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Medical Applications of Laser Induced Breakdown Spectroscopy

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Abstract. Sedentary lifestyle of human beings has resulted in various diseases and in turn we require a potential tool that can be used to address various issues related to human health. Laser Induced Breakdown Spectroscopy (LIBS) is one such potential optical analytical tool that has become quite popular because of its distinctive features that include applicability to any type/phase of samples with almost no sample preparation. Several reports are available that discusses the capabilities of LIBS, suitable for various applications in different branches of science which cannot be addressed by traditional analytical methods but only few reports are available for the medical applications of LIBS. In the present work, LIBS has been implemented to understand the role of various elements in the formation of gallstones (formed under the empyema and mucocele state of gallbladder) samples along with patient history that were collected from Purvancal region of Uttar Pradesh, India. The occurrence statistics of gallstones under the present study reveal higher occurrence of gallstones in female patients. The gallstone occurrence was found more prevalent for those male patients who were having the habit of either tobacco chewing, smoking or drinking alcohols. This work further reports *in-situ* LIBS study of deciduous tooth and *in-vivo* LIBS study of human nail.

1. Introduction

Increasing incidence of gallstone formation in gallbladder is of great concern for medical practitioners which is a major cause of morbidity and mortality in developed as well as developing countries. Analysis of chemical composition of the different kinds of gallstones can provide a significant clue for the treatment and prevention of their recurrence. The presence of gallstones in the gallbladder results from changes in bile components. Bile is yellowish fluid, formed by liver, to digest and absorb the fats from food which has been broken by enzymes. These enzymes are secreted from pancreas and the intestine. Usually, gallstones form when bile stored in the gallbladder hardens into pieces of stone-like materials. Gallbladder is capable of considerable distension in some pathological conditions [1].The gallbladder mucocele or empyema occurs when a stone obstructs the neck of the gallbladder. Mucocele is a non-inflammatory condition of the gallbladder with increased mucus production from the gallbladder epithelium. The empyema of gall bladder appears to be filled with pus that may be a sequel of acute cholecystitis or the result of a mucocele being infected. As these gallstones are capable of obstructing the neck of gallbladder, thus our interest, in the present study, is to explore the elemental differences between these gallstones under these conditions. Bio-materials such as teeth and nails are excellent bio-monitors because they can show the level of deposition of essential minerals over weeks to years due to the habitat, nutrition, and other environmental conditions [2-3]. Dental related problems (like caries) are major oral health problems throughout the world. The elemental analysis of teeth samples can give information about the causes of caries in human teeth. Laser Induced Breakdown Spectroscopy (LIBS) is an excellent analytical tool suitable for the elemental analysis of any kind of materials irrespective of their phases [4-6].This manuscript also presents an excellent example of *in-situ* LIBS analysis of deciduous tooth and *in-vivo* LIBS study of human nail.

2. Materials and methods

Gallstones samples along with patient history were obtained from Opal Hospital, Varanasi, India. After the surgery of the patients the stone samples were washed with de-ionized water to remove the blood and other adherent contaminants/ impurities and brought it in the Laser Spectroscopy Laboratory, Department of Physics, University of Allahabad, Allahabad, in sealed pots. The tooth samples from patients were supplied by Vinayak Hospital, Allahabad, India. The tooth samples were washed in distilled water to remove all the contamination from its outer surface and dried at room temperature. Firstly, tooth samples were visually examined by trained examiners/Doctors and then used for the LIBS diagnosis. The nail samples obtained from different age group volunteers have been used for *in-vivo* analysis and results related to volunteer aged 32 is discussed.

The LIBS spectra of various gallstone samples were recorded using LIBS experimental set up described in detail in review by Pathak et al. [6]. The laser pulse energy and pulse repetition rate were optimized and better signal to background and signal to noise ratio were observed for gallstone and nail samples at 20 mJ energy and 10 Hz repetition rate. For tooth samples the averaged (10 laser shots) LIBS spectra having best signal-to-noise (S/N) ratio and signal-to-background (S/B) ratio was found using 30 mJ of laser energy per pulse @ 10 Hz. *In-vivo* LIBS spectra of nails and *in-situ* LIBS spectra of deciduous tooth (collected from hospital mentioned above) were recorded by directly focusing the laser beam on the surface of the samples.

