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RESEARCH PAPER

Fast Analysis of Gallstones at Women Patients Using Nanotechnology of Laser-Induced Breakdown Spectroscopy

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ABSTRACT

Laser-induced breakdown spectroscopy (LIBS) is a powerful tool for elemental characterization of complex materials, including biomedical samples like gallbladder stones. In this study, single-pulse LIBS was used to analyse the elemental composition of gallstones collected from women in Hillah City, complemented by scanning electron microscopy (SEM) for morphological insights. A passively Q-switched Nd:YAG laser (1064 nm, 10 ns pulse duration) generated plasma, and a high-resolution CCD spectrometer captured emitted spectra in the 200–630 nm range. Major elements identified include calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and phosphorus (P), along with trace elements such as iron (Fe), zinc (Zn), copper (Cu), and chlorine (Cl). Calcium predominantly because this is the major component of calcium stones besides the trace metals that may signify metabolic or environmental influence. SEM exposed structural variation as per elemental compositions: Crystals identified calcium-based stones, and smooth surface identified homogenous surface cholesterol-based stones. 1064 nm laser ablation was able to provide effective ablation through reduced water absorption and, as a result, improved spectral quality. This finding provides a new twist to the classification of gallstones that may be in harmony with potential individualized treatment to prevent reoccurrence. The comparison demonstrates the potential of LIBS and SEM used in a complementary study to discuss the elemental and morphological characteristics of gallstones. This potential may still be extended even further to larger collections of data to the direction of cholelithiasis diagnosis and treatment.

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INTRODUCTION

Laser-induced breakdown spectroscopy is the multi-elemental analysis technique that gained much popularity in the 1980s [1]. The basic concept of laser-induced breakdown spectroscopy is akin to that of conventional plasma atomic emission spectroscopy. A major difference between LIBS and traditional atomic emission spectroscopy, however, is sample transportation to the plasma which is not required in LIBS. The medium can be designed as vacuum air and low-

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pressure inert gases for running laser-induced breakdown spectroscopic experiments [2,3]. LIBS has a sensitivity level at ppm or sub-ppm concentration levels hence modifications were made to enhance its performance. Among them are the use of an additional energy source or coupling with other analytical methods, e.g., laser-induced fluorescence, spark discharge assistance LIBS, surface-enhanced LIBS, among others that will sensitize its sensitivity [4]. This analytical method has received much attraction due to many advantages it possesses such as a requirement for a small amount of sample, relatively simple sample preparation, possibility of multi-element analysis, and cheap equipment used [5]. For these reasons, LIBS found broad applications in metal, plastics, petrochemical and food industry and environmental analyses increasingly applied in biomedicine [6]. Laser-induced breakdown spectroscopy (LIBS) is intensely researched regarding materials analysis from a very broad application background whereas interest in this technique increased due to technical advantages compared to other analytical methods7. Advantages include readiness to work with small sample sizes, non-dependence on vacuum conditions, little or no sample preparation, fast analysis time, multi-elemental detection capability, and à distance [8,9] along with online sample analysis. Among others, LIBS offers a chance of the determination of the light elements including hydrogen (H), carbon (C), nitrogen (N), and oxygen (O)[10, 11]. These are the significant components of the organic compounds.The objective of the work is to work out the elemental composition of gallbladder stones gathered in women of Hillah city using Laser-Induced Breakdown Spectroscopy-1064 nm laser. Major and trace elements of the stones will also be addressed to their help in classification and personalized dietary and medical interventions to avoid the recurrence; it would also play an important role in this research of the ability of LIBS power to be viewed as one potent weapon when deployed specifically on biomedical samples; this would be the case in Gallstone characterization.

The gastrointestinal disease manifests itself in cholelithiasis. It is accompanied by the presence of stones in the gallbladder. The surgical alternative of management of the gallstones is to have the gallbladder and the stones surgically removed in a procedure referred to as cholecystectomy. There are numerous variants of different strategies that have been employed to comprehend the ways how their formations occur in order to achieve a successful prevention of their reoccurrence. Non-spectroscopic methods like calorimetric and enzymatic methods are however, not specific, sensitive, or accurate enough. Conversely, Spectroscopic methods include Optical Photography, Radiography, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy (RS), Photoacoustic spectroscopy (PAS) and X-ray

