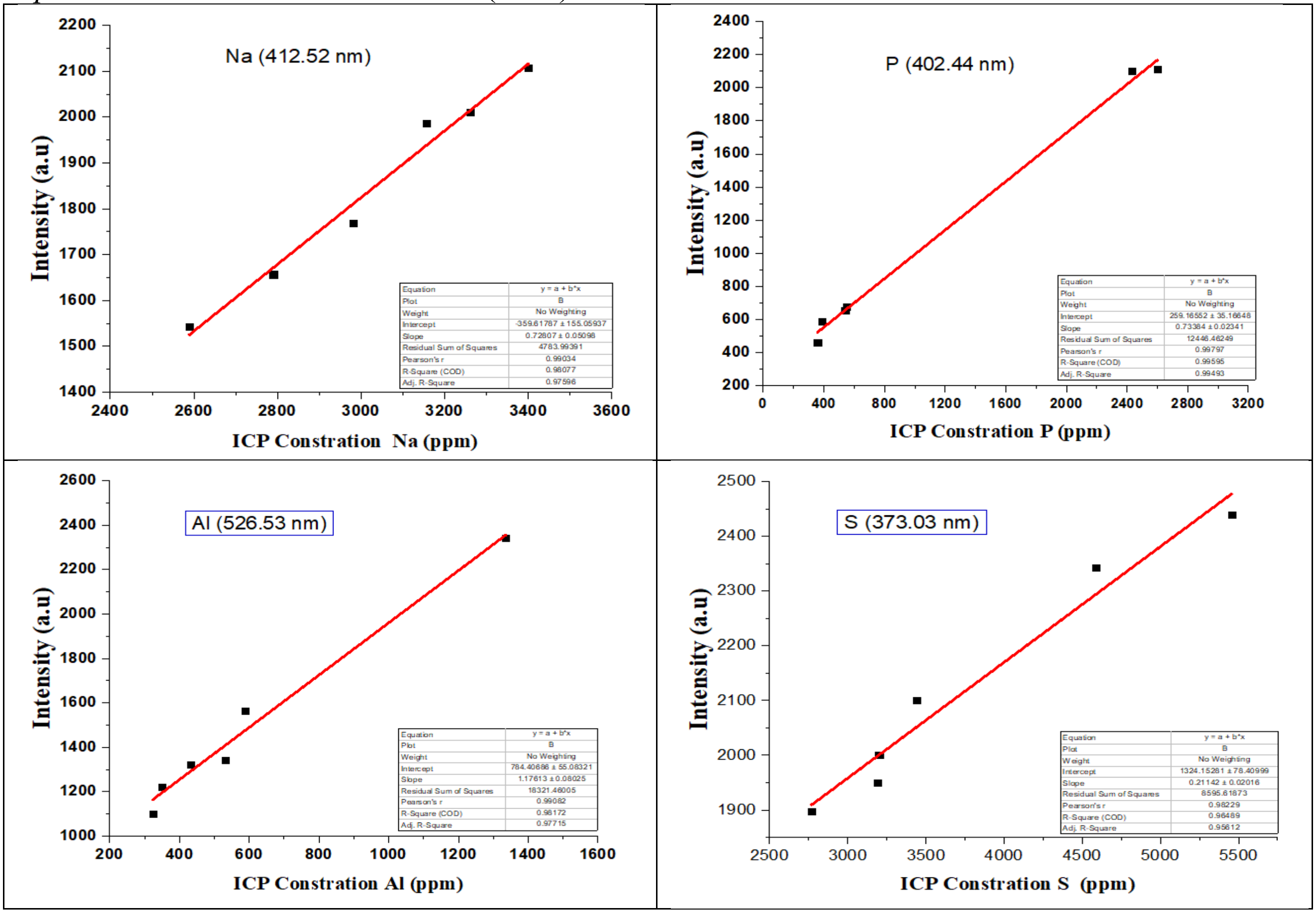
Table 1 Concentrations of elements in gallstone samples determined by ICP-OES (ppm).