3. Results and discussion

3.1. Occurrence statistics of gallstones

A total of 92 patients of gallstone disease were reported during March 2008 to July 2009 at Opal hospital, Varanasi, Uttar Pradesh, India. This section studies the risk factors for gallstone disease on the basis of database (age, gender, diet & BMI) collected to these 92 patients [7].

Table 1 shows the age and gender distribution of gallstone patients (N= 92). From the Table 1, it is observed that maximum number of gallstone patients belong to age group 35-65, irrespective of their gender. Further, it is also observed that occurrence of gallstone is almost twice in female as compared to male patients. Figure 1 clearly depicts the Gender (male & female), Diet (Veg & Non-veg) and Body Mass Index in kg/m^2 (BMI) distribution and number of gallstone occurrence for 92 patients. Though it is observed from the data of Table 1, the possibility of gallstone formation is less in male patients but on the other hand the occurrence of gallstone in male patients having habit of either chewing tobacco, smoking or drinking alcohols are more as compared to normal.

Table 1. Age and Gender distribution of Gallstone patients (N=92)

Age group (years)	**<35**	**35-65**	**Over 65**
Number of Patients	18(19.56 %)	69(75.0 %)	05(5.4 %)
Females	15(16.3 %)	49(53.3 %)	02(2.2 %)
Males	03(3.2 %)	20(21.7 %)	03(3.2 %)

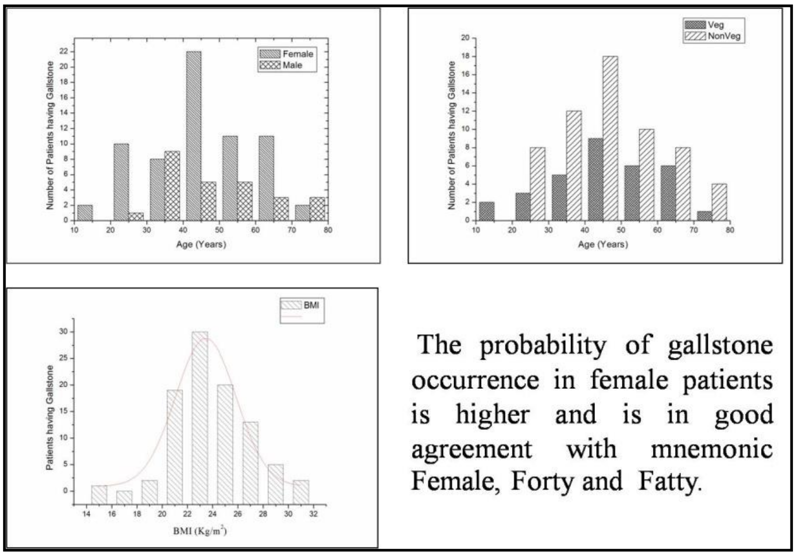

Figure 1. Bar diagram showing Age & Gender, Diet & BMI distribution of gallstone occurrence.

3.2. Study of gallstone related to empyema and mucocele state of gallbladder

We have reported studies related to different type of gallstones using LIBS [8-10] and this manuscript describes the gallstone formed under empyema and mucocele of gallbladder. Out of ninety two patients of gallbladder stones only eight (9.8%) gallstone samples belong to empyema of gallbladder and four samples (4.4%) belong to mucocele of gallbladder whose specifications are mentioned in Table 2. It is observed from Table 2, that the outer parts of the most of the gallstones belonging to empyema (E-1to E -8) and mucocele (M-1to M-4) of gallbladder are of colours yellow, white and green and the morphological classification suggests that outer parts of these may be dominated with cholesterol[6].

Table 2. Specifications of gallstones associated to empyema and mucocele (N=12)

Empyema	**Color of gallstone**	**Patient Age(Years) Gender**	**Remarks**
E-1	Black	56 , Female	
E-2	Outer yellow with inner brown	55, Female	Obese,Type-2 diabetes and hypertension
E-3	Outer white with light yellow inner	29, Female	
E-4	Outer green with inner brown	40, Male	Habit-Smoking
E-5	Outer green with inner brown	48, Female	
E-6	Outer brown with inner black	40, Female	
E-7	Outer yellowish white with inner brown	42, Female	
E-8	Outer yellow with inner brown	45, Female	

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Mucocele	**Color of gallstone**	**Patient Age(Years) Gender**	**Remarks**
M-1	Outer white with inner black	36, Female	
M-2	Outer white with inner brown	40, Female	
M-3	Outer greenish brown with inner brownish white	60, Male	Habit-Alcohol
M-4	White	70, Female	Type-2 diabetes and hypertension with cardiovascular disease