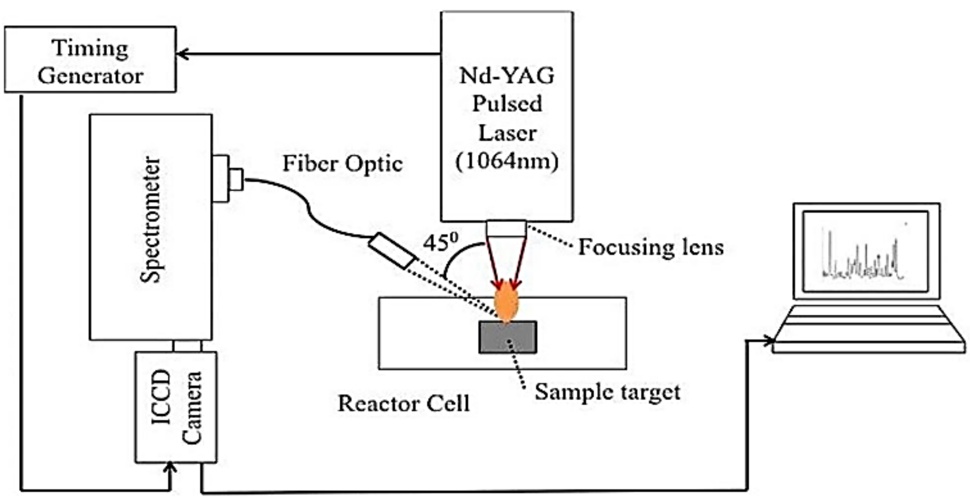

Fig. 1. Schematic diagram of LIBS experimental setup for the analysis of gallstone samples.

Diffraction (XRD) have also been widely used in structural and molecular studies of gallstones [13]. Among them, Laser-Induced Breakdown Spectroscopy (LIBS) can be listed as one of the largest diagnostic methods in defining the elemental constituency of gallstones [15,16]. LIBS is extremely beneficial as it identifies the general emissions produced by the plasma caused in case of laser ablation and that would generate a detailed data on the elemental composition of the sample [13].

It is highly crucial that a 1064 nm laser is chosen as a source in excitation in LIBS analysis because it falls under the near-infrared wavelength and does not interact with water strongly. This is why it is better to use it with biological samples; as an example, even the stones of the gall bladder may be high in moisture. A 1064 nm is enough to provide sufficient energy that will ablate the surface of the sample and therefore, produce plasma that will cause the intensification of the strong and clear spectral responses. Effects of self-absorption may distort peaks, especially of elements that have a high concentration. Also, due to the elaborate structure of the gallbladder stones, the broadening or overlap of the peaks may occur, making it difficult to identify some of the traces. These challenges can be addressed through proper calibration and the use of reference materials, ensuring more accurate and reliable results.LIBS offers then a powerful and versatile approach for analysing the elemental composition of gallstones, contributing valuable insights into their formation and potential prevention strategies.

MATERIALS AND METHODS

Sample collection Statistical analysis

Gallstones were collected from five women aged between 30 and 50 years and weighing between 65-80 kg as shown Table 1, which surgically removed from them in Al-Jumhuriya Hospital in Hillah City, Iraq, the stones were washed with deionized water to remove traces of urine, blood, and other potential contaminants, then dried and stored in sealed containers

Experimental set up

The spectra of the collected gallstones were recorded using single-pulse laser-induced breakdown spectroscopy (SP-LIBS). This method entails the creation of a plasma plume by using a passively Q-switched solid-state (ND:YAG) laser which has a pulse duration of 10ns and fundamental wavelength of 1064nm 13-16. In the experiment, a high-resolution charge-coupled device (CCD) spectrometer was also used, which has a spectral range of 200-800 nm and a delay time of the spectrometer is 400 ns and a laser energy of 40 mJ, as shown in Fig. 1. This arrangement was optimal in regards to elemental emission detection of the plasma plume produced during the ablation process.

RESULTS AND DISCUSSION

LIBS Spectrum Analysis

The majority of the spectral lines indicating the constituting elements in the five samples of gallbladder stones were found in the specific wavelength range as shown in Fig. 2. The identified essential nutrient elements were Ag, C, K, Cu, Al, Ca, P and Na, Si, S, Cl, I, Mg, O, N, Fe, Mn, Zn and Ag as revealed in Fig. 2. All these factors are presented in Tables 2-4 that gives a detailed account of spectral assignment of induced spectra. The tables consist of the concentration of the elements, the wavelength of the elements, the intensity of the elements and the full width half maximum (FWHM). This information leads to the full analysis of the elemental composition of the gallbladder stones under analysis.

