Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon /College of pharmacy

Laser in medical applications -A Review

Submitted to the council of college of pharmacy – Babylon University
As partial of the requirement for BSc degree of pharmacy

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2022 A.D 1443 A.H

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ} [المجادلة: ١١]

صدق اللَّهُ العظَيم

الشكر والتقدير

أول مشكور هو الله عز وجل، ثم والداي على كل مجهوداتهم منذ ولادتي إلى هذه اللحظات، أنتم كل شيء أحبكم في الله أشد الحب .

يسرني أن أوجه شكري لكل من نصحني أو أرشدني أو وجهني أو ساهم معي في إعداد هذا البحث بإيصالي للمراجع والمصادر المطلوبة في أي مرحلة من مراحله، وأشكر على وجه الخصوص استاذتنا الفاضلة الدكتورة (رفل احمد عبد الكاظم) على مساندتي وإرشادي بالنصح والتصحيح وعلى اختيار العنوان والموضوع، كما أن شكري موجه الى ادارة كلية الصيدلة/جامعة بابل للمجهودات المبذولة من قبل أساتذتنا الكرام في الجامعة والحمد لله رب العالمين .

الإهداء

الحمد والشكر لله سبحانه وتعالى، والصلاة والسلام على من بلغ الرسالة وأدى الأمانة ونصح الأمة إلى نبي الرحمة العالمين سيد الخلق أجمعين نبينا محمد صلى الله عليه وعلى آلة الطيبين الطاهرين .

إذا كان الإهداء يعبر ولو بجزء من الوفاء، فالإهداء . . .

إلى من أُفضّلها على نفسي، ولِمَ لا؛ فلقد ضحّت من أجلي

ولم تدّخر جُهداً في سبيل إسعادي على الدّوام

(أُمّي الحبيبة) .

نسير في دروب الحياة، ويبقى من يُسيطر على أذهاننا في كل مسلك نسلكه

صاحب الوجه الطيب، والأفعال الحسنة .

فلم يبخل عليّ طيلة حياته

(والدي العزيز) .

إلى أصدقائي، وجميع من وقفوا بجواري وساعدوني بكل ما يملكون، وفي أصعدة كثيرة

أُقدّم لكم هذا البحث، وأتمنّى أن يحوز على رضاكم .

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Abstract

LIBS is an atomic spectroscopy technique which is based on the analysis of the spectral emission from laser-induced plasmas produced by high-power laser pulses of short duration applied to the surface of the target material, probably the most versatile method of elemental analysis currently in use for many biomedical applications. Over the last few decades the use of lasers had become standard for the treatment and diagnosis of many diseases including the treatment of a range of ophthalmological and dermatological conditions, The applications of lasers in medicine can be categorized into two major disciplines, namely diagnostic and therapeutic, LIBS have many advantages and One the unique advantage of LIBS in allowing the study of a broad variety of samples without sample preparation is attractive for the analysis of biological samples. Therefore, the LIBS technique is being utilized promisingly for the analysis of biomaterials. LIBS also has certain limitations, As Difficult to get suitable matrix-matched standards, which makes the technique qualitative or at best semiquantitative.

1.0 Introduction

The characterization of biomaterials is both interesting and challenging for analytical scientists. There are many analytical techniques based on the emission of electromagnetic radiation produced after excitation of atoms, ions and molecules present in the target materials. These techniques generally employ some kind of energy source to excite the species present in the sample to higher energy levels from where they return to lower levels emitting the characteristic radiation which can be collected and sent to a wavelength selector and finally detected. However, most emission techniques cannot be applied directly to intact samples because they require treatment before analysis, and this limits the use of these analytical techniques in environmental analysis, forensic analysis, archaeological analysis, biological analysis and many other areas of applied science as these samples are very sensitive to their surrounding atmosphere. Trace mineral elements play an important role in biologically active materials because minute variations in the amounts of these minerals may adversely affect the metabolism processes in all living creatures. Thus detection and quantification of such minerals in biomaterials is essential to monitor metabolism. Further, the presence of small amounts of toxic and heavy metals in food and food products can adversely affect human health and consequently the detection and analysis of these metals present at trace levels is of the utmost importance.