As these gallstone samples are capable of obstructing the neck of gallbladder, thus we were interested to investigate how these gallstones are different. For this, we have focussed laser beam on outer and inner parts of these gallstones to record the LIBS spectra. Typical LIBS spectra of gallstones belonging to empyema and mucocele are shown in Figure 2. The LIBS spectra have been analyzed and spectral lines of the elements like C, Ca, Mg along with C_2 bands are observed in both types of samples. The analysed LIBS spectra of outer parts associated with empyema and mucocele gallbladder stone have prominence presence of atomic spectral signature of carbon C lines at 247.8 nm and 229.6 nm and molecular signatures of C_2 bands at 469.7 nm, 471.4 nm, 473.6 nm, as compared to inner parts of these gallstone. Thus the outer parts of these gallstone samples resemble cholesterol gallstone.

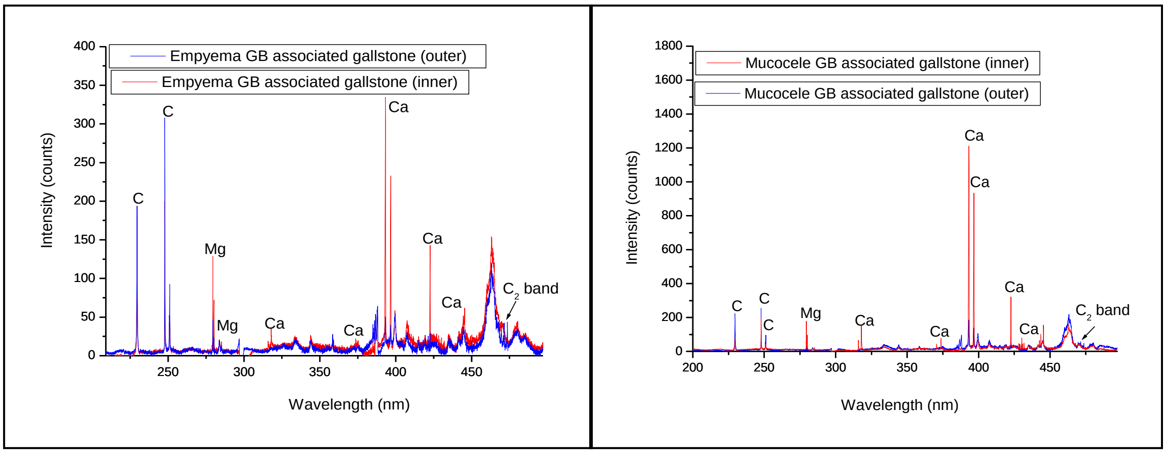

Figure 2. Typical LIBS spectra of gallstone.

In order to get the idea of hardness, we have calculated intensity ratio of ionic line of C (229.2 nm) to the corresponding neutral atomic line C (247.2 nm) for outer and inner parts of gallstones related to empyema and mucocele of gallbladder. The ratio[C(229.2)/C(247.2)] for outer parts are calculated as 1.26±0.06 and 1.55±0.13 but this ratio for inner parts are calculated as 0.75±0.05 and 0.45±0.0.24, for empyema and mucocele gallstones, respectively. Thus we found that the outer parts of both the gallstones related with empyema and mucocele state of gallbladder are having higher ionic to neutral spectral intensity ratio as compared to inner part. Based on the report by Tsuyuki et al. [11] in which he described that the intensity ratio of ionic to neutral atomic line of calcium is proportional to the compressive strength of target sample because in hard target, speed of shock wave produced is more in comparison to the soft target which results in enhancement of the ionization. Therefore from LIBS study we can conclude that outer surface of the gallstone is harder than inner part which may be the probable cause why these gallstones obstruct the neck of gallbladder.

