

Gallbladder Stones Analysis Using Pulsed UV Laser Induced Breakdown Spectroscopy

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Abstract—A laser sensor based on Laser Induced Breakdown Spectroscopy (LIBS) was developed for analysis of human gallbladder stones specifically for determination of heavy metals. In addition to qualitative analysis, the concentrations of the heavy metals were determined in different gallbladder stones samples using LIBS technique for the spectral lines of Cr I at 425.4 nm, Pb I at 368.3 nm, Cd I at 291.5, Ni at I 361.9 nm and Hg I at 366.3 respectively. The limit of detection of our LIBS system was determined as well for these five elements. The concentrations of above mentioned elements detected with Our LIBS system were in good agreement with ICP-results. In addition, the plasma parameters like temperature and electron density were also estimated using the so called Boltzmann plot and Boltzmann distribution of upper energy levels and Stark broadening, respectively. The plasma temperature and electron density data for gallbladder stones was essential to testify that the laser induced plasma fulfils Local Thermal Equilibrium condition which is highly critical for the quantitative LIBS analysis.

Index Terms—laser technology and its applications, laser induced breakdown spectroscopy, gallbladder stones analysis, toxic metals

I. INTRODUCTION

Laser Induced Breakdown Spectroscopy (LIBS) has many advantages [1]-[7] over the other existing optical techniques as it is a fast, portable, in-situ and remote analyzing method. It is capable for analysis of all elements concentration present in samples like solid, liquid or gaseous phase with little or no sample preparation. LIBS is based on the detection of spectral emission collected from plasma generated at the surface of the test sample by the incident laser beam. When a high power laser pulse impacts on the surface of a material, the irradiation at the focal spot leads to the removal (ablation) of some material. The ablated material compresses the surrounding atmosphere and leads to the formation of shock waves. During this process, a wide variety of phenomena including rapid local heating, melting and intense evaporation take place. Then, the evaporated material is ionized and create plasma because of high temperature. This plasma contains excited electrons, ions of the ablated material. As the plasma

decays or cools (~1.0 μ sec after the laser pulse), excited atoms in the plasma emit light of characteristic wavelengths distinct to the element. All elements emit light of characteristic wavelength called the finger print wavelength. These finger print wavelengths can be used to identify the elements present in test sample [7]-[8]. In addition to the determination of presence of elements in a test sample, LIBS also allows the determination of concentration of every element in the sample by measuring the intensity of the peak corresponding to the element and then comparing it with the concentration by drawing the so called calibration curve [3], [4].

The gallbladder stone is very common problem nowadays, and there are two types of these: a) cholesterol stones and b) pigment stones which mainly consist of cholesterol and calcium salts and bilirubin respectively [8]. These stones in some cases contain toxic metals such as chromium, lead, nickel, cadmium and arsenic which could be due to the exposure of people to environmental pollution, industrial waste and smoking [8]-[12]. Previous research showed that the contamination of drinking water with toxic metals such as chromium, lead and cadmium increase their level in the biliary and eventually to the gallbladder stone [8]. The rise of heavy metals toxicity is associated with the carcinoma of gallbladder or other organs [9]. It is reported that the constituents of the gallbladder stones are Ca, Na, Cl, K, Mg, Mn, Fe, P, Cu, Pb, Ti, Zn, Al, Ni and Cr using either x-ray fluorescence or Neutron Activation Analysis.

In this work, carcinogenic metals like Cr, Pb, and Cd were detected in the two different test samples of gallbladder stones using LIBS technique by recording their finger-print wavelengths. In addition, we determined their concentrations in the two test samples using calibration curves drawn from standard test samples with known concentrations of these elements. To validate our LIBS results, the samples were also analyzed using the standard technique Inductively Coupled Plasma-Mass Spectrometer ICP-MS. Plasma temperature and electron density were also estimated using Boltzmann plot [13]-[17]. This work is a continuity of our work on the environmental applications of LIBS [1]-[7].