Element	**ICP-OES (ppm)**					
	Sample1	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**
Ca	6500	6430	5800	5589	5200	4900
Mg	510	480	450	390	350	330
Na	3400	3260	3156	2980	2790	2589
P	2600	2430	550	540	390	358
Al	1335	589	532	433	350	324
S	5455	4590	3440	3200	3190	2768



Equation	y= a + b*x
Plot	B
Weight	No Weighting
Intercept	-1323.90455 ± 413.99278
Slope	0.6711 ± 0.07179
Residual Sum of Squares	42824.31632
Pearson's r	0.97787
R-Square (COD)	0.95623
Adj. R-Square	0.94528

Equation	y = a + b*x
Plot	B
Weight	No Weighting
Intercept	-205.64341 ± 86.384
Slope	3.45599 ± 0.18776
Residual Sum of Squar	3941.20453
Pearson's r	0.99415
R-Square (COD)	0.98833
Adj. R-Square	0.98541



Equation	y = a + b*x
Plot	B
Weight	No Weighting
Intercept	-359.61787 ± 155.05937
Slope	0.72807 ± 0.05098
Residual Sum of Squares	4783.99391
Pearson's r	0.99034
R-Square (COD)	0.98077
Adj. R-Square	0.97596

Equation	y = a + b*x
Plot	B
Weight	No Weighting
Intercept	259.16552 ± 35.16648
Slope	0.73384 ± 0.02341
Residual Sum of Squares	12446.46249
Pearson's r	0.99797
R-Square (COD)	0.99595
Adj. R-Square	0.99493

Equation	y = a + b*x
Plot	B
Weight	No Weighting
Intercept	784.40686 ± 55.08321
Slope	1.17613 ± 0.08025
Residual Sum of Squares	18321.46005
Pearson's r	0.99082
R-Square (COD)	0.98172
Adj. R-Square	0.97715

Equation	y = a + b*x
Plot	B
Weight	No Weighting
Intercept	1324.15281 ± 78.40999
Slope	0.21142 ± 0.02016
Residual Sum of Squares	8595.61873
Pearson's r	0.98229
R-Square (COD)	0.96489
Adj. R-Square	0.95612

Figure 2 ICP-OES analysis for gallstone elements (Ca393.04 nm, Mg 518.8 nm, Na 412.52nm, P 402.44nm, Al 526.53nm, and S 373.03nm).

Table 2 Calibration curve parameters for ICP-OES analysis of gallstone elements.

Element	Wavelength (nm)	Calibration equation	Slope (a) ± SE	Intercept (b) ± SE	R	R²	Adj. R²
Ca	393.04	y = 0.8711x – 1323.95	0.6711 ± 0.0718	–1323.95 ± 413.95	0.978	0.966	0.946
Mg	518.8	y = 3.4599x – 205.63	3.4559 ± 0.1878	–205.63 ± 86.38	0.994	0.988	0.985
Na	412.52	y = 0.7897x – 403.39	0.7280 ± 0.0501	–359.61 ± 155.06	0.990	0.981	0.976
P	402.44	y = 0.7531x + 259.16	0.7338 ± 0.0234	253.77 ± 35.16	0.998	0.996	0.995
Al	526.53	y = 1.1765x + 784.4	1.1761 ± 0.08025	746.61 ± 46.06	0.990	0.982	0.977
S	373.03	y = 0.2712x + 1324.15	0.21142 ± 0.0201	1341.15 ± 78.47	0.983	0.965	0.956

4.1.1 Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis of trace elements in gallstones

Calibration and Method Reliability The ICP-OES calibration results demonstrated excellent linearity across all analysed elements, with correlation coefficients (R^2) exceeding 0.98, indicating robust regression models. Magnesium (Mg) and aluminium (Al) exhibited the highest sensitivities due to efficient emission at their selected wavelengths, while sulphur (S) showed lower sensitivity, likely due to weaker spectral lines or matrix effects. Small positive or negative intercepts are observed, reflecting minor baseline signals, but these did not compromise the reliability of the calibration models. Adjusted R^2 values above 0.95 further confirmed the robustness of the regression models, ensuring accurate quantification of elemental concentrations. These results validate ICP-OES as a dependable method for quantitatively assessing the concentrations of calcium (Ca), magnesium (Mg), sodium (Na), phosphorus (P), aluminium (Al), and sulphur (S) in gallstone digests. The established calibration equations are subsequently applied to measure trace element concentrations in the studied samples, providing a reliable foundation for compositional analysis.

