بسم الله الرحمن الرحيم

{ الا ان يشاء الله 0 نرفع درجات من نشاء 0 وفوق كل ذي علم عليم }

صدق الله العلي العظيم

سوره يوسف/الايه ٧٦

الاهداء

الى الرسول الأعظم مبلغ رسالات ربه ومستودع الوحي والتنزيل محمد صلى الله عليه وعلى اله الطيبين الطاهرين .

الى من يؤمنون بك حين يخذلك الجميع الى أصحاب الكلمات التي سارت بي نحو النجاح الى من ساندوا خطاي المتعثرة الذين اغدقوا عليَّ الدعوات والحب امي وابي .

الى الذين جعلهم الله عضدا لي اخوتي واخواتي .

الى أصدقاء الطرق جميعا والى رفاق الأيام بكل تفاصيلها الى الذين تقاسموا معي السنوات وتناثرت أيامنا على دروب العلم أصدقائي .

الى منفذي وصايا الله ورسوله في طلب العلم وتعليمه والذين افنوا سنواتهم كالسراج ليضيئ طريقنا والذين عبَدوا لنا طرق الحياة لتتسدد خطواتنا اساتذتي الاجلاء . . .

شكـــــــر وتـقــديـــر

لا يسعنا بعد الانتهاء من اعداد بحث التخرج الا ان

نتقدم بجزيل الشكر وعظيم الحب الى الاستاذه العزيزه

الدكتورة رفل احمد

التي تفضلت بالاشراف على اعداد البحث

حيث قدمت لنا النصح والإرشاد طيله أيام اعداده فلها منا

الموده والاحترام . . . .

LIST OF CONTENT

I - 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.

Also ,Type 2 diabetes drug tablets containing voglibose having dose strengths of 0.2 and 0.3 mg of various brands have been examined, using laser-induced breakdown spectroscopy (LIBS) technique. The statistical methods such as the principal component analysis (PCA) and the partial least square regression analysis (PLSR) have been employed on LIBS spectral data for classifying and developing the calibration models of drug samples. The experiment has been performed in air and argon atmosphere, respectively, and the obtained results have been compared. The present model provides rapid spectroscopic method fordrug analysis withhigh statistical significance for online control and measurement process in a wide variety of pharmaceutical industrial applications.

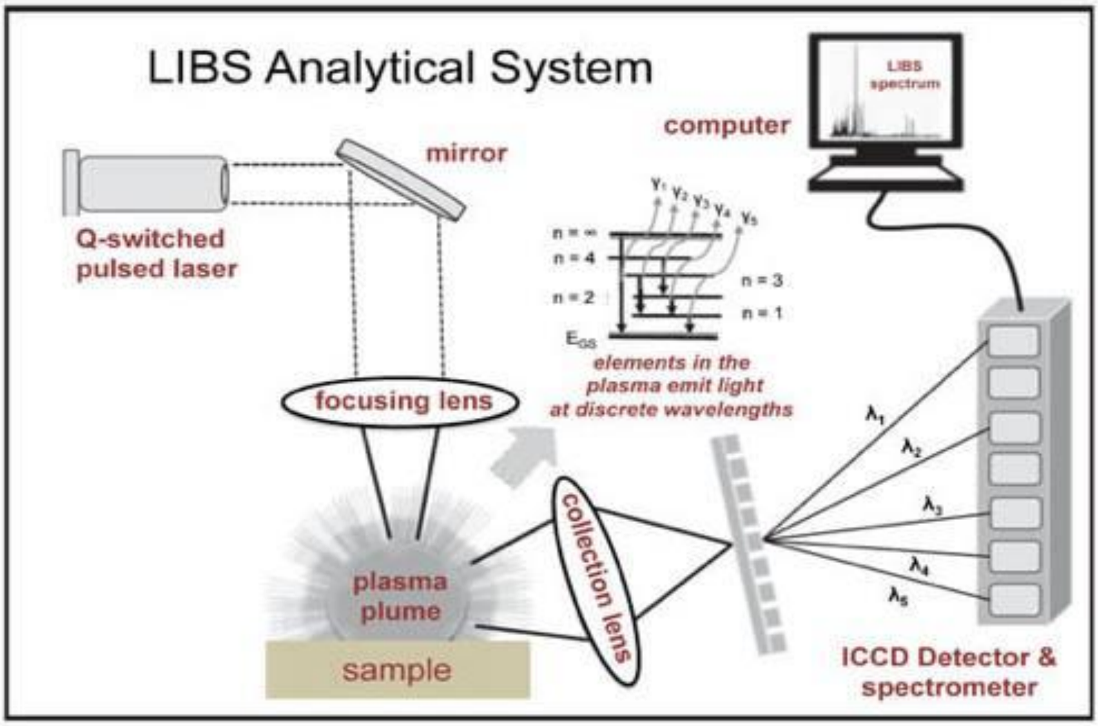
1- Introduction

Laser-Induced Breakdown Spectroscopy:

Atomic emission spectroscopy is a technique for chemical analysis to determine either the presence or the mass fraction of an element present in a sample based on measurement of the intensity of light emitted from a flame, spark, arc, or plasma [1,2].

Laser-induced breakdown spectroscopy (LIBS) is an established, straightforward, reliable, and versatile form of atomic emission spectroscopy that has broad capability for rapid, in situ elemental detection in any material (solid, liquid, or gas), and quantitative analysis by LIBS is possible using either conventional calibration methods or calibration-free approaches [3,4,5,6,7].