Several analytical techniques have been applied in an attempt to address these problems. But these methods often require laboratory-scale equipment and sophisticated sample treatment protocols. Recently laser-induced breakdown spectroscopy (LIBS) has emerged as a powerful analytical technique for direct spectrochemical analysis of a variety of solids, liquids and gases with no or little sample pretreatment.

In LIBS, intense laser pulses at UV, visible or infrared wavelengths are used to ablate the target material to produce a luminous plasma plume which emits characteristic radiation that helps to determine the target composition. The emitted radiation from the plasma is analysed using a high-resolution spectrometer and sensitive detector. The LIBS spectrum of the target material yields qualitative and quantitative information which can be correlated with the sample identity.

LIBS is probably the most versatile method of elemental analysis currently in use for many biomedical applications [1]. In the biomedical field, LIBS is particularly used to diagnose and classify cancers in vivo by determining the intensity ratios of trace elements in normal and cancerous material. Generally, cancer diagnosis and classification rely upon subjective interpretation of biopsy material, but with the use of LIBS the diagnosis of cancer is easier. Although LIBS has been traditionally considered as an elemental analysis technique it is also being used successfully for molecular identification of materials including biomaterials.

Over the last couple of years there has been an exponential growth in the areas of utilization of LIBS which is reflected by an increasing number of publications, and thus its utility as an analytical technique has been proved[2].

2.0 The laser and its applications in biomedicine

In 1917, Albert Einstein first theorized about the phenomenon of stimulated emission which is the backbone of the laser [3]. The first working laser, the pulsed ruby laser, was invented by Theodore H. Maiman in 1960. However, there were no potential applications known and in fact it was popularly referred to as a tool looking for applications [4]. Within a few years of the development of the laser its medical application particularly in urology was reported in 1968 by Mulvaney and Beck who used the ruby laser to fragment urinary calculi [5]. They were able to ablate the calculi, but the continuous wave ruby laser generated excessive heat and so its clinical use was not extended. By the mid-1980s the use of the laser to treat stone disease had become established, and the era of laser lithotripsy had begun [6]. Thus the development of potential applications of the laser in medical science has given a new direction to analytical scientists. Over the last few decades the use of lasers had become standard for the treatment and diagnosis of many diseases including the treatment of a range of ophthalmological and dermatological conditions [7-9]. There are many medical disciplines where lasers are successfully used for a variety of purposes. However, there is a necessity for further research in laser applications in medicine in order to achieve optimal outcomes [10].

To improve the medical applications of laser based techniques an understanding of the kinetics and dynamics of laser interactions with biological tissues is essential. Knowledge of laser–tissue interactions will guide the identification of optimal laser parameters to achieve more efficient and safer outcomes [11]. The applications of lasers in medicine can be categorized into two major disciplines, namely diagnostic and therapeutic. The vast majority of applications are in the therapeutic field. In recent years, there has also been much interest in the use of the laser as a diagnostic tool and this has resulted in some exciting developments across all

medical specialities [12]. Gaining clinical diagnostic information by the use of a laser probe, for example for the analysis of tissue and biomaterials,