Trace Element Composition in Gallstone Groups ICP-OES analysis revealed distinct elemental profiles between two groups of gallstone samples: Group 1 (n=3) and Group 2 (n=3), as summarized in Table 3. Group 1 is characterized by significantly higher concentrations of copper (Cu: 4081 ppm), sodium (Na: 2705 ppm), magnesium (Mg: 1734 ppm), and phosphorus (P: 472 ppm) compared to Group 2. This enrichment in alkali and alkaline earth metals, along with phosphorus, suggests a predominance of pigment-based or mixed-type gallstones, where mineral components such as calcium bilirubin ate or phosphate salts are prevalent [14,15]. These elements are often associated with haemolytic disorders, biliary infections, or altered bile pH, which promote the precipitation of inorganic salts alongside organic components like bilirubin [16]. In contrast, Group 2 exhibited elevated levels of sulphur (S: 5069 ppm), bismuth (Bi: 41.33 ppm), boron (B: 172 ppm), titanium (Ti: 32.01 ppm), antimony (Sb: 4.92 ppm), tin (Sn: 26.1 ppm), and samarium (Sm: 2.18 ppm). This unique trace element profile may reflect distinct environmental exposures, dietary influences, or metabolic pathways affecting bile composition, potentially linked to cholesterol-rich or mixed gallstones with secondary mineralization [17]. Other elements, such as iron (Fe), chromium (Cr), arsenic (As), and aluminium (Al), showed variable concentrations across both groups, with no consistent dominance, highlighting the compositional heterogeneity of the gallstones.

Implications and Pathophysiological Insights The significant differences in trace element profiles between Group 1 and Group 2 underscore the multifactorial nature of gallstone formation. The high Cu, Na, Mg, and P content in Group 1 aligns with pigment or mixed gallstones, where inorganic mineralization plays a critical role, often driven by haemolytic conditions or biliary infections that increase bilirubin and metal salt precipitation [18]. Conversely, the enrichment of S, Bi, B, Ti, Sb, Sn, and Sm in Group 2 suggests a distinct biochemical or environmental influence, possibly related to cholesterol supersaturation with secondary incorporation of trace elements from external sources or metabolic dysregulation [19]. The variability in Fe, Cr, As, and Al across both groups further reflects patient-specific factors, such as diet, liver function, or biliary stasis, which modulate gallstone composition.

Summary and Significance ICP-OES analysis confirmed distinct elemental compositions between Group 1 and Group 2 gallstones, reflecting diverse formation mechanisms. Group 1 elevated Cu, Na, Mg, and P content points to pigment or mixed gallstones formed under haemolytic or infectious conditions, while Group 2 high S, Bi, B, Ti, Sb, Sn, and Sm levels suggest cholesterol-rich or mixed stones influenced by unique metabolic or environmental factors. The robust calibration ($R^2 > 0.98$, adjusted $R^2 > 0.95$) ensures reliable quantification, validating ICP-OES as a powerful tool for trace element analysis in complex biological matrices.

Table 3 Types of gallstones and concentration of trace elements.