Thus, LIBS has the potential for widespread use for rapid chemical detection and analysis outside the research laboratory. At its core, an LIBS analytical system (Figure 1) consists of just a few components:



(i) a solid-state, short-pulsed, Q-switched laser operating at 1064 nm (or one of its frequency-multiplied harmonics) used to create a microplasma on the target,

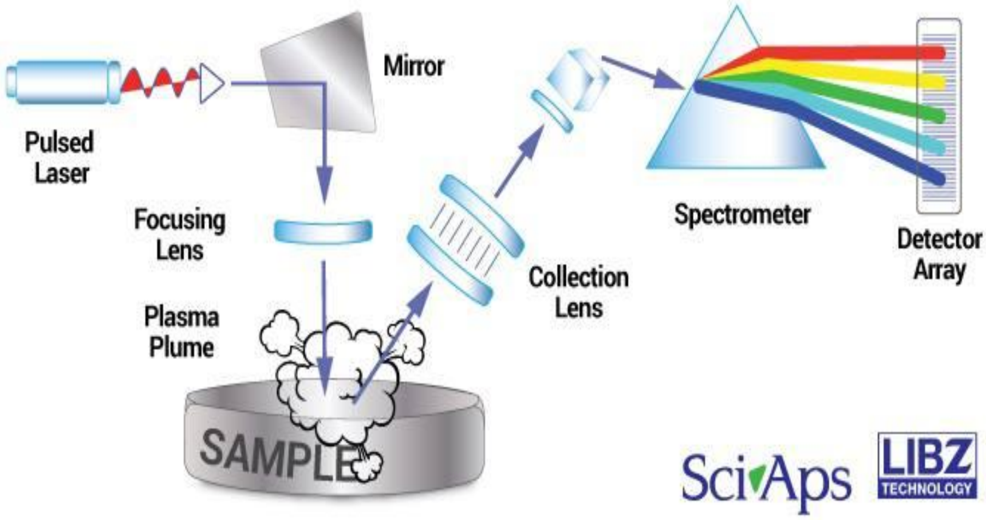
(ii) a set of optics to focus the laser light onto the target and to collect the light emitted as the plasma cools,

(iii) a coupled fiber-optic and spectrometer/detector system for acquisition of the plasma light emission and spectral resolution of the light spectrum, and

(iv) a computer for system control and data processing

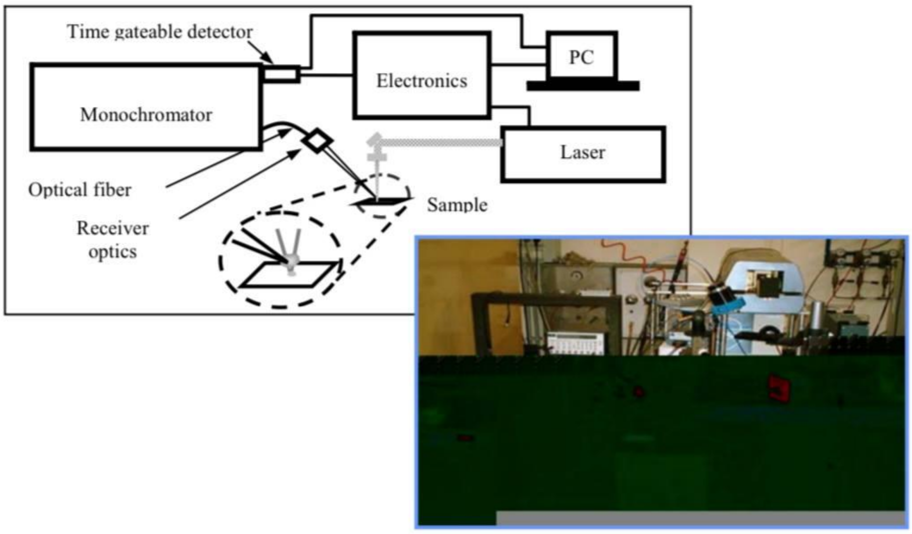
1.1-

- **LIBS is also advancing as a technology as new commercial instruments are becoming available. The core attributes of**
- (1) real-time analysis
- (2) no sample preparation
- (3) high sensitivity
- (4) high specificity for materials identification;
- (5) sensitivity to all chemical elements in each laser shot
- (6) uncommon versatility of point, standoff, as well as underwater-sensing provides a strong argument that LIBS will make a significant impact on science and society.

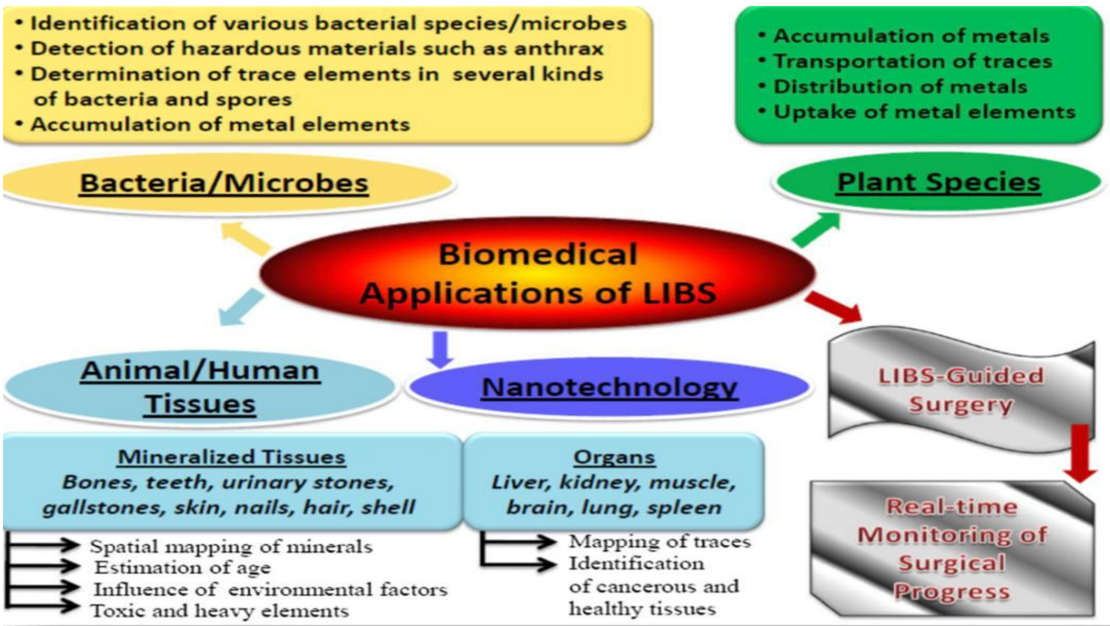


1.2 -Constituent:

The main constituents of a LIBS set-up are a pulsed laser source, a spectrometer (dispersive element plus multichannel detector) with suitably designed optical systems (laser focusing and collecting plasma emission) and electronics components (trigger and delay generators) (8)



MEDICAL APPLICATION



2-Medical application:

Laser-induced breakdown spectroscopy (LIBS) is a fast multielemental analytical technique which is based on the spectroscopic analysis of the radiation emitted by laser- induced plasma . This technique has opened up many applications in different fields of science and technology from thin film deposition to elemental analysis of target materials(9).