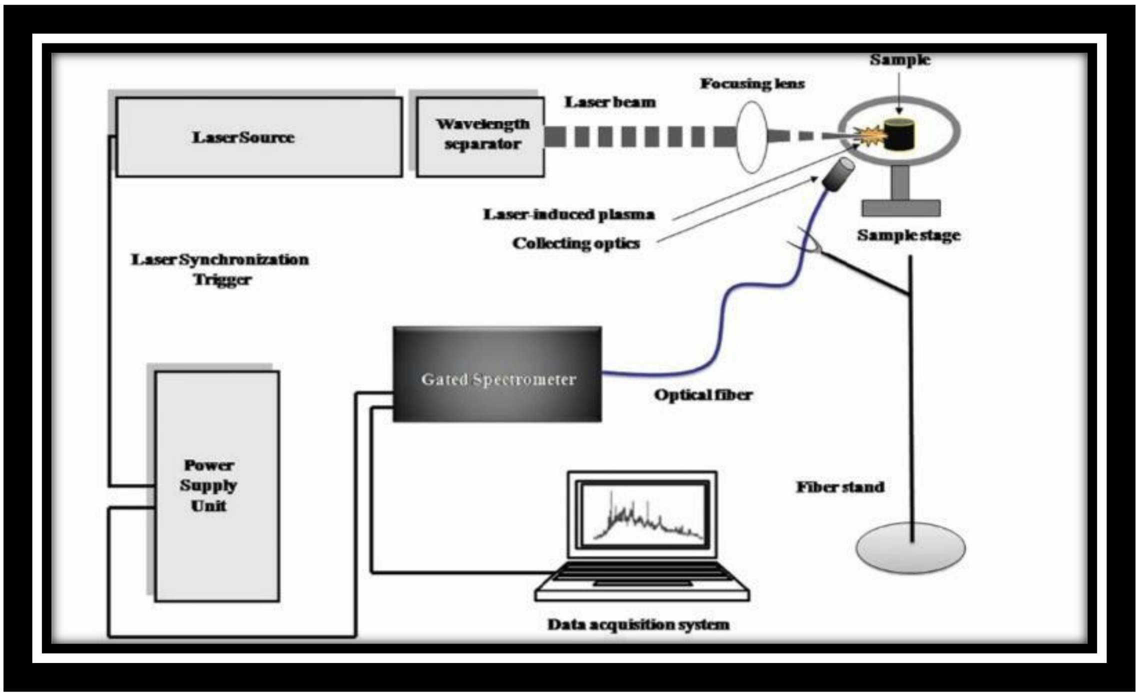
may better guide treatment and may also be helpful in optimizing the therapeutic technique [13].

3.0 Laser-induced breakdown spectroscopy

Shortly after the invention of the laser device using a ruby crystal, Brech and Cross demonstrated the first useful laser-induced plasma produced on the surface of the target [14]. This was the "birth" of the LIBS technique, and in subsequent years significant milestones were reached in the development of this method. LIBS is an atomic spectroscopy technique which is based on the analysis of the spectral emission from laser-induced plasmas produced by high-power laser pulses of short duration applied to the surface of the target material.
Generally, atomic emission spectroscopy (AES) uses an external energy source to excite atoms in their ground state. The atoms spontaneously emit radiation when

they revert back to the lower energy state, with the emission intensity being proportional to the concentration of atoms in the ground state [15].