3.3. Study of healthy and caries affected parts of deciduous tooth

Early childhood caries are becoming more common problem because of frequent intake of fast foods and sugars. The health of deciduous teeth (Primary dentition) should be also taken care because the health of permanent teeth also depends on them [4]. Therefore we have studied the caries and healthy parts of enamel of tooth sample of a child (aged 5 years) affected with early childhood caries. LIBS spectra of caries and healthy parts of the deciduous tooth sample were recorded in the spectral range 200-900 nm. LIBS spectra of the healthy part and caries affected part of the enamel of deciduous tooth sample in spectral range 225-330 nm & spectral range 575-800 nm are shown in Figure 3. Since the major constituents of the tooth's crystalline enamel structure is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ and we observed the persistent emission lines of the elements Ca, P ,H and O which are the matrix elements of the tooth. Our result reveals that the intensities of the atomic lines of Ca and P are higher in healthy parts than that of caries affected parts of the tooth samples (Figure 3) which indicates that the concentrations of Ca & P are decreased in caries affected part of the tooth enamel. In contrast to the above, the intensity of atomic lines of the trace elements i.e. Mg, K, and C are higher in the caries than that of healthy part of the tooth sample and thus the concentration of these elements are increased in caries affected part in comparison to healthy part (Figures 3). We have calculated intensity ratios of different analyte emission lines of C at 247.8 nm, Mg at 285.2 nm, and K at 766.4 nm to the background were estimated using LIBS spectra of caries affected part and healthy part of the tooth sample. It is clear from Fig. 4 that the normalized intensity of C (247.8 nm), Mg (285.2 nm), and K (766.4 nm) are higher in caries affected parts of the tooth sample than that of the healthy parts which reveals that the concentration of the non-matrix elements like C, Mg, and K increases. The changes in the concentration of matrix elements may be due to fact that calcium bound to the hydroxyapatite are ionized and subsequently washed out from the tooth and are replaced by other elements like Mg, C and K. Demineralization of main matrix elements (Ca & P) occurs for several reasons, but one of the most important causes is the ingestion of sugars. In addition to, foods high in starches, such as potato chips, crackers, breads, and cakes may also be a cause to damage teeth. Our result related to caries and healthy parts of deciduous tooth are in agreement with the results reported for permanent tooth [5].

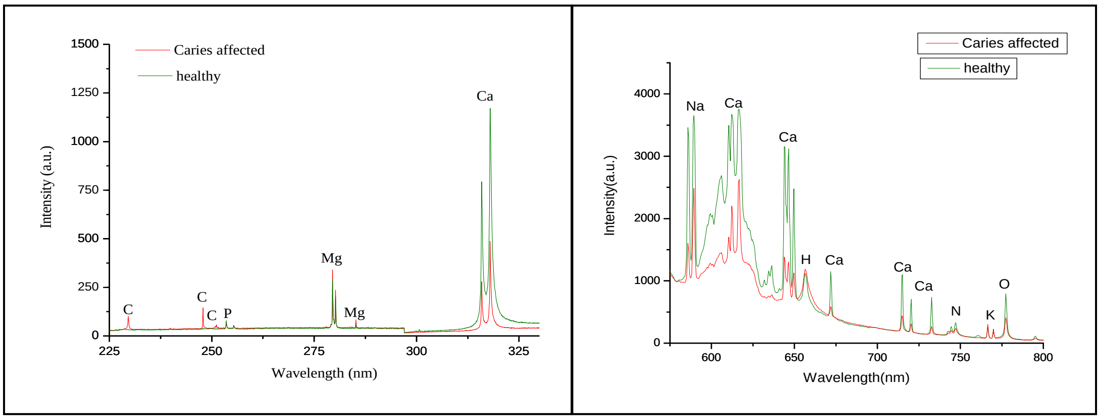

Figure 3. LIBS spectra of caries and healthy enamel of tooth.

We have also compared the hardness of healthy and caries affected part of the tooth samples. For this purpose we have used spectral line of Calcium and we have calculated intensity ratio of ionic lines Ca II (373.6 nm), to neutral lines Ca I (422.6 nm) from the LIBS spectra of the caries enamel and healthy enamel. For deciduous tooth this ratio is calculated and found as 1.11 ± 0.20 for healthy enamel but 0.63 ± 0.10 for caries enamel. This indicates that hardness of the tooth enamel decreases in the case of caries. Thus it is observed that the ionic to atomic spectral intensity ratio is higher for healthy enamel as compared to caries enamel. We have repeated this measurement for the permanent tooth sample belonging to different age group of patients and also found similar trends. Therefore the ionic to atomic LIBS spectral line intensity ratio is also an important indicator to distinguish healthy part and caries affected part of tooth.