Nowadays, it is being used for both qualitative and quantita- tive analyses with the possibility of fast and on-site measure- ments of samples in the form of solids, liquids, gels, gases, plasmas, and biological materials like teeth, bones, leaf, and blood, etc.

Due to the new demonstrations of Laser-induced breakdown spectroscopy (LIBS) applicability in a surprisingly wide variety of applications, the use of LIBS as a medical diagnostic tool is steadily gaining momentum(10). Especially in different cancer diseases, LIBS has the potential to become a fast and valuable analytical tool.

We addressed LIBS equipment and quantitative analytical procedures, and signal enhancement techniques for improving element detection. For detailed aspects of applications, we reviewed the recent progress of LIBS in different cancer diseases.

This overview of the different cancers by LIBS is meant to summarize the research performed to date and suggest some suitable advanced chemometrics techniques and effective LIBS devices, if successfully implemented, would be significantly beneficial to the medical field in the future(11)

Kidney stones were analyzed using laser-induced breakdown ،(spectroscopy (LIBS

The results are beneficial in understanding kidney stone formation processes, which can lead to preventive therapeutic strategies and .treatment methods for urological patients(12).

2.1- bones and teeth

LIBS has emerged as a promising technology for the analysis and characterization of a broad variety of objects due to its advantages such as no need for laborious sample preparation, fast analysis, and in situ analysis capability. LIBS is capable to detect lighter elements like C, H, N, and O, useful for material identification, depth profiling, and elemental sur- face mapping, so it can solve numerous industrial, environ- mental, biological, and scientific problems in real time(13) . It is important to note that in some cases, the sensitivity of LIBS to detect ultra-trace amounts of elements may be insufficient but it can provide spatial information of elements present in bones and teeth, which is not possible by conventional elemental analysis techniques (e.g. , inductively coupled plasma (ICP), AAS, etc.) without sample prepara- tions such as ashing or acidic dilution . The information of the spatial distribution of elements serves to establish compo- sitional interrelationships of the elemental constituents in the biological samples. Moreover, LIBS analysis can be under- taken directly from the solid sample, often in situ and at "remote" locations, and even in vivo analysis may be possible, when dealing with living organisms . Detection limits of LIBS for solid samples are of the order of a few ppm(14) . However, it can be improved by applying modified LIBS techniques such as Double-Pulse (DP) LIBS or combining laser ablation with laser-induced fluorescence spectrometry (LIFS) . An improvement of signal-to-noise (S/N) ratio over an order of magnitude can be realized by reheating the existing microplasma with a second laser pulse (DP LIBS) and is advantageous in terms of lower sample load(15) .

The authors showed that bone ash could be used as a suitable standard reference material for calcified tissue cali- bration using LIBS with a 266-.nm excitation wavelength

Recently, LIBS has emerged as a promising technique for the analysis of bioarcheological materials such as calcified tissues, namely, teeth and bones

A) LIBS analysis of bones

developed a remote LIBS system for investigating a calcified tissue sample at a distance of up to 6 m and tested in the laboratory. The sample used was a transverse section of a seventeenth- to eighteenth-century [luetic shinbone (tibia bone) cut and polished for analysis. The system used for the present study was a Galilean tele- scope and a flat-topped laser beam profile at 532 nm. Initial test were made on a slice of calcified bone placed in air 6 m away from the telescope. The system was sufficiently sensi- tive to detect major elements like Mg and P and minor ele- ments such as Na, Zn, and Sr at the semiquantitative levels of milligrams per kilograms. Trace elements were not easily recorded LIBS results were validated by the results obtained from LA-ICP-MS(16). The variation of elements along a trans- 1 verse radial cross section was also found. Variations in the bone porosity, and a rather large 1-mm diameter ablation crater, lead to variable results on the concentrations .of ele- ments sensitive to bone growth kasem et al.

studied the influence of biological deg- radation and environmental effects on the interpretation of archeological bone from three different ancient Egyptian dy- nasties using LIBS. Authors demonstrated that postmortem effects must be taken into consideration on studying dietary i habits(17). Also, a clear correlation between the degradation of the tissues in the archeological bones with the absence of CN and C_2 .molecular band in the LIBS spectra was found

Essential trace elements	Normal level (ppm of bone ash)	Nonessential/Toxic elements	Normal level (ppm of bone a
Copper (Cu)	25	Aluminum (Al)	1–10
Iodine (I)	<1	Arsenic (As)	0–5
Iron (Fe)	500	Barium (Ba)	10–20
Manganese (Mn)	10	Lead (Pb)	<70
Zinc (Zn)	200	Mercury (Hg)	<1
		Strontium (Sr)	100–200

Trace element in human bones

B) LIBS analysis of teeth

The human teeth consist of a unique composition of three distinct .segments namely enamel, dentin, and pulp(18)

$$Ca_{10}(PO_4)_6(OH)_2(s) + 8H^+(aq) \rightarrow 10Ca^{2+}(aq) + 6HPO_4^{2-}(aq) + 2H_2O$$

By means of this reaction, the enamel can be demineralized within a few days only. Calcium bound to the hydroxyapatite is ionized and washed out by saliva. This process turns the hard enamel into a very porous and .permeable structure

The authors successfully prepared enamel and dentine p cavities using this Er:YAG laser. Since then, this laser has to been used for caries removal and cavity preparation, soft tissue minor surgery, and scaling . Now, lasers have been routinely used to fix dental problems such as cavities. treat gum disease, and canker sores including teeth whitening. di Novel dental applications of lasers have been proposed and the results have been published by Niemz(19) .

2.2- LIBS ANALYSIS FOR DIFFERENT CANCER

2.2.1- Liver Cancer

In humans, the liver is the sixth most common primary cancer site, and it is frequently linked with cirrhosis and inflammation(20) . Kumar et al.(21)used LIBS for the first time to classify a dog's liver tissue to identify liver cancer. The findings demonstrate that the ratios of calcium (Ca), copper (Cu) and sodium (Na) to potassium (K) line concentrations are higher in a malignant sample than those in a normal one. They also used inductively coupled plasma emission spectroscopy (ICPES) to compare the results with LIBS and achieved good approximation results of both methods.