II. EXPERIMENTAL METHODS

A schematic diagram of the LIBS setup developed at our lab for analysis of gallbladder stone is depicted in Fig. 1. Here a pulsed Nd:YAG laser having a wavelength of

266 nm, and 1064nm, pulse width = 8ns, frequency of 20 Hz and energy between 12.5 and 50 mJ was employed for pulsed laser ablation and generation of LIBS signal. A quartz convex lens of 50 mm focal length focused the laser energy on the test sample surface, while the sample was kept on a movable table in order to prevent craters formation and shifting in the focal length in the sample. The LIBS induced spectra in the 270-800 nm region were recorded where most of the spectral lines of gallbladder stone were present using a high resolution (.1 FWHM) spectrometer (Andor SR 500 i-A) with a grating of 600 line/cm groove density and an optical fiber at an angle of 45°. The ICCD camera (Andor iStar) was synchronized with the incident laser pulses in order to control the delay time between the laser pulse and the ICCD shutter (gate) opening time. In this experiment, the delay time was in the region 25-500 ns, and the gate pulse width was 40 μ s. The accumulation number was optimized to be 20. Many other parameters in the LIBS system can affect the induced plasma properties such as the target matrix, geometrical setup and other ambient conditions. The variation in these parameters strongly affect the emission spectral lines intensities hence the accuracy of LIBS system. In this experiment, two gallbladder stones were taken surgically from different patients were polished, cleaned by ethanol and kept at the focal point of the convex lens on rotary tab.

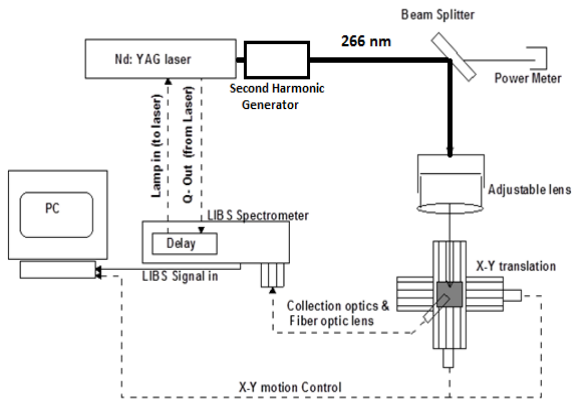


Figure 1. Schematic diagram of Laser induced breakdown spectrometer for analysis of gall bladder stones using 266nm (4th harmonic of Nd:YAG laser) and 1064 nm (fundamental wavelength of Nd:YAG).

III. RESULTS AND DISCUSSION

A. LIBS Spectrum Analysis

The LIBS spectra of the two gall bladder stone samples were recorded in the 270 -850 nm region under the experimental conditions of 400 ns delay time, 40 mJ laser energy and 1064 nm and 266 nm laser wavelengths. Most of the spectral lines of the constituting elements in the two gallbladder stones samples were identified in this region as depicted in Figs. 2–3. The identification of the atomic transition lines was carried out using the National Institute of Standards and Technology (NIST) data base [16]. The existence of the each constituting element was confirmed by identifying at least three finger-print

spectral lines or more of that element present in the LIBS spectra. The constituting elements are divided in two categories, essential nutrient elements (Ca, Na, Mg, K, Fe, Zn, Cl and Cu) and toxic elements (Cd, Ni, Cr, Pb and Hg). Table I summarizes the spectral assignments of the induced spectra which were used in calculating plasma temperature and electron density as well as the elements concentrations.

