Biomedical applications of Laser-Induced Breakdown Spectroscopy (LIBS)

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ABSTRACT

LIBS has been proven to be a robust elemental analysis tool attracting interest because of the wide applications. LIBS can be used for analysis of any type of samples i.e. environmental/physiological, regardless of its state of matter. Conventional spectroscopy techniques are good in analytical performance, but their sample preparation method is mostly destructive and time consuming. Also, almost all these methods are incapable of analysing multi elements simaltaneously. On the other hand, LIBS has many potential advantages such as simplicity in the experimental setup, less sample preparation, less destructive analysis of sample etc. In this paper, we report some of the biomedical applications of LIBS. From the experiments carried out on clinical samples (calcified tissues or teeth and gall stones) for trace elemental mapping and detection, it was found that LIBS is a robust tool for such applications. It is seen that the presence and relative concentrations of major elements (calcium, phosphorus and magnesium) in human calcified tissue (tooth) can be easily determined using LIBS technique. The importance of this study comes in anthropology where tooth and bone are main samples from which reliable data can be easily retrieved. Similarly, elemental composition of bile juice and gall stone collected from the same subject using LIBS was found to be similar. The results show interesting prospects for LIBS to study cholelithiasis (the presence of stones in the gall bladder, is a common disease of the gastrointestinal tract) better.

Keywords: LIBS, Elemental analysis, Calcified tissue, Cholelithiasis

1. INTRODUCTION

Laser-induced breakdown spectroscopy (LIBS)/laser-induced plasma spectrometry (LIPS)/laser spark spectrometry (LSS) is a total multi-elemental analytical technique based on atomic emission spectroscopy (AES). LIBS technique can be used for elemental analyses of solid, liquid or gas samples, qualitatively as well as quantitatively[1-3]. It utilizes a high-energy laser pulse as the vaporization, atomization, and excitation source to create a high-temperature micro-plasma at the surface of the target. This plasma while relaxing emits electromagnetic radiation. The emitted light can be collected by a spectrograph and recorded, usually with an Intensified Charge Coupled Device (ICCD). The short laser pulse breaks up the chemical bonds in the sample to elementary atoms and ionizes them.

Cholelithiasis, the presence of stones in the gall bladder, is a common disease of the gastrointestinal tract. Most of the patients with this condition require surgical treatment called cholecystectomy. Over the past several years, there have been continuous efforts aimed at understanding morphology, pathogenesis, formation, and compositions of gall stones. The gall stones are classified in to two main categories namely cholesterol and pigment stones[4] based on the composition of cholesterol and bile pigments, cholesterol is the main composition of cholesterol stones (70%) and bile pigments in pigment stones. The bilirubin, bile acids, fatty acids, triglycerides, phospholipids, glycoprotein, mucin are the other chief constituents of gall stones[5]. Many of these are normally found in the bile and serum. Chemical composition and appearance of each of these stones differ from case to case indicating their different mechanism of formation. It has been reported that many elements viz. sodium, potassium, calcium, magnesium, copper, iron, phosphate, and chloride are constantly present in these stones[6]. So, the elemental analysis gives important evidence on metabolic basis of their formation which helps in the identification of certain etiological agents/protein factors that predispose certain individuals

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to the calculi formation. Also it is known that, mineralized tissue, i.e. bones and teeth have been found to be excellent 'archives' related to living habits, nutrition and mobility of the ancient as well as the modern human.

Non-spectroscopic techniques, such as enzymatic and calorimetric methods, have been used to determine chemical composition of gallstones, which lacks both sensitivity and specificity. X ray diffraction, scanning electron microscopy, FTIR spectroscopy and LIB spectroscopy are some of the other techniques currently being used to detect the various elements present in the gall stones. The LIBS technique showed real time elemental detection of any type of samples as mentioned earlier[7-9]. The present study aimed to find out the elemental composition of gallstones and correlation between the components of gall stones and bile juice, in order to understand the mechanism of their formation. Also the preliminary experiments carried out on calcified tissues or teeth for trace elemental detection, yields interesting inferences.

2. MATERIALS AND METHODS

2.1 Experimental

5 gall stones samples (Figure 1) were collected from patients with cholelithiasis from the Department of Surgery, Kasturba Medical College, Manipal. Gall stone samples were used directly to record the LIBS spectra. The bile juice sample used for this study was subjected to lyophilization and then the pellets were made. These pellets were later used to record the LIBS spectra. Similarly, calcified tissue samples utilized for this study were obtained from oral surgery department, Manipal college of dental sciences, Manipal.