2.2.2- Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is a rare subtype of head and neck cancer with a highly unbalanced endemic spread (22).used LIBS combined with chemometric methods. In this research, a total of 160 serum samples was used, including 100 healthy controls and 60 NPC patients.The major elements (Na, Mg, K, and Zn) from LIBS spectra were selected for diagnosis and further classification purposes. They concluded that, the variable importance of three lines (K I 766.49, K I 769.90, and Na I 589.59 nm) with RF extraction are much greater than the average variable importance(23).

2.2.3- Cervical Cancer

used paraffin-embedded tissue samples of normal and cervical cancer patients. They coupled LIBS with chemometric methods PCA and SVM for classification purposes. It was hard to distinguish between normal and cervical cancer tissue using the raw spectra. PCA was performed, but the result of PCA was overlapped, hard to differentiate the cervical and normal sample. Then for normal tissues and cervical cancer tissues identification, SVM and PCA-SVM were used. The findings found that the PCA-SVM identification accuracy is much better than SVM and increased from 93.06 to 94.44%. They also concluded that LIBS technology has a lot of potential for cancer diagnosis in real-time(24). To achieve the best calcification and discrimination results the sample

2.2.4- Stomach Cancer

For gastric cancer identification,used spark discharge assisted laser-induced breakdown spectroscopy (SD-LIBS) in investigating the possibility of distinguishing neoplastic (cancerous) from non-neoplastic (normal) stomach tissues (25). In this study, they also found the difference between neoplastic and non-neoplastic gastric tissues emission spectra. The study concentrated on an in vitro comparison of elemental concentrations in distinct tissues. Depending on these observations, the intensities of Ca and Mg in cancerous spectra are higher than the normal sample spectra obtained from the same person. Likewise, they also concluded that the number of samples used in this study was insufficient to draw a firm conclusion, and more research is needed to generalize this concept

2.2.5-Ovarian cancer

For further LIBS analysis, they collected blood samples from mice at different ages of 8, 12, and 16 weeks. A total of 56 blood plasma samples .(were used in the experiment, 28 of each class (normal and cancerous

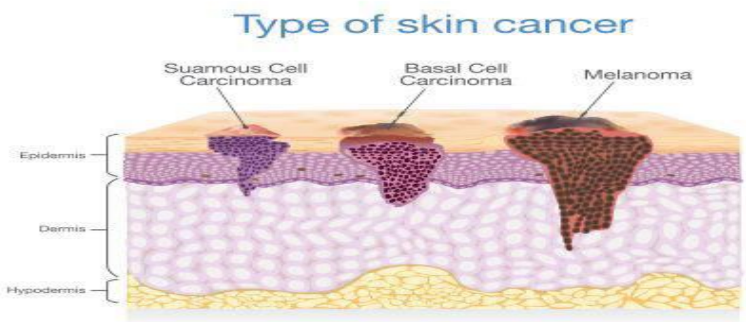
This finding backs up the theory that as the tumour load in the animals grows, plasma specimens will deviate more from control specimens.(26) When all six data are combined, however, the accuracy of classification for each age group drops. We believe this drop is because the LIBS chamber has to be re-opened after collecting LIBS spectra from one set of blood plasma samples to load the new blood plasma samples(27). They concluded that more research is needed to classify ovarian cancer and identify atomic and ionic lines in the ablated samples

2.2.6- Lung Cancer

used LIBS combined with machine learning techniques to differentiate lung boundaries from lung tissues. For this purpose, they used 90 tissue samples of lung tumor and lung boundary from 45 patients, of which 20 men and 25 were female, respectively(28). They used SVM and Boosting Tree classification models that employ PCA and RF to enhance accuracy, sensitivity and specificity. The RF-Boosting tree outperformed the competition in classification and recognition.m. The combination of LIBS with an RF-Boosting Tree model to detect lung tumour boundaries is a rapid and accurate method

2.2.7- Skin cancer

concentrated on the feasibility of LIBS to differentiate between lesions in the surrounding dermis from melanoma(29). Using homogenized pellet extracts from melanoma-implanted mice, and performed a qualitative and quantitative elemental analysis of melanomas and the underlying dermis.collect and compare the blood serum and tissue homogenate LIBS spectra harvested from a pre- clinical model of melanoma. The use of four distinct classification algorithms (LDA, FDA, SVM, and Gradient Boosting) is effective. The results of these four algorithms were compared, and Gradient Boosting was found to provide the best precision for classification.recently used LIBS combined with chemometric methods to examine and discriminate against human melanoma tissue samples instead of animal samples, and achieved good classification results(30)



2.3- LIBS APPLICATION OF GALLSTONES

Firstly, Singh et al. applied LIBS technique to investigate major and minor constituents of cholesterol gallstones obtained after surgery. The authors used a neodymium: yttrium–aluminum–garnet (Nd:YAG) laser (Continuum Surelite III-10), operating at 532 nm wavelength and capable of delivering a maximum energy of 425 mJ over a pulse duration of 3–4 ns (full-width at half-maximum) at a maximum pulse repetition rate of 10 Hz. A good signal-to-noise ratio and signal-to-background ratio were found at 20 mJ laser energy and 10 Hz repetition rate. The elements detected in the center and in the shell part were calcium (Ca), carbon (C), copper (Cu), hydrogen (H), magnesium (Mg), nitrogen (N), sodium (Na), oxygen (O), and potassium (K), but Cu was absent from the surface of the cholesterol gallstones(31).

The results revealed that Ca was a major constituent of cholesterol gallstones. They also showed that the concentrations of Ca, Cu, and Mg were high in the center in comparison with the shell. The authors also recorded the LIBS spectra of colored parts and discolored parts on the surface of the cholesterol gallstones. The concentrations of Na and K were higher in the nonpigmented (colored) part than in the pigmented part (discolored/pigment), which showed that the deficiency of Na and K playeda key role in the formation of discoloration at the different locations on the outer surfaces of the cholesterol gallstones. Finally, they concluded that LIBS is a quite suitable technique for the analysis of gallstones without any sample preparation. Pathak et al. applied LIBS technique for the identification of cholesterol and

pigment gallstones on the basis of atomic lines of different elements and molecular bands of C2 molecules present in their LIBS spectra. They also applied chemometric technique such as principal component analysis (PCA) to LIBS data for rapid identification/classification of different gallstone samples(32). Recently, Pathak and Singh (58) used LIBS technique to analyze gallstones obtained from patients from the northeast region of India (Assam).