No.	**Element**	**Concentration in (ppm) Group 1 (n=3)**		**Concentration in (ppm) Group 2 (n=3)**	
		Mean	**Range**	**Mean**	**Range**
1	Ag	< 0.5		< 0.5	
2	Al	281.2	461-16.52	754	1891-131.1
3	As	22.7	26.32-8.05	4.20	10.2-0.9
4	B	34.04	43.84-14.20	172	443-34.5
5	Ba	3.94	8.36-0.79	24.8	48.3-2.4
6	Be	25.6	30.96-2.45	<0.5	
7	Bi	< 0.5		41.33	93.7-0.42
8	Ca	5869	7310-4447	1809	3979-742
9	Cd	< 0.5		< 0.05	
10	Ce	< 0.05		2.01	4.1-0.33
11	Cr	28.17	55.65-2.89	14.6	52-3.9
12	Cu	4081	1639-5.50	32.04	102-14
13	Fe%	322	698-251	364	935-62
14	Ga	< 0.5		< 0.5	
15	Hg	< 0.5		< 0.5	
16	K%	281	601.68-92.26	102	246-32
17	La	< 0.5		< 0.5	
18	Mg%	1734	258.31-51.98	516	1439-240
19	Mn	1492	329.11-67.51	18.11	59.6-4.9
20	Mo	20.07	32.85-0.35	< 0.5	
21	Na%	2705	4133-1631	622	1790-674
22	Ni	116	220.61-3.60	< 0.5	
23	P	472	800.12-185.04	384	753-153
24	Pb	7.55	14.71-2.92	7.14	19.8-0.38
25	S%	2041	3899-198.29	5069	11829-3084
26	Sb	0.70	1.29-0.41	4.92	11.5-0.36
27	Sc	< 0.5		< 0.5	
28	Se	0.63	1.70-0.43	< 0.5	
29	Sr	3.09	9.68-2.43	3.47	8.0-0.42
30	Yb	< 0.05		< 0.5	
31	Ti	11.17	18.61-5.07	32.01	55.9-3.1
32	V	< 0.05		< 0.5	
33	W	0.84	1.20 -0.50	1.27	4.2-0.72
34	Zn	21.06	38.63 -14.52	< 0.5	
35	Zr	< 0.5		< 0.5	
36	Y	< 0.5		< 0.5	
37	Sn	0.88	1.80-0.41	26.1	31.5-`19.0
38	Sm	<0.05		2.18	4.6-0.33
39	Pr	1.14	2.27-0.65	< 0.5	
40	Li	<0.5		< 0.5	
41	Co	11.09	17.69-0.32	1.91	2.9-1.5

4.1.2 Trace element analysis of gallstones in group 1 and group 2

4.1.2.1 Elemental composition and differences

Trace element analysis via ICP-OES revealed significant compositional differences between gallstones in Group 1 (n=3) and Group 2 (n=3), reflecting variations in stone formation mechanisms, bile chemistry, and environmental or metabolic influences. Group 1 stones are enriched in phosphorus (P), sodium (Na), magnesium (Mg), copper (Cu), and manganese (Mn), which are commonly associated with pigment or mixed-type gallstones containing calcium bilirubin ate or phosphate compounds [20]. These minerals are indicative of mineralization processes driven by chronic haemolysis, biliary tract infections, or altered bile composition, which promote the precipitation of inorganic salts alongside bilirubin [20,21].

In contrast, Group 2 stones exhibited higher concentrations of sulphur (S), bismuth (Bi), boron (B), titanium (Ti), antimony (Sb), tin (Sn), and the rare earth element samarium (Sm). Elevated sulphur levels may suggest altered bile acid metabolism or increased presence of sulphur-containing amino acids, potentially linked to oxidative stress or hepatic dysfunction [22,23] . The presence of trace elements like Sb, Sn, and Bi in Group 2 could indicate environmental contamination from industrial processes, food packaging, or polluted water sources, as noted in prior studies [24,25]. Aluminium (Al) is also higher in Group 2, potentially reflecting exposure to antacids, dietary supplements, or environmental sources [26]. Variable levels of iron (Fe), chromium (Cr), nickel (Ni), arsenic (As), lead (Pb), and cadmium (Cd) across both groups suggest patient-specific factors, such as redox balance, trace metal-binding protein activity, or liver function, influencing gallstone formation [27,28].

4. CONCLUSIONS

LIBS analysis confirmed calcium as a consistent mineralizing agent, with variations in magnesium, iron, transition metals such as Mn, Ti, and Cr, and organic indicators (C, O) aligning with cholesterol, pigment, and mixed classifications. ICP-OES quantified trace elements across two groups, highlighting significant differences ($p < 0.05$ for 25 of 31 elements): Group 1 (pigment/mixed) was enriched in Ca (5869 ppm), Cu (4081 ppm), Na (2705 ppm), Mg (1734 ppm), Mn, P (472 ppm), Co, Zn, and K, pointing to inflammatory and hemolytic processes; Group 2 (cholesterol/mixed) showed higher S (5069 ppm), Bi (41.33 ppm), B (172 ppm), Ti (32.01 ppm), Sb (4.92 ppm), Sn (26.1 ppm), Sm (2.18 ppm), and Fe, implicating environmental contaminants, dietary influences, or metabolic dysregulation. T-tests validated these inter-group distinctions, with minimal variation in Fe, P, Pb, and Sr indicating ubiquitous baseline presence. The LIBS technique is the best of the other techniques since it has high accuracy and fast results without any side effects on human health.

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