Quantitative Analysis of heavy metals in gallstone Using LIBS

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Abstract. Concentrations of heavy metals in gallstones samples gathered from different hospital were estimated via laser induced breakdown spectroscopy (LIBS). Quantitative and qualitative analysis of the gallstone samples were achieved by using the locally developed LIBS set up equipped with Nd: YAG laser (Q-switched) at 1064 nm, pulse duration 9 ns and pulse energy 300 mJ. Quantitative analysis was performed for Cr, Cd, Zn, Pb, Cu and As based on calibration curve constructed from the correlation between the intensity of the LIBS emission lines and the concentration of each element measured by AAS. It was noticed that the percentage of copper, zinc, and manganese was higher in pigment samples higher than in mixed samples, and that it was not present in the cholesterol samples. However, chromium and cadmium elements were present in a high percentage in cholesterol samples and higher than other types of gallstones. The most important conclusion was that heavy elements such as Pb and As were present in the samples of smoking patients. The relative errors for all samples were in the range of (1.5-7.8) %.

1. Introduction

Gallstone formation in human tissues such as the gallbladder, bile duct, and liver is a common digestive disease, rising a major health problem worldwide [1- 3]. The major three constituents of gallstones are cholesterol, bilirubin, and calcium. Cholesterol is the most important and may amount to more than 70%, whereas calcium content will decide the hardness of stone [4]. There are several techniques for examining the concentrations of elements for gallstones and kidney stones, especially heavy substances. Omer et al. (2011) utilized ICP to analyze thirty gallstones, they classified the examined stone by FTIR analysis and concluded that the amount of trace elements in pigment stones was higher than that in cholesterol or mixed stones [5]. Heavy metals were quantified from patients of different age groups and observed trace and heavy metal in gallstones using FTIR and WD-XRF also compared the results with AAS for five samples. They observed that the center of stones was dark brown, black, and dark yellowish. [6] Concerning the necessity for a fast and accurate analysis technique. Laser induced breakdown spectroscopy (LIBS) has been proven to be a well-established non-destructive technique for any type of materials [7-9]. Brij Bir S. Jaswal et al. Utilized LIBS and WD-XRF to study spectroscopic analysis of heterogeneous gallstones they deduced that the presence of trace element in different types of gallstones and observed that pigment gallstones were rich in minerals and heavy metals [11]. Oztoprak et al. (2012) utilized LIBS to analyze five different types of kidney stones, they easily detected the lighter elements such as N,C and H by LIBS but not by XRF and used these results to classify the examined stones [12]. Recently, Gondal et al. (2016) developed LIBS set up for analysis of human gallstones for determination of heavy metals, the spectral lines of Cr, Pb, Cd, Ni and Hg were
detected using UV Pulsed laser, their contents were measured from the calibration curves and compared to ICP analysis[13]. The present work demonstrates the use of LIBS to detect and identify concentrations of heavy metals including Cr, Cd, Zn, Pb, Cu and As using the calibration curves that constructed based on these concentrations.

2. Experimental setup

Fig. 1 displays a Lab-built LIBS set up used to recorded plasma emissions from gallstones sample surface. LIBS analysis systems contain (1) plasma emission collection system (2) Laser source; and (3) spectral analysis device. In Table 1 lab-built system used in this work are presented in a details.

| Table 1. The - set up of experimental applied for gallstone samples |
|---------------------------------------------------------------|
| **Collection system**                                       | multi-mode optical fiber cable diameter (400 µm) |
|                                                               | 10 cm distance from the focused spot. |
|                                                               | Located at 45º angle with the normal |
| **Laser source**                                             | Nd: YAG laser |
|                                                               | operating at wavelength (λ=1064nm) |
|                                                               | Lens focal length (10cm) |
|                                                               | Pulse duration (τ=9ns) |
|                                                               | Laser Energy 300 mJ |
| **Spectral analysis device**                                 | Optical spectrometer |
|                                                               | equipped with CCD camera Sample matrix |
|                                                               | Spectral range (200-1100) nm |

3. Sample collection

Twelve gallstones referred to as GS1-GS12 collected from different hospitals and different ages, these samples are divided into two groups. The first group listed in Table. ٢ includes the samples GS1- GS6 and were used to construct the calibration curves. The second group listed in Table. ٣ includes the samples GS7-
GS12 and used as unknown targets. All samples are cleaned with deionized water, and then dried in furnace at 50°C for two hour. The samplers are stored in numbered sealed pots.