Several elements, including Ca, Mn, Mg, Cu,Si, P, Fe, Na, and K, were detected in the gallstones. Lighter elements, including C, H, N, and O, were also detected in gallstones. This capability of LIBS demonstrates its superiority over other existing analytical techniques. They applied LIBS technique to investigate the evolution of C2 swan bands and CN violet bands in the LIBS spectra of the gallstones in air and an argon atmosphere. The authors differentiated the dark and light layers of the gallstones on the basis of the presence and intensities of the spectral lines for C, H, N, O, and Cu. They made a good attempt to correlate the presence of major and minor elements in the gallstones with the .common diet of the population of Assam

Figure 1 Gall stone samples used for LIBS studies

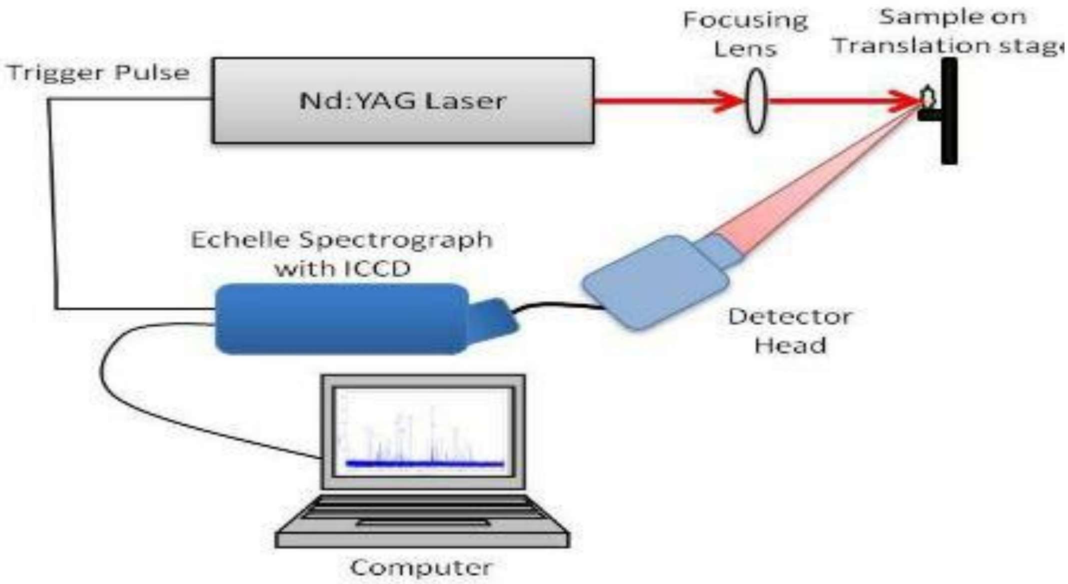


Figure 2 LIBS set-up used for the current study

2.4- Applications of Lasers in Urology

2.4.1- Utility of LIBS to Urinary Stones

Within a few years of the development of a workable laser, studies on their ability to fragment urinary stones had been reported. In vitro destruction of urinary stones was studied by Mulvaney and Beck using a 694-nm ruby laser(33) . In their study, the urinary stones seemed to be vaporized by the laser energy with a considerable heating effect. Vaporization was enhanced by staining the stones blue or black. Mulvaney and Beck noted that "apatitic and struvite stones broke easily; oxalates with difficulty" and "significant shock waves are produced and can be lessened by using air instead of water as a medium." However, the accompanying thermal effect precluded the ruby laser from clinical use because of the potential for tissue damage. Anderholm addressed the problem of tissue-damaging heat production and showed that the effects of the laser were related not only to .(thermal energy but also to the development of shock waves

The search began for a suitable laser for in vivo fragmentation of urinary stones. A continuous-wave (CW) carbon dioxide laser was used by Tanahashi et al. to drill holes through stones.

Watson et al. emphasized that all CW lasers had a thermal action only on stones. They noticed that the area of the stone that absorbed the .laser energy became hot, melting and then vaporiz- ing

In 1987, Watson et al. reported the results of an in vitro study of stone fragmen- tation using a tuneable flashlamp pumped dye laser with a variety of dyes as the lasing medium emitting at 445, 504, and 577 nm. They concluded that ureteroscopy itself was a more significant source of potential ureteric damage than the modality of fragmentation. In 1987, Dretler et al. were the first to report the clinical use of the pulsed dye laser for stone fragmentation(34). This was a very promising new technology enabling endoscopic more than 80-95% fragmentation of stones.

There was no significant ureteral injury attributable to the laser energy.

Dye lasers have proved their ability to fragment stones; however, their application is associated with several other difficulties, including very high initial costs and expensive disposables (coumarin dye) and trouble with fragmenta- tion of notoriously hard stones composed of calcium oxalate monohydrate (COM), brush- ite, and cysteine

2.4.2- Applications of Lasers in Urology and Utility of LIBS to kidney Stones:

Kidney stones were analyzed by using a commercially available LIBS instrument with a 6-channel CCD spectrometer (RT100-EC LIBS instrument. Applied Spectra, Inc., Fremont, CA). The RT100-EC integrates a Nd:YAG laser with maximum of 50 mJ energy at 1064 nm and with 5 ns pulse duration (FWHM). The system is capable of 20 Hz maximum repetition rate. Gate delay of 1 us was selected to optimize the signal-to-noise (S/N) ratio. The samples were placed into a chamber that could be purged with different buffer gases. To maintain a consistent sample height and therefore, consistent laser spot size, the system uses auto height adjustment to account for morphological variation of the sample surface. The spot size of 150 µm was maintained at each sampled location and the laser fluence was estimated to be 113 J/cm^2(35). For each sample, fifty single-shot LIBS spectra were acquired from four different random locations, except for sample 5 due to its small size

This paper demonstrates LIBS as a promising, simple, and accurate analytical method for the characterization of kidney stones. LIBS emission lines characteristic of elements present in five different types of kidney stones were detected at different locations of the stones, producing elemental mapping. The lighter elements critical to kidney stone classification such as C, H. and N were also detected with ease by LIBS and not by XRF. The classification of the stones was conducted using the spectral line intensity ratios of C, H, Ca, Mg, and P elements.