Figure 1 Gall stone samples used for LIBS studies

The setup for LIBS analysis is shown in Figure 2. The 3rd harmonic (λ = 355 nm) of a Nd:YAG laser (Spectra Physics PRO 230-10) was used as radiation source. The pulsed beam of 6 ns duration and 10 Hz repetition frequency with energy 30 mJ is focused on to the sample. A bi-convex lens of 10 cm focal length was used to focus the laser beam on to the gall stone/tooth samples. The sample was fixed on a moveable x-y-stage. It was moved during measurement to ablate material from an area not influenced by the laser beam. The light collecting system consisted of lenses/mirrors that focus the emitted radiation to an optical fiber. The optical fiber guides the collected light to the entrance slit of a high resolution, cross-dispersion echelle spectrograph-ICCD system (Mechelle ME5000-DH734-18U-03PS150). The detector was kept in synchronization with the laser using a delay-generator to allow time-resolved measurements. The spectrum was measured in the range from 250 nm to 850 nm at a wavelength resolution of 0.05 nm.

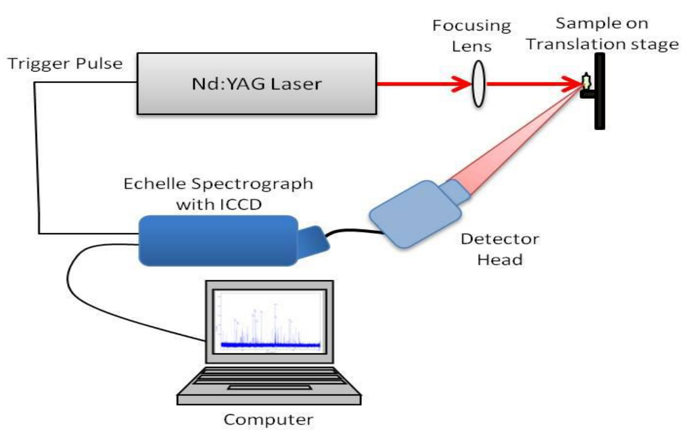

Figure 2 LIBS set-up used for the current study

3. RESULTS AND DISCUSSION

LIBS spectra have been recorded (laser line intensity 4.38 x 10^9 W/cm^2; gate delay 700ns and gate width 6µs) from all five gallstone samples.

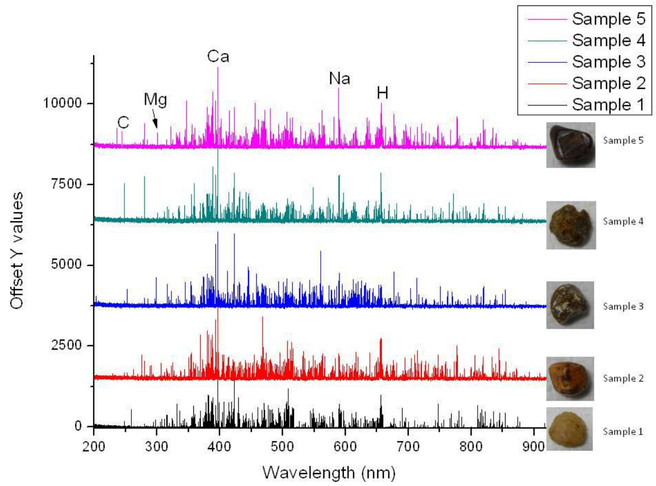

Figure 3 LIBS spectra generated from gall bladder stone samples with major elements identified and marked

Also, recorded LIBS spectrum of gallstones for higher delays (2000ns) and it was found that the number of emission lines significantly reduce at longer delays. Another point to be noted here is that the strongest lines found at shorter delays, viz. 393.36 nm and 396.84nm, which are the ionic emission lines of Ca, are missing at higher delays as expected. Similarly C and H emission lines, which were prominent at shorter delays, are completely missing for longer delays

With an ultimate aim to understand the mechanism behind the formation of gall stones, we have recorded LIBS spectra of bile juice and gall stone samples collected from the same subject (Figure 4). Initial analysis gives rise to interesting results as you can see from Figure 4 that all the major elements identified from bile juice and gall stones using LIBS are same. However, further studies need to be done using more bile and gall stone samples from the same subject to confirm these results and to understand the chemical composition and difference in appearance of each of these stones from case to case. Also, efforts are on to analyze the recorded LIBS spectrum carefully by choosing those elemental emission lines which are present in bile spectrum but missing in the gall stone spectrum.