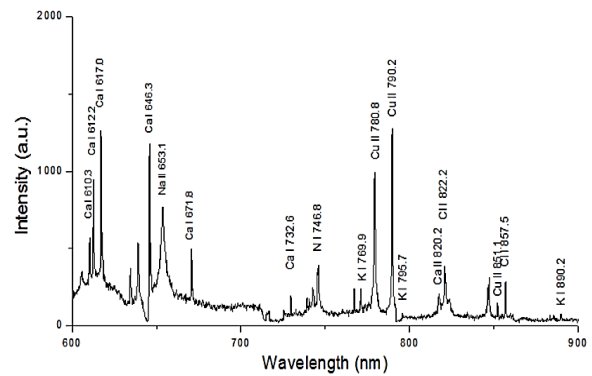
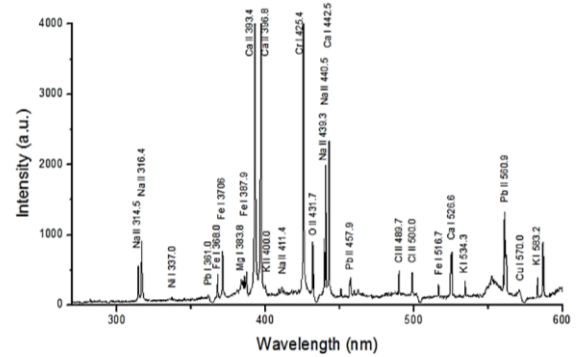
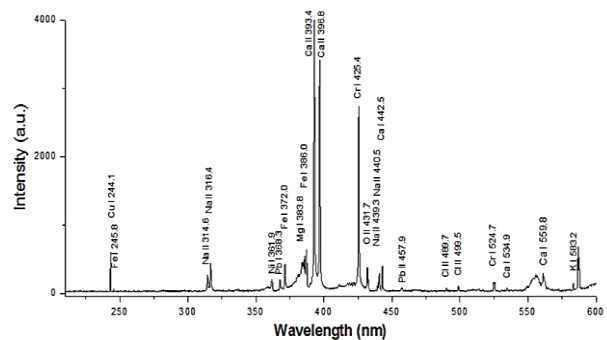
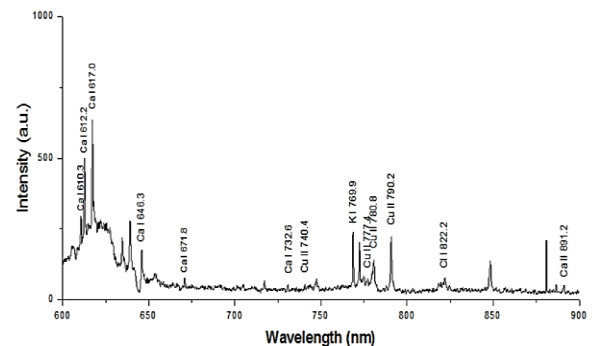


Figure 2. Typical LIBS spectrum of a gallbladder stone test sample using 1064 nm laser as an excitation source.



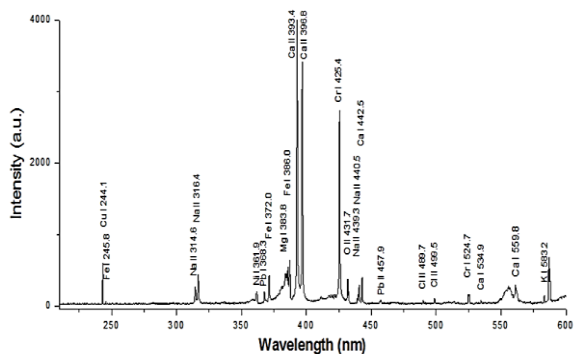


Figure 3. LIBS spectrum of a gallbladder stone test sample using 266 nm laser as an excitation source.

TABLE I. SELECTED WAVELENGTH FOR CHARACTERISTICS ATOMIC TRANSITION LINES OF NEUTRAL VARIOUS ELEMENT OTHER PARAMETERS USED FOR BOLTZMANN'S PLOT AND IDENTIFICATION OF ELEMENTS