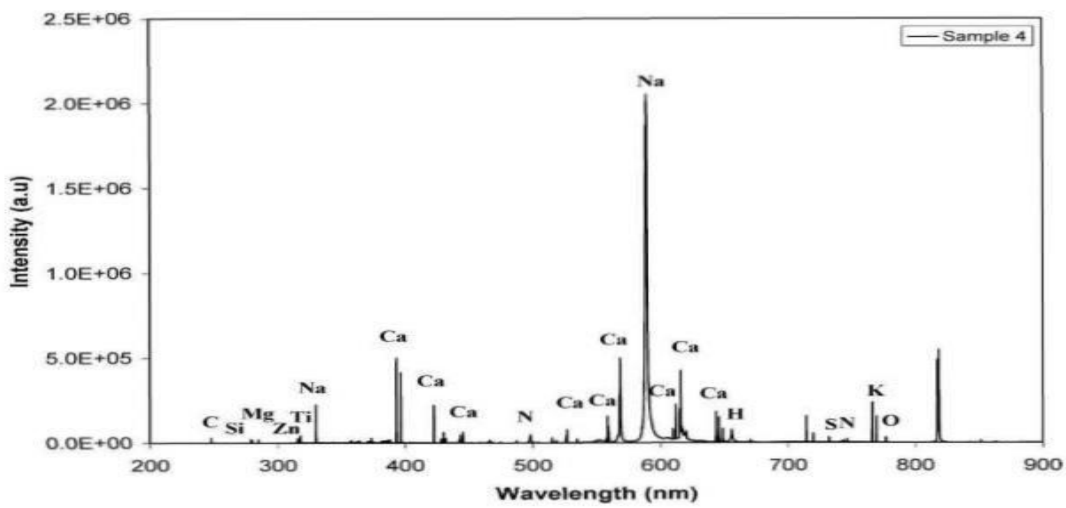
The 3D view of the PCA chart showed clustering characteristics with respect to whether a stone was of organic/ inorganic nature or mixed.

A LIBS system has advantages for rapid analysis of kidney stones compared to XRD and XRF LIBS also provides a measure of spatial distribution of elements in the samples

Heterogeneous kidney stones were analyzed by LIBS using a short-pulsed high power laser and high resolution multi-channel CCD with LIBS, a laser beam can be focused on a sample ‹spectrometers surface with a spot size less than a few tens of micrometers to achieve high spatial resolution. Formation of kidney stones takes place over a long period of

The results are beneficial in understanding kidney stone formation processes, which can lead to preventive therapeutic strategies and treatment methods for urological patients(36)

The ability of LIBS to determine the type of kidney stone without any sample preparation, with high speed, and in situ under a clinical setting is expected to significantly aid the medical community in selecting proper treatment and preven- tion methods for patients. LIBS gives an advantage of carrying out simultaneous elemental analysis and classification of kidney stones using a single instrument, whereas this range of information would only be possible by carrying out separate measurements of XRF and XRD



3- Pharmaceutical application:

LIBS offers sev- eral remarkable features like in-situ and real-time monitoring of chemical species, dosage strength identification and clas- sifications of pharmaceutical samples . LIBS is a non/ minimal distractive technique which can detect all integral parts (organic and inorganic) of pharmaceutical sample in a single step. In essence, LIBS is an elemental analysis tech- nique for rapid and spot analysis of wide range of samples. It offers the capability to analyse different phases, i.e. solid, liquid, gas, aerosol, powders, biological material, polymers. etc., of samples under any atmospheric condition . This technique does not require additional chemicals, which makes it safe and environment friendly. LIBS can play a major role in elemental analysis of surface and internal dis- tribution of drug samples. In the present study, we have examined five uncoated drug samples named by A, B, C, D and E which con- tain only voglibose as API, of dose strength 0.3, 0.2, 0.3, 0.2 and 0.3 mg. respectively, using LIBS. The above five samples belong to three different brands; A and B belong to one brand. C and D belong to the second brand and E belongs to third brand. Six spectra of each tablet have been recorded and each spectrum has an accumulation of 50 laser shots. Five tablets of each sample have been investigated for the study. Voglibose (C10H1NO) was dis- covered in Japan in 1981 and it is a class of competitive alpha-glucosidase inhibitors (a-GIs) which is used for the treatment of type 2 diabetes.

Table 1 Spectral peaks of different elements observed in the LIBS spectra of voglibose

S.N.	Elements	Wavelength (nm)
1	Carbon	247.8
2	Hydrogen	656.4
3	Silicon	250.7, 251.4, 251.6, 252.8, 288.1
4	Magnesium	279.5, 280.2, 285.2, 382.9, 383.2516.7, 517.3, 518.3
5	Sodium	589.0, 589.6
6	Potassium	766.4, 769.8
7	Calcium	393.3, 396.8, 397.2
8	Nitrogen	742.3, 744.2, 746.8, 818.4, 818.8, 821.6, 824.2
9	Oxygen	777.3, 844.8

*Analysis of film coating tablet by using LIBS:

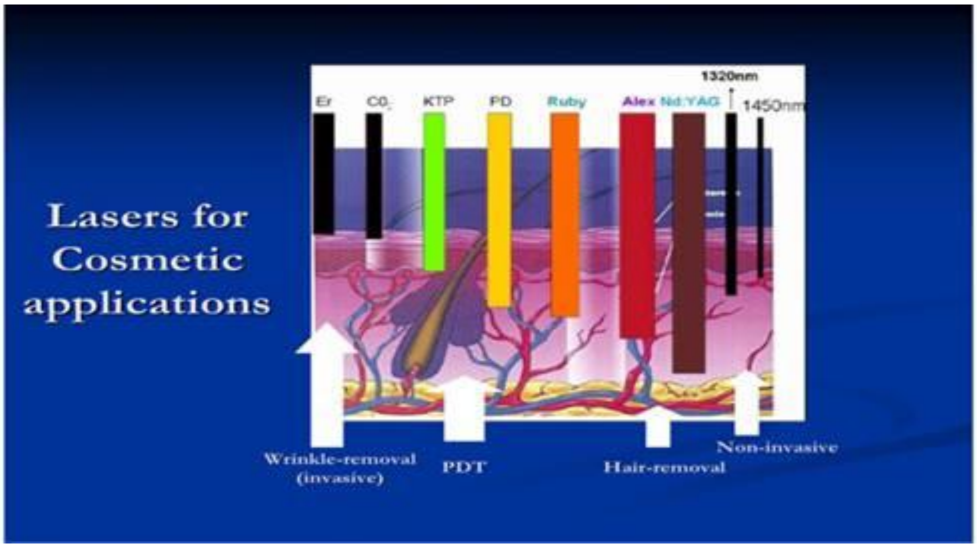
The majority of pharmaceutical tablets on the market are film-coated, which serves several purposes, including taste masking, dissolution modification, and the protection of active pharmaceutical ingredients (APIs) against air, moisture and light. The photo-stability of film-coated tablets often depends on the thickness of the coating pigment. LIBS analysis was applied to tablets in this experiment to demonstrate effective monitoring of coating composition, thickness and uniformity with minimum sample preparation. (37)

4- Ophthalmology lasers :

As shown by Table 3, various lasers have been used for various ophthalmic applications including retinal photocoagulation using argon blue-green laser (488/514 nm), double-YAG green laser (at 532 nm), krypton laser (at 647 nm) and diode lasers (at 806-810 nm). Photocoagulation process was also used to seal leak blood vessels for The treatment of age-related macular degeneration (AMD).(38-39)

5- Cosmetic and dermatology lasers

Figure 4 shows various cosmetic lasers and their applications for invasive and non-invasive uses defined by their tissue penetration depth (d). Lasers with small depth (d<0.1 mm) suitable for invasive wrinkle or tattoo removal, whereas large depth (with d> 2 mm) suitable for non-invasive simulation or hair removal. Figure 4 can be compared with Figure 2 for the penetration depth. Figure 4 shows the commercial laser for hair removal (using a diode laser at 810 nm with large penetration depth about 4 mm) and hair growth device (using a red, 635 -690 nm, LED or laser with smaller penetration depth and low power for surface stimulation). Figure 5 shows a UV (308 nm) light device for the treatment of psoriasis; and a pen-type blue laser (at 405 nm) combined with a red laser (at 660 nm) for the treatment of acne.(40-41)



6- Conclusion:

The ratio-based classification and regression of pharmaceuti- cal samples show that LIBS along with statistical analysis has the capability of fast online assessment of identification and classification of pharmaceutical drugs of different dosages and brands. Calibration models are developed to predict relative intensity ratios of different elements(0). Our study claims that H/ C ratio analysis can be applied for rapid measurement of rel- ative concentrations as well as identification of dose strength. It is found that H/C ratios are almost constant in the LIBS spectra recorded in air and argon atmosphere; thus, it will be cost-effective to perform experiment in air(0). LIBS can be used to identify the counterfeit in generic pharmaceutical drugs. Thus, the proposed methodology can be implemented for on- line, in-situ monitoring of .active ingredients in pharmaceutical drug samples

5- Reference:

1) Noll, R. Laser-Induced Breakdown Spectroscopy: Fundamentals and .Applications; Springer: Berlin, Germany, 2012

2) Cremers, D.; Radziemski, L. Handbook of Laser-Induced Breakdown .Spectroscopy; Wiley: Hoboken, NJ, USA, 2013

3) Lee, Y.; Song, K.; Sneddon, J. Laser-induced Breakdown .Spectrometry; Nova Publishers: Hauppauge, NY, USA, 2000

4) Miziolek, A.; Palleschi, V.; Schechter, I. (Eds.) Laser Induced Breakdown Spectroscopy; Cambridge University Press: Cambridge, UK, .2006

5) Singh, J.; Thakur, S. (Eds.) Laser-Induced Breakdown Spectroscopy; .Elsevier: Amsterdam, The Netherlands, 2007

6) Musazzi, S.; Perini, U. (Eds.) Laser-Induced Breakdown Spectroscopy, ‹Theory and Applications; Springer: Berlin

Germany, 2014

7) Rusak, D.; Castle, B.; Smith, B.; Winefordner, J. Fundamentals and applications of laser-induced breakdown spectroscopy. Crit. Rev. Anal. Chem. 1997, 27, 257–290

8) Dahmani, F. (1992), Experimental study of laser and wavelength plasma coupling, transport, and ablation –dependences of laser processes in planar gold targets, Phys

9)McCaughey RG, Chlebicki C, Wong BJ (2010) Novel wavelengths for .laser nerve stimulation. Lasers Surg Med 42: 69-75

10)Lin JT (2016) Combined analysis of safety and optimal efficacy in UV-light-activated corneal collagen crosslinking. Ophthalmology Research 6:

11)Murday JS, Siegel RW, Stein J, Wright JF (2009) Translational . 1-14 nanomedicine: status assessment and opportunities. Nanomedicine 5: 251-273

12)West JL, Halas NJ (2003) Engineered nanomaterials for biophotonics applications: improving sensing, imaging, and therapeutics. Annu Rev Biomed Eng 5: 285-292

13) Samek O, Beddows DCS, Telle HH, Kaiser J, Liˇska M, C'aceres JO, Gonz'ales Ure˜na A (2001) Quantitative laser-induced break- down .spectroscopy analysis of calcified tissue samples

14)Spectrochim Acta B 56:865–875

-Sighinolfi GP, Sartono S, Artoli YG (1993) Chemical and mineral

ogical studies on hominid remains from Sangiran, Central Java

Indonesia). J Hum Evol 24:57–68)

15) ‹Stamoulis KC, Assimakopoulos PA, Ioannides KG, Johnson E Soucacos PN (1999) Strontium-90 concentration measurements in

human bones and teeth in Greece. Sci Total Environ 229:165–182

16)Vandeweert E, Bastiaansen J, Philipsen V et al. The detection of Sr sputtered from metallic and biological matrices by double-resonant photoionization mass spectrometry. In RIS 2000: Laser ionization and applications incorporating RIS (ed. J. E. Parks and J. P. Young