### Table 2. The gallstone samples used for calibration curve

| Sample | Gallstones Sample | Age | Gender | Type –FTIR | Sample code |
|--------|-------------------|-----|--------|------------|-------------|
| GS1    | ![Image](image1)  | 43  | Female | Mixed      | Mi1-f       |
| GS2    | ![Image](image2)  | 35  | Male   | Mixed      | Mi2-m       |
| GS3    | ![Image](image3)  | 45  | Female | Cholesterol| C3-f        |
| GS4    | ![Image](image4)  | 52  | Male   | Cholesterol| C4-m        |
| GS5    | ![Image](image5)  | 63  | Female | Pigment    | P5-f        |
| GS6    | ![Image](image6)  | 65  | Male   | Pigment    | P6-m        |

### Table 3. The gallstone samples used for quantitative analysis.

| Sample | Gallstones | Age | Gender | Type | Sample code |
|--------|------------|-----|--------|------|-------------|
| GS7    | ![Image](image7) | 61  | Female | Mixed | Mi7-f       |
4. Results and Discussion

4.1 Qualitative analysis of gallstone

After optimizing the LIBS parameters for maximum signal intensity, spectra of twelve gallstone samples are recorded at spectral region of 250-650 nm for qualitative and quantitative analysis. The LIBS spectra for gallstone sample GS\textsuperscript{6} are plotted as shown in Fig 2. Amongst many detected lines appeared in examined samples, the represented emission lines of Cr at (534.5 nm), Cd at (441.56 nm), Pb at (357.27 nm), Mn I (380.67 nm), Zn I (307.27 nm) and As I (249.29 nm) with maximum peak intensity have been identified. These lines were relatively free from other spectral interferences and extracted with the help (NIST) database [14]. The recorded peak intensities for the selected elements emission lines were further analyzed to established calibration curves for the gallstone samples.
4.2. Quantitative analysis of gallstone
Initially, the tested gallstone samples have been divided in two groups, the first consisted of six samples was used for constructing the calibration curves (C.C) based on known elements concentration determined by AAS analysis. Calibration curves (C.C) for Cd, Pb, Cr, Cu, Mn As and Zn are established based on gallstone samples. The intensity of LIBS signals belong to Cr at (534.5 nm) and Cd at (441.56 nm) Cu I at (450.59 nm) Pb at (357.27 nm) Mn I (380.67 nm), Zn I (307.27 nm) and As I (249.29 nm) were plotted as a function of their actual concentrations. The linearity of correlation between the peak intensity of LIBS signal of elements and their corresponding concentrations for six samples are shown in Fig 3& 4. They show linear relationship for both Cr, Cd, Pb, Cu, Zn, Mn and As with regression coefficients $R^2$ of 0.93and 0.97 respectively. Since the characteristic line strength of the element ( heavy metal) is proportional to the concentration in the gallstone sample. According to these calibration curves, the remaining unknown concentration in the last six samples (second group) have been measured.
Figure 3. Calibration curves the intensity of emission lines of Cr I (534.5 nm), Cd II (441.56 nm), Pb I (357.27 nm) and Cu I (450.59 nm) versus AAS concentration in ppm.

Figure 4. The intensity of emission lines (Calibration curves) of Mn I (380.67 nm), Zn I (307.27 nm) and As I (249.29 nm) versus concentration in ppm by AAS Tech.
The measured concentrations of elements HM such as (Zn, Cd, Cr, Pb, Cu, Mn and As) are listed in Table 4. It can be noticed that in all kinds of stones the elemental content of both Cr and Cd have been detected; same results have been reported early [15]. Furthermore, Cr and Cd levels in cholesterol were higher than others stones. On the other hands, Concentrations of elements in pigment including (Zn, Mn and Cu) are comparatively higher than those in other stones indicating that the elemental content of various elements differ greatly reliant on the type of gallstone. And finally the elements of As and Pb are found especially in smoker patients.

Table 4. Elemental analysis, AAS and LIBS results for Cd, Zn, Cr, Pb, Cu and As in six gallstone.