Element	Wavelength (nm)	Transition Assignment	Energy of Upper Level (cm ⁻¹)	Transitional Probability (10 ⁸ s ⁻¹)
Ca I	610.3	³ S ₁ - ³ P ₀	31539.5	0.096
	612.2	³ S ₁ - ³ P ₁	31539.5	0.29
	646.3	³ F ₃ - ³ D ₂	35818.7	0.47
	442.5	³ D ₁ - ³ P ₀	37748.2	0.50
Cr I	425.4	⁷ P ₄ - ⁷ S ₃	23498.8	0.32
Cu I	244.2	⁴ P _{1/2} - ² S _{1/2}	40943.7	0.020
Fe I	372	⁵ F ₃ - ⁵ D ₄	26874.6	0.16
	386	⁵ D ₄ - ⁵ D ₄	25900.0	0.097
K I	769.9	² P _{1/2} - ² S _{1/2}	12985.2	0.37
Mg I	383.8	³ D ₃ - ³ P ₂	47957.1	1.68
Ni I	361.9	¹ F ₃ - ¹ D ₂	31031.0	0.66
Pb I	368.3	² S _{1/2} - ² P _{3/2}	34959.9	1.5
Pb II	560.9	² P _{3/2} - ² S _{1/2}	77272.6	

Optically thin plasma and Local Thermal Equilibrium (LTE) conditions for our LIBS were satisfied due to high electron density and Maxwellian distribution of the velocity as written below. Optically thin plasma is considered only under the absence of reabsorption of the emission lines in the plasma [13], [17]. The electron temperature and electron density estimated by recording the strongest lines of Na and Ca in gall bladder stone samples was 17000 K and 9x10¹⁸ cm⁻³ respectively.

Standard samples in known concentration were prepared to draw calibration curves of spectral line intensity versus concentration in ppm for the lines Cd I 291.5, Pb I 368.3, Cr I 425.4, Ca II 393.4, Ni I 361.9 and Hg I 366.3. Next, the elemental concentration of these elements was determined from the lines intensity of the two gallbladder stones samples by locating the line intensity on the calibration curve. The concentrations of these elements were also measured using Inductively Coupled Plasma-Mass Spectrometer ICP-MS technique to validate our LIBS results. The limit of detection which

is the minimum detectable concentration of the element was calculated from the equation $LOD=2\sigma/S$, where σ is the standard deviation of the background noise and S is slope of the spectral line that is used to detect the element of interest [13]. The limit of detection of our LIBS analyzer was in ppm range for most of the elements detected in gall bladder stones.

B. Influence of Laser Energy on LIBS Signal Intensity

In order to determine the optimum laser energy for the LIBS signal intensity of various elements present in the test samples, with all other parameters such as the LTE, time delay and laser beam diameter were kept constant in accordance with the previous findings. The effect of laser energy on the LIBS signal intensity was investigated in our experiment in the range of 10-50 mJ/pulse.. All of the investigated spectral lines showed linear dependence of the signal intensity on the laser energy. Fig. 4 shows typical plot of linear dependence of incident laser energy of Ca I 649.4 spectral line for gall balder stone samples.

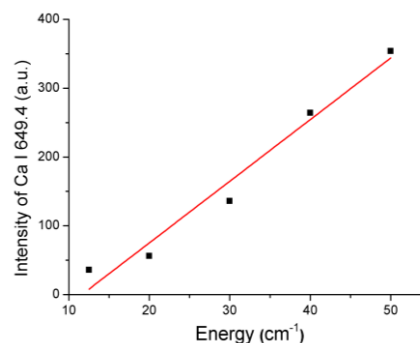


Figure 4. Intensity of Ca I 649.4 spectral line versus the incident laser energy at 266 nm wavelength.

IV. CONCLUSION

A laser induced breakdown spectrometer for detection of carcinogenic metals in gallbladder stones was developed using a pulsed laser beam with wavelengths of 266 nm and 1064 nm as excitation sources and our spectrometer was equipped with a gated ICCD camera. The spectral lines intensity decreases linearly as the increase in delay time or the decrease in laser energy. The atomic transition lines were identified using spectroscopic data published by NIST, and the emission spectral lines of the toxic elements Cr, Pb, Ni, Cd and Hg were detected. Their concentrations were calculated from the calibration curves and matched with ICP-MS analysis. The plasma temperature and electron density were calculated to satisfy optically thin plasma and local thermal equilibrium conditions. The rise of heavy metals in the body presents a potential risk to human health and possible precursors in the pathophysiology of chronic diseases and cancers.

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