The analysis of Tables 2-4 reveals several significant observations regarding the elemental composition of the gallbladder stones, as detailed below:

1. Major Elements:

• Silver (Ag) : The presence of silver is confirmed

Table 1. Specifications of the sample of patients with gallstones.

Gender	Age	Length(cm)	Weight (kg)
Female	45	155	81
Female	55	151	74
Female	40	160	70
Female	36	157	65

Table 2. The characteristics of atomic transition lines of different neutral elements and element identification.

Peaks table			
Wavelength [nm]	Intensity (a.u)	FWHM [nm]	Element
211.62	485.37	--	Ag II
232.73	5770.8	0.99097	
211.64	825.64	--	
211.56	777.15	--	
211.43	657.22	--	
215.19	462.2	--	P I
215.17	755.11	--	
529.06	426.15	--	Cu I
229.14	647.19	--	
615.51	1637.8	2.8046	
578.84	1187.4	--	
221.99	500.54	2.0912	Si I
222.1	119.6	0.68231	
252.19	5196.4	2.302	
252.04	3800.6	1.5664	
263.06	1490.9	0.67313	Fe II
262.98	1269.9	1.1807	
259.92	1515	--	
263.13	2452.8	0.66682	
432.18	1680.4	--	Fe I
374.11	3930.1	--	
373.84	6434.5	--	
386.57	17143	2.935	
627.68	450.57	--	Cu II
629.74	98.066	1.3645	
629.36	563.78	--	
628.2	810.4	2.3178	
470.81	2132.1	2.6454	O II
470.8	1979.1	2.2248	
470.71	2644.5	2.6065	
470.84	3188.9	2.5387	
624.82	461.37	--	Al II
624.91	239.51	0.40654	
624.88	781.53	--	
624.74	777.88	--	
316.28	1356.9	--	Na II
301.31	534.66	2.773	
300.88	976.74	3.3294	
318.55	6998.3	1.5128	

with a major peak at 232.73 nm, exhibiting an exceptionally high intensity of 5770.8 a.u. Smaller peaks are also observed around 211.62 nm with an intensity of 485.37 a.u. The strong emission at 232.73 nm is often used for the spectroscopic identification of silver.

• Copper (Cu) : Copper exists in two phases : Cu I and Cu II.

• Cu I Phase : Peaks are observed at 529.06 nm (intensity: 426.15 a.u.) and 324 nm (intensity: 3743.5 a.u.), with a notable FWHM of 1.4086 nm , indicating relatively sharp peaks.

• Cu II Phase : Significant peaks include those at 627.68 nm (intensity: 450.57 a.u.), 628.2 nm (intensity: 810.4 a.u.), and 277.2 nm (intensity: 41343 a.u.). The peak at 277.2 nm is the most intense, suggesting a stronger presence of ionized copper (Cu II) compared to neutral copper (Cu I).

• Oxygen (O) : Several strong peaks for O II are observed in the range of 470.8–470.84 nm, with the highest intensity at 3188.9 a.u. The FWHM values range from 2.22 to 2.64 nm, indicating well-defined and narrow emission lines characteristic of oxygen.

• Sodium (Na) : Sodium exhibits classic doublet lines at 588.73 nm (intensity: 2966.5 a.u.) and 588.75 nm (intensity: 1558.6 a.u.). Additionally, Na II shows strong peaks at 318.55 nm (intensity: 6998.3 a.u.) and 370.97 nm (intensity: 2915.1 a.u.), confirming the presence of ionized sodium.

• Magnesium (Mg) : A dominant peak appears at 517.86 nm with an intensity of 8584.2 a.u., along with multiple peaks in the 517–518 nm range. These peaks have relatively sharp FWHM values of 2.88–3.25 nm, making magnesium's spectral signature a reliable marker in spectroscopic analysis.

• Calcium (Ca) : Calcium's well-known H and K lines are evident at 393.61 nm (intensity: 32477 a.u.) and 396.97 nm (intensity: 20922 a.u.). These strong, sharp peaks are characteristic of ionized calcium and are commonly used in both astronomical and laboratory analyses.