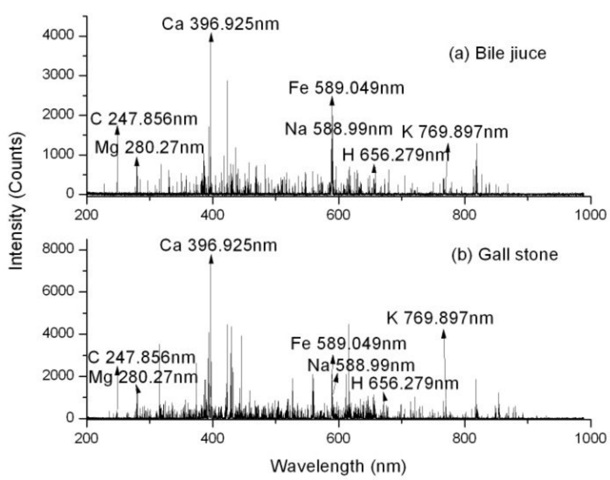

Figure 4 LIBS spectra of (a) bile juice and (b) gall stone collected from same subject

As mentioned previously, elemental analysis of calcified tissue after hard tissue Osteotome has been tried using LIBS. The sample was made into 3 sub-regions where the elemental composition of the teeth is expected to vary. Generally in teeth, the calcium and phosphorus concentrations decrease from enamel to root but the magnesium concentration increases. To verify this, each region of the sample was exposed to laser (irradiance ~10^{11}W/cm^2) and LIBS spectra were recorded for 500ns gate delay, and 3 μs gate width. A typical spectrum is shown in Figure 5.

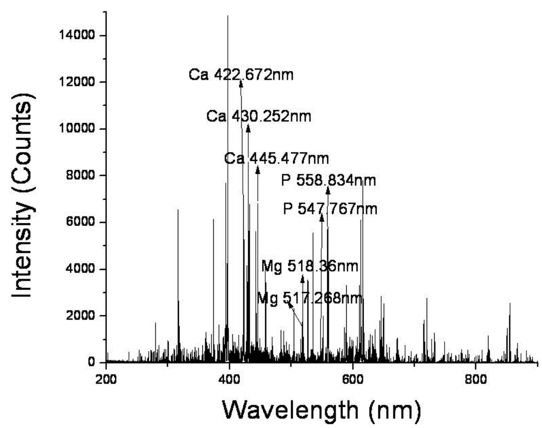

Figure 5 LIBS spectrum of a calcified tissue or teeth

Figure6 shows the LIBS intensity changes in calcium and magnesium in a single spectrum as we move from one region to other (Here region 1: enamel, region 2: intermediate area between enamel-root and region 3: root). The results clearly demonstrate how the elemental concentration varies from one region to other in calcified tissues.

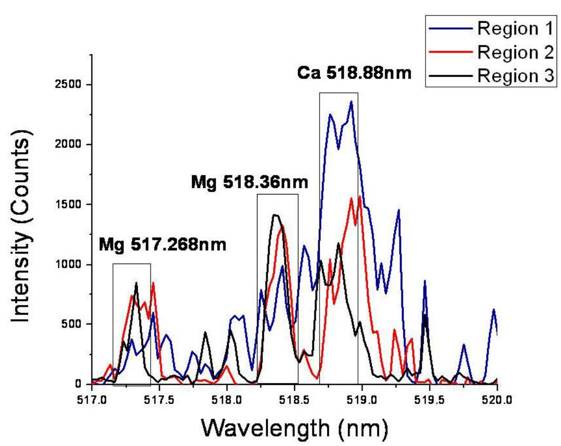

Figure 6 LIBS spectral variations of calcium and magnesium at different regions in calcified tissue sample

4. CONCLUSION

LIBS studies have been done using gallstone samples from different subjects. Characteristic emission lines of Ca, Na, C, H and Fe were observed from all the five samples. Mg lines were present in all samples except Sample 2. Similarly K lines were found to be weaker in most of the samples and were missing in Sample 1 and Sample 3. Ca, Na and Fe are present due to important steps in stone nucleation and formation. C and H lines are significant because cholesterol is the main composition of these stones. Elemental composition of bile juice and gall stone collected from the same subject using LIBS was found to be similar. The presence of all these elements in bile juice confirms the fact that they are derived from bile. The results show interesting prospects for LIBS to understand cholelithiasis better. Also from the studies conducted on calcified tissues show that the presence and relative concentrations of major elements (calcium, phosphorus and magnesium) can be easily determined using LIBS technique. The relevance of this study comes in anthropology where tooth and bone are main samples from which reliable data can be easily retrieved.

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