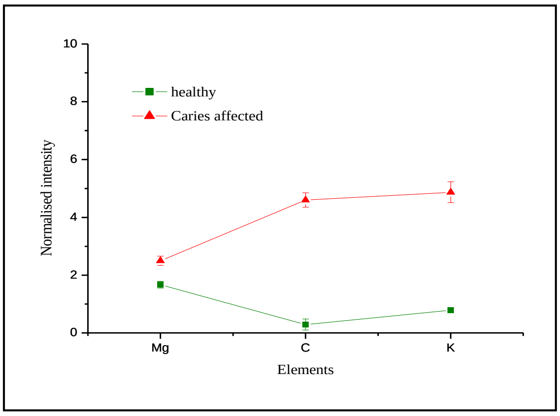

Figure 4. Variation of concentration of non-matrix elements.

3.4. In-vivo human nail study

In-situ LIBS study of the finger nail clippings belonging to normal nail sample and the healed injured nail sample is reported [12] and it is shown that main matrix of the nail are not affected much but mineral elements like Ca, Na & K changed much in injured/ reshaped nail. In this study we present the *in-vivo* LIBS analysis of human nails. In order to know the variations and similarities between left-hand and right-hand nails, the LIBS spectra of mineral profiles of left-hand and right-hand nails were recorded. The LIBS spectra were recorded by focusing the laser beam directly on each finger nail (Thumb finger nail:TFN, Index finger nail:IFN, Middle finger nail:MFN, Ring finger nail:RFN, Smallest finger nail:SFN) of left hand and right hand belonging to different volunteers.

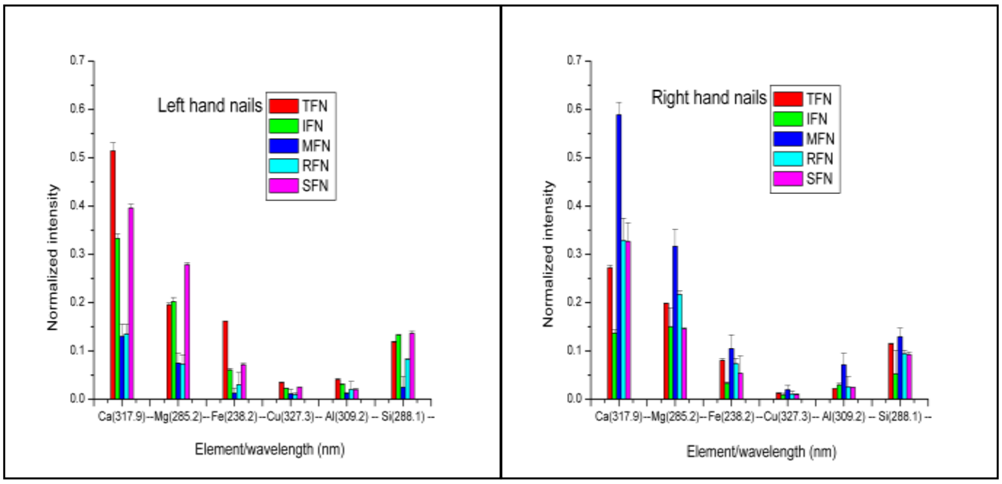

Figure 5. Bar diagram showing intensities of the spectral lines of elements in LIBS spectra.

The normalized intensity of selected spectral lines of Ca (317.9 nm), Mg (285.2 nm), Fe (238.2 nm), Cu (327.3 nm), Al (309.2 nm) and Si (288.1 nm) were evaluated by dividing intensity with corresponding background signal for each finger nails belonging to Left hand and Right hand. The *in-vivo* analysis belonging to left hand and right hand nails of volunteer aged 32 years is shown in Figure 5. It is observed from Fig. 5 that even the left hand and right hand mineral profiles are not exactly same. We have also found similar pattern of nail profiles for other volunteers. Thus *in-vivo* nail study can give instant information regarding changes in elemental profile of nail. We are also continuing our study for large sample data to find further correlation in different nail samples and the study is in progress.

4. Conclusion

The present study demonstrated how laser induced breakdown spectroscopy has been implemented to understand the role of various elements in the formation of gallstones (formed under the empyema and mucocele state of gallbladder).On the basis of database from patient history, the occurrence statistics of gallstones revealed higher occurrence of gallstones for female patients. Though the gallstone occurrence was less in male patients but cases were more prevalent for those male patients who were having the habit of either tobacco chewing, smoking or drinking alcohols. The work also reported *in-situ* study of deciduous tooth and *in-vivo* study of human nail.

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