• Potassium (K) : Peaks are observed at 630.11 nm (intensity: 373.89 a.u.) and 579.05 nm (intensity: 360.56 a.u.), with a sharp FWHM of 1.249 nm for the latter. These sharp peaks provide distinct emission characteristics often used as

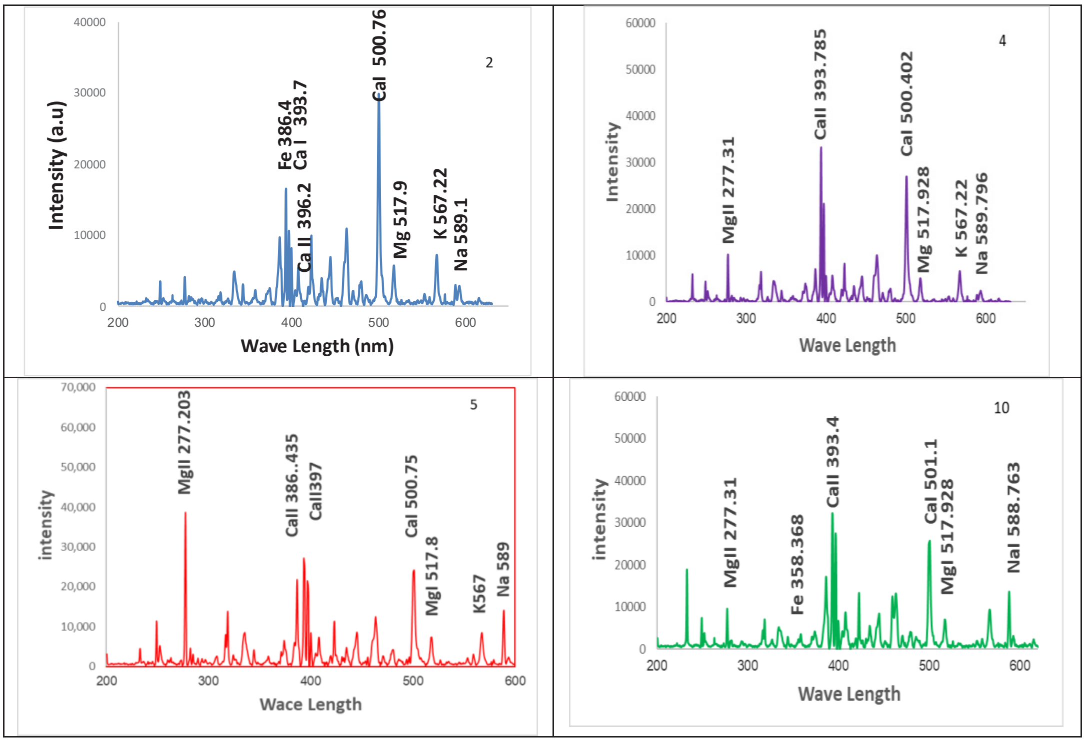

Fig. 2. Typical LIBS spectrum of a gallbladder stone test sample using 1064 nm laser as an excitation source for the five samples (A, B, C, and D).

reference points for potassium.

2. Minor Elements:

- Chlorine (Cl II): Chlorine exhibits a peak at 541.93 nm with an intensity of 576.46 a.u, characterized by a relatively high FWHM of 3.1186 nm .
- Sulfur (S II): A significant peak is observed at 544.98 nm with an intensity of 1077.9 a.u, while other sulfur peaks are relatively minor.
- Nitrogen (N I / N II): Nitrogen peaks are detected at 399.9 nm (intensity: 9407.1 a.u.), 400.04 nm (intensity: 6637.3 a.u.), and 399.96 nm (intensity: 8165.2 a.u.). These indicate the presence of nitrogen in both neutral (N I) and ionized (N II) states.

3. Trace Elements:

- Iron (Fe) : Iron is present in two phases : Fe I and Fe II.
- Fe I Phase : Peaks are observed at 432.18 nm (intensity: 1680.4 a.u.), 374.11 nm (intensity: 3930.1 a.u.), and a dominant peak at 373.84 nm (intensity: 6434.5 a.u.).
- Fe II Phase : Intense peaks are seen at 263.13 nm (intensity: 2452.8 a.u.) and 252.19 nm (intensity: 5196.4 a.u.), with relatively narrow FWHM values of approximately 0.67–2.3 nm . The coexistence of Fe I and Fe II suggests high-temperature or high-energy conditions during plasma formation.
- Zinc (Zn) : Zinc shows intense peaks at 334.09

Table 3. The characteristics of atomic transition lines of different neutral elements and element identification.