AIP Conf. Proc. 584 (2001), 301-304

17)Kasem MA, Gonzalez JJ, Russo RE, Harith MA (2013) LIBS

analysis of artificial calcified tissues matrices. Talanta 108:53–58

18)Niemz MH (2007) Laser-tissue interactions: fundamentals and ap- plication. Springer Verlag, Berlin

19)LeGeros RZ, Ito A, Ishikawa K, Sakae E, LeGeros JP (2009) Fundamentals of hydroxyapatite and related calcium phosphates. In: Basu B, Katti DS, Kumar A (eds) Advanced biomaterials: fundamentals, processing and applications. Wiley, New York, pp 19–52

20)Li X, Ramadori P, Pfister D, Seehawer M, Zender L, Heikenwalder M, The Immunological and Metabolic Landscape in Primary and Metastatic Liver Cancer, Nat Rev Cancer (2021) 21:541–57. doi:10.1038/s41568-021-00383-9

21)Kumar A, Yueh F-Y, Singh JP, Burgess S. Characterization of Malignant Tissue Cells by Laser-Induced Breakdown Spectroscopy. Appl Opt (2004) 43(28): 5399. doi:10.1364/ao.43.005399

22)Chen Y-P, Chan ATC, Le Q-T, Blanchard P, Sun Y, Ma J. Nasopharyngeal Carcinoma. Lancet (London, England) (2019) 394(10192):64–80. doi:10.1016/ b978-0-12-814936-2.00001-8

23)Chu Y, Chen T, Chen F, Tang Y, Tang S, Jin H, et al. Discrimination of Nasopharyngeal Carcinoma Serum Using Laser-Induced Breakdown Spectroscopy Combined with an Extreme Learning Machine and Random forest Method. J Anal Spectrom (2018) 33(12):2083–8. doi:10. 1039/c8ja00263k

24)Wang J, Yang LP, Zhu YY, Hao MZ, Li X. Identification of Cervical Cancer Using Laser-Induced Breakdown Spectroscopy Coupled with Principal Component Analysis and Support Vector Machine. Lasers Med Sci (2018) 33:1381–6. doi:10.1007/s10103-018-2500-2

25)Seifalinezhad A, Bahreini M, Hassani Matin MM, Tavassoli SH. Feasibility Study on Discrimination of Neo-Plastic and Non-neoplastic Gastric Tissues Using Spark Discharge Assisted Laser Induced Breakdown Spectroscopy. J Lasers Med Sci (2019) 10(1):64–9. doi:10.15171/jlms.2019.10

26)Is W, Cancer O. What Is Ovarian Cancer? (2021). 1–14.

27)Melikechi N, Markushin Y, Connolly DC, Lasue J, Ewusi-Annan E, Makrogiannis S. Age-specific Discrimination of Blood Plasma Samples of Healthy and Ovarian Cancer Prone Mice Using Laser-Induced Breakdown Spectroscopy. Spectrochimica Acta Part B: At Spectrosc (2016) 123:33–41. doi:10.1016/j.sab.2016.07.008

28)Lin X, Sun H, Gao X, Xu Y, Wang Z, Wang Y. Discrimination of Lung Tumor and Boundary Tissues Based on Laser-Induced Breakdown Spectroscopy and Machine Learning. Spectrochimica Acta Part B: At Spectrosc (2021) 180: 106200. doi:10.1016/j.sab.2021.106200

29)Han JH, Moon Y, Lee JJ, Choi S, Kim Y-C, Jeong Differentiation of Cutaneous Melanoma from Surrounding Skin Using Laser-Induced Breakdown Spectroscopy

30)Gaudiuso R, Ewusi-Annan E, Melikechi N, Sun X, Liu B, Campesato LF, et al. Using LIBS to Diagnose Melanoma in Biomedical Fluids Deposited on Solid Substrates: Limits of Direct Spectral Analysis and Capability of Machine Learning. Spectrochimica Acta Part B: At Spectrosc (2018) 146:106–14. doi:10.1016/j.sab.2018.05.010

31)I.S. Kim, S.J. Myung, S.S. Lee, S.K. Lee, M.H. Kim, "Classification and nomenclature of gallstones revisited", Yonsei medical journal, 44, 561-.(570 (2003

32)P. Chandran, N.K. Kuchhal, P. Garg, C.S. Pundir, "An extended chemical analysis of gallstone", Indian Journal of Clinical Biochemistry, 22, 145-150 (2007

33) S.H. Lee, H.S. Shim, J.H. Yoo, R.E. Russo, S.H. Jeong. ‘‘Analysis of the . Absorption Layer of CIGS Solar Cell by Laser-Induced Breakdown Spectroscopy’’. The 3rd North American Symposium on Laser-Induced .Breakdown Spectroscopy. Florida, USA: NASLIBS, 2011. July 18-20, 2011

34)B. Genc Oztoprak, E. Akman, M.M. Hanon, M. Gunes, S. Gumus, E. Kacar, O. Gundogdu, M. Zeren, A. Demir. J. Opt. Laser Technol. 2012

35)M. Tran, Q. Sun, B.W. Smith, J.D. Winefordner. ‘‘Direct Determination of Trace Elements in Terephthalic Acid by Laser Induced Breakdown .Spectroscopy’’. J. Anal. Atom’’. Spectrom. 2001. 16: 628-632

36)C. Lo´pez-Moreno, S. Palanco, J.J. Laserna, F. DeLucia, A.W. Miziolek, J. Rose, R.A. Walters, A.I. Whitehouse. ‘‘Test of a Stand-Off Laser-Induced Breakdown Spectroscopy Sensor for the Detection of Explosive Residues on Solid Surfaces’’. J. Anal. Atom. Spectrom. 2006. 21: 55-60

37)Madamba, M., Mullett, M. W., Debnath, S., Kwong, E. Characterization of Tablet Coatings Using Laser- Induced Breakdown Spectroscopy, AAPS PharmSciTech, 2007, 8, E1-E7

38)Lin JT (1992) Multiwavelength solid state laser for ophthalmic .applications. Proc SPIE 1644: 266-275

39)Lin JT (2016) Combined analysis of safety and optimal efficacy in UV-light-activated corneal collagen crosslinking.

40)Tsuyuki K, Miura S, Idris N, Hendrik K, Jie T, Kagawa K 2006 Appl. Spectrosc.60 61