| Element | Fingerprint wavelength (nm) | AAS (ppm) | LIBS (ppm) | Error % |
|---------|-----------------------------|-----------|------------|---------|
| Cd      | 441.56                      | 0.038     | 0.035      | 7.8     |
| Cr      | 534.50                      | 0.650     | 0.690      | 5.7     |
| Pb      | 357.27                      | ----------| ----------  | --------|
| Cu      | 450.59                      | 0.015     | 0.016      | 6.6     |
| Zn      | 380.67                      | 0.030     | 0.028      | 6.6     |
| Mn      | 307.27                      | 0.019     | 0.020      | 5.2     |
| As      | 249.29                      | ----------| ----------  | --------|
| Cd      | 441.56                      | 0.031     | 0.029      | 6.4     |
| Cr      | 534.50                      | 0.590     | 0.620      | 5.1     |
| Pb      | 357.27                      | ----------| ----------  | --------|
| GS8(Mi8-f) | Cu      | 450.59                      | 0.021     | 0.020      | 4.7     |
| Zn      | 380.67                      | 0.050     | 0.048      | 4.0     |
| Mn      | 307.27                      | 0.020     | 0.019      | 5.0     |
| As      | 249.29                      | ----------| ----------  | --------|
| Cd      | 441.56                      | 0.055     | 0.053      | 3.6     |
| Cr      | 534.50                      | 0.720     | 0.680      | 5.5     |
| Pb      | 357.27                      | ----------| ----------  | --------|
| GS9(C9-f) | Cu      | 450.59                      | ----------| ----------  |        |
| Zn      | 380.67                      | ----------| ----------  |        |
| Mn      | 307.27                      | ----------| ----------  |        |
| As      | 249.29                      | ----------| ----------  |        |
| Cd      | 441.56                      | 0.049     | 0.051      | 4.1     |
| Cr      | 534.50                      | 0.710     | 0.680      | 4.2     |
| Pb      | 357.27                      | ----------| ----------  | --------|
| GS10(C10-f) | Cu      | 450.59                      | ----------| ----------  |        |
| Zn      | 380.67                      | ----------| ----------  |        |
| Mn      | 307.27                      | ----------| ----------  |        |
| As      | 249.29                      | ----------| ----------  |        |
| Cd      | 441.56                      | 0.034     | 0.036      | 5.8     |
| Cr      | 534.50                      | 0.589     | 0.610      | 3.5     |
| Pb      | 357.27                      | ----------| ----------  | --------|
| GS11(P11-f) | Cu      | 450.59                      | 0.044     | 0.047      | 6.8     |
| Zn      | 380.67                      | 0.540     | 0.510      | 5.5     |
| Mn      | 307.27                      | 0.065     | 0.061      | 1.5     |
| As      | 249.29                      | ----------| ----------  |        |
The concentrations of heavy metal measured by LIBS are found to be close to that measured by AAS. Furthermore, the relative errors for all samples were in the range of (1.5-7.8) %. This indicates the potential of LIBS as an extremely valuable technique for accurate quantitative analysis. It is worth mentioning that the concentration of elements detected in this work exceeds the permissible limits [16].

To get an idea of the distribution of heavy elements in gallstones, a PCA diagram shows that the following shows three groups. The first group representing the type of cholesterol contained higher levels of chromium and cadmium compared to the other elements. The second group was characterized by high levels of arsenic and lead. These samples are of male smokers, and this is likely due to inhaling high amounts of tobacco [6]. As for the third group, it was characterized by high concentrations of manganese, copper, and zinc elements in the samples of stones of the chromosomal and mixed type, with varying proportions.

In short, the presence of heavy elements in gallstones varies according to the nature of the food, geographical location, and issues related to pollution of the surrounding environment in which the patient lives.

| Element | LIBS Concentration | AAS Concentration | Relative Error |
|---------|--------------------|-------------------|----------------|
| Cd      | 441.56             | 0.030             | 0.029          | 3.3 |
| Cr      | 534.50             | 0.660             | 0.690          | 4.5 |
| Pb      | 357.27             | 0.049             | 0.052          | 6.1 |
| Cu      | 450.59             | 0.041             | 0.039          | 4.8 |
| Zn      | 380.67             | 0.490             | 0.460          | 6.1 |
| Mn      | 307.27             | 0.060             | 0.058          | 3.3 |
| As      | 249.29             | 0.069             | 0.067          | 2.8 |

Figure 5: PCA results for heavy metal concentrations in twelve samples of gallstones from people of different ages and classes, some of whom were smokers.
The results obtained confirmed the ability of the LIBS technique to determine the concentrations of heavy elements with high accuracy, which helps in early detection of the causes of gallbladder diseases such as the diet system and the polluted environment and providing the necessary advice to avoid these diseases.

5. Conclusion

It was possible to prove the capability of LIBS technique for direct and multi-elements quantitative analysis of gallstones samples. With locally developed LIBS setup, the Cr, Cd, Cu, Zn, Mn and As elements were examined simultaneously in six gallstone samples. Calibration curves for each element were constructed based on gallstone samples with known element concentrations determined previously by AAS measurement. It was noticed that the percentage of copper, zinc, and manganese was higher in pigment samples higher than in mixed samples, and that it was not present in the cholesterol samples. However, chromium and cadmium elements were present in a high percentage in cholesterol samples and higher than other types of gallstones. The most important conclusion was that heavy elements such as Pb and As were present in the samples of smoking patients.

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