Peaks table			
Wavelength [nm]	Wavelength [nm]	Wavelength [nm]	Wavelength [nm]
293.21	911.82	2.5969	
293.16	892.02	1.9211	
293.41	1898.5	--	Mn II
293.27	1850.4	--	
334.09	4838.1	4.1732	
334.46	4489.6	4.5961	Zn I
334.98	8416.1	4.457	
397.05	10476	1.2614	
393.61	32477	1.4669	
396.97	20922	1.6973	Ca II
393.63	32099	1.6663	
630.11	373.89	--	
500.6	26918	2.8566	
611.43	965.91	--	K II
611.59	1277.1	--	
404.4	2207.7	--	Mn I
404.6	4226.8	--	
578.94	602.75	--	
579.05	360.56	1.249	
534.78	1251.1	--	K I
534.93	1297.8	--	
517.83	5655.6	3.1498	
517.89	5101	3.0812	
517.86	7375.2	2.8815	Mg I
517.92	7016.5	3.131	

nm (intensity: 4838.1 a.u.) and 334.46 nm (intensity: 4489.6 a.u.), with the most intense peak at 334.98 nm (intensity: 8416.1 a.u.). The broad FWHM of 4.457 nm for this peak may indicate potential blending with nearby emissions.

The spectral analysis indicates the existence of both major and minor elements, i.e. silver, copper, oxygen, sodium, magnesium, calcium, and potassium, and chlorine, sulphur and nitrogen, respectively. Also, some trace elements like iron and zinc are detected, which are useful in the deep examination of the elemental composition of the gallbladder stones. This information may contribute to the study of the process of formation of such stones and can inform the further therapeutic interventions.

Discussion of LIBS Spectrum Results for Gallbladder Stone Analysis

The spectral peaks obtained from analysis of the gallbladder stone are investigated and correspond to the specific elements detected in the sample. Ca, Mg, P as well as the trace metals Fe and Zn are often assigned to these peaks. Perhaps the most apparent is calcium, because it is a part of building calcium salts such as calcium carbonate, which you often see in gallbladder stones. The intensities of the peaks in the spectrum are associated with the abundance of the corresponding elements in the sample. Higher intensities of peaks show higher

(a) (b)

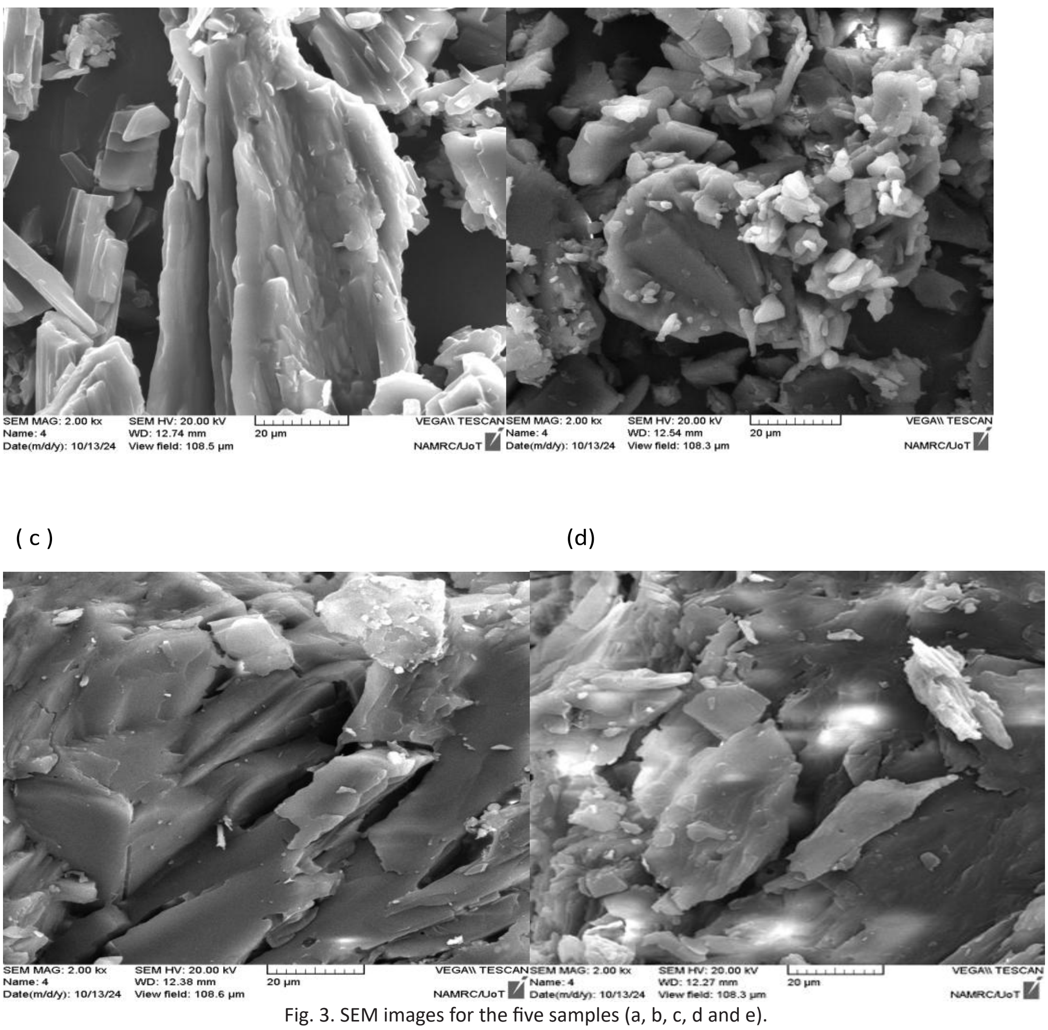

Fig. 3. SEM images for the five samples (a, b, c, d and e).

concentrations of that element. Though calcium can be a heavy ingredient if the moonstone is heavy in color whereas the other peaks are lighter. Alternatively, trace elements such as zinc or iron might appear as smaller peaks, indicating that they are detected in smaller quantities. The chemical composition of gallstones can be dependent on physiological, metabolic, and nutritional

Table 4. The characteristics of atomic transition lines of different neutral elements and element identification.

Peaks table			
Wavelength [nm]	Wavelength [nm]	Wavelength [nm]	Wavelength [nm]
524.03	519.04	--	I II
307.78	2582.8	3.3078	
534.94	1113.2	4.5246	
615.7	1130.2	3.2576	Ca I
615.94	642.54	1.8356	
559.1	3058.2	2.341	
615.77	2307.8	3.0255	
541.93	576.46	3.1186	
541.87	179.06	1.8247	Cl II
544.98	1077.9	--	
386.42	24460	2.6475	
544.83	765.52	--	S II
544.81	455.12	0.85623	
415.45	604.84	1.4236	
567.18	7207.1	3.2032	Si II
567.17	6584.5	3.1208	
567.19	8491.7	3.1941	
567.3	9382.8	3.3415	
588.73	2966.5	1.9739	Na I
588.75	1558.6	2.0785	
603.21	592.29	--	P II
603.1	300.55	1.1029	
603.19	745.81	--	
603.29	1043.7	--	
574.59	272.03	1.0085	N I
399.96	8165.2	1.2723	N II
400.04	6637.3	1.3461	
399.9	9407.1	1.4177	
578.86	1694.8	1.7715	Cu
588.75	14018	1.7834	C II
588.8	13564	1.8973	
588.73	22308	1.775	
308.5	743.01	--	Al I
236.87	832.87	--	

circumstances. Using the elemental profile, type of stone (pigment, cholesterol, or mixed stone) can be classified by the LIBS spectra:

- Cholesterol Stones : These stones may exhibit less intense peaks for calcium and phosphorus since they are primarily composed of cholesterol.
- Pigment Stones (Bilirubin-Based) : These stones often show prominent peaks for calcium and iron, as bilirubin stones are typically associated with calcium bilirubinate.
- Mixed Stones : These stones may display peaks corresponding to multiple components, reflecting the presence of both cholesterol and calcium-based compounds.

The primary clinical importance of the knowledge of the elemental composition of GB stones is appreciated. Knowledge of the predominant composition of the stone could be useful to objectively devise tailored treatment and diet guidelines. A high level of calcium, for example, could signal changes in the diet to avoid the precipitation of calcium salt into bile, while the specific presence of certain metals could signal metabolic abnormalities that increase stone formation. The elemental composition of gallbladder stones can be essential. LIBS is a fast, non-destructive analytic technique and has the advantage that elemental emission can be detected from a plasma plume that is generated from the material of interest. The composition of the stone is known through the resulting spectrum, which has peaks that correspond to the emission wavelengths of the elements included in the sample. A few similarities and differences between our results and the previous works on gallbladder stone analysis performed by LIBS. The results of the current study corroborate previous research that repeatedly found calcium, Magnesium and phosphorus are important parts of gallbladder stones. This idea is supported by a study (15), which found that calcium was the most common element in most samples. Similar results have been seen in earlier research that reported trace metals like iron and zinc, showing a consistent pattern in what makes up gallbladder stones across different populations. However, differences in where the samples come from, geographic locations, and eating habits can lead to different findings. These variations show how metabolism and environment influence stone formation. Besides, this study used a laser with a wavelength of 1064 nm, which has some advantages over other studies that used lasers with different wavelengths. The 1064 nm laser is less affected by water absorption, leading to clearer and more accurate spectral data. This improvement emphasizes LABS's potential as a powerful tool for exploring the detailed makeup of gallbladder stones and helps us better understand how they develop.

SEM Investigations

By examining the SEM images of 5 gallstone samples (Fig. 3) a variety of shapes and surface textures can be observed which indicates the different elemental compositions of the samples. On a closer look, you see patterns that are both clear crystalline and grainy textures, and more amorphous and compact forms in these pictures... The physical structure of the stones and their elemental composition are closely connected, with the close correlation existing between these morphological differences and the chemical elements identified by LIBS, such as the strong peaks in the Ca in the LIBS spectra at 393.61 nm and 396.97 nm, indicating a high calcium content of the sample. Typically, it happens that well-organized crystalline structures are observed in the SEM pictures of samples that are rich in calcium, which are strongly indicated by the strong peaks of the Ca in the LIBS spectra at This is consistent with the presence of calcium salts, such as calcium phosphate or carbonate, which at a molecular scale can be seen as crystals that are recognizable. Conversely, samples containing a large proportion of cholesterol give smoother, more homogenous morphologies, typical of lipid-rich stones, with the peaks of calcium being less conspicuous in the LIBS spectra.Other trace elements such as copper (Cu), zinc (Zn), and iron (Fe) identified in the LIBS spectra appear as small particles or aggregates in the SEM images, and appear to be concentrated in specific areas of the samples. The various patterns of distribution of trace metals imply that they could contribute to the process of crystals formation and growth within gallstones. By way of example, the regions with high levels of iron, indicated by the peaks of Fe I and Fe II in LIBS spectra, are frequently presented in SEM images in the form of small dots or nodules. These are potential deposits of complexes composed of ferric iron and bilirubin. On the whole, the comparison of SEM images and LIBS information reveals that the two techniques are very compatible with each other. SEM allows us to glimpse the form and physical structure of these elements in the stones whereas LIBS gives us accurate data on the presence and the quantity of the elements. This single method is able to not only recognize whether a stone is predominantly a cholesterol, pigment, or a combination of both, but also enhance our knowledge of how the gallstones are formed, and how they proliferate. Such information can provide helpful hints to the prevention and treatment of issues with gallstones.

CONCLUSION

The elemental composition of the gallbladder stones was further explained with the assistance of the laser-induced breakdown spectroscopy (LIBS). Through the method, vital components that were made were calcium, phosphorus and trace metals like iron, zinc among others. Because of the capacity of the 1064 nm laser to effectively ablate and promote spectral clearness, LIBS is very practical in the examination of wet biological samples. The results will make it possible to categorize gallstones as cholesterol, pigment, or mixed stones and, therefore, will help develop gallstones and specific treatment plans. The electron microscopy (SEM) scanned the surface of the stones providing detailed images and it is possible to notice that the differences in textures are consistent with the elemental compositions. To illustrate, a calcium rich rock was obviously divided into crystals but cholesterol stones were smoother and homogenous to the naked eye. As they complement each other, LIBS and SEM provide a more detailed perspective on gallstones and the diagnosis of them is more accurate. To make LIBS and SEM even more powerful in biomedical studies in the future, we might consider developing our datasets and applying the newest advanced techniques that would enable the latter to do the same. Overall, LIBS is a new technology-it is speedy, non-invasive and would translate to better diagnostic tests and prevention and treatment interventions against the complications caused by gallstones.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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