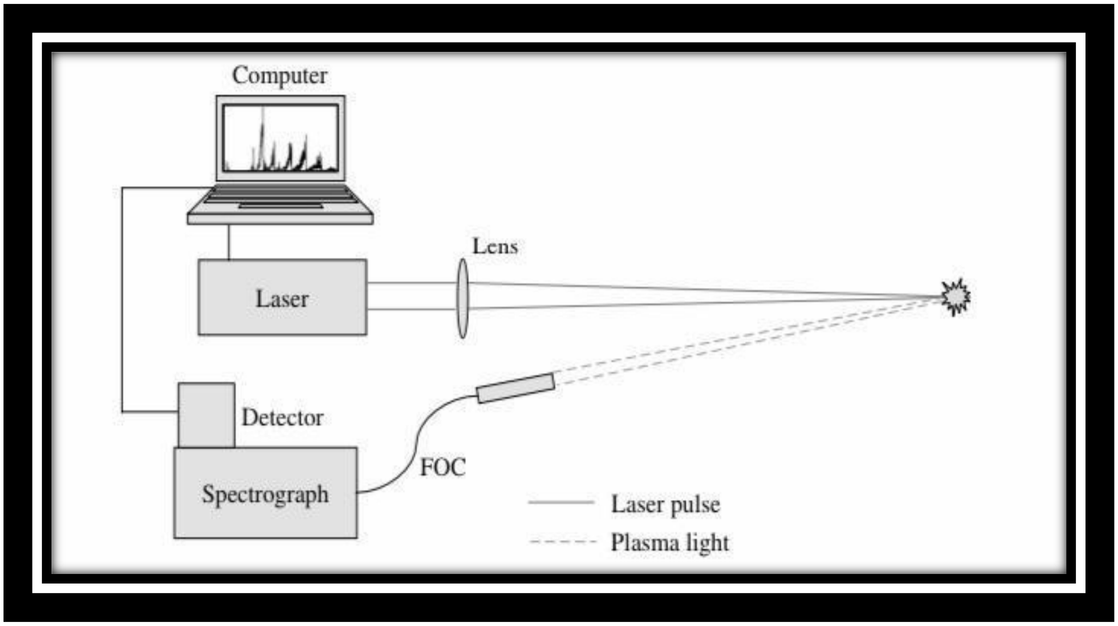
In the LIBS technique, high-power pulsed lasers are used as the excitation source (Fig. 1) [16].



"Fig. 1 Schematic diagram of a simple LIBS system comprising the essential components to produce laser-induced plasma and of the detection system" [16].

Laser-induced breakdown spectroscopy (LIBS), also sometimes called laser-induced plasma spectroscopy (LIPS) or laser spark spectroscopy (LSS) has developed rapidly as an analytical technique over the past two decades.

As most commonly used and shown schematically in Figure 2[17], the technique employs a low-energy pulsed laser (typically tens to hundreds of mJ per pulse) and a focusing lens to generate a plasma that vaporizes a small amount of a sample. A portion of the plasma light is collected and a spectrometer disperses the light emitted by excited atomic and ionic species



"Figure 2 A schematic of a simple apparatus for laser-induced breakdown spectroscopy illustrating the principal components" [17].

in the plasma, a detector records the emission signals, and electronics take over to digitize and display the results. The book cover shows a LIBS spectrum with certain strong spectral features standing out from the continuous background plasma light [17].

During the 1980s, the neodymium-doped yttrium aluminium garnet (Nd:YAG) laser was the most common laser system used in most applications involving LIBS. The Nd:YAG laser became popular for LIBS because it was easily configured to produce the megawatt peak power levels required for reliable laser plasma generation from the target materials. The Nd: YAG is a four-level laser system that produces a very high power emission. The energy levels of the Nd^{3+} ion are responsible for the fluorescent properties and are thus suited for the amplification process.

3.1 Advantages and some limitations of LIBS

The main attributes that make LIBS a very powerful and attractive analytical tool are: its in situ measurement capability, simultaneous multi elemental detection, and real-time analysis of materials in the laboratory or in the field. There are several significant advantages that make LIBS more applicable than other techniques, as follows:

1. The need for little or no sample preparation results in less need for toxic chemicals usually required for sample preparation.
2. Versatile sampling of all media (solids, liquids, gases as well as biomaterials), including both conducting and nonconducting materials.
3. Very small amounts of sample (order of micrograms) are vaporized.
4. Extremely hard materials that can be difficult to get into solution can be analysed (e.g. ceramics, glasses and superconductors).
5. With a spatial resolving power of the order of 100 μm, micro regions can be analysed.
6. Multiple elements can be determined simultaneously.
7. The direct determination of aerosols or ambient air is possible.
8. The analysis is simple and rapid.
9. Point detection capability enables the analysis of any kind of material including biomaterials.

10. Remote sensing is possible with the use of fibre optics.

11. Samples can be analysed in a hostile environment.

12. Underwater analysis is possible.

13. Development of field instruments is possible.

14. Stand-off detection is also possible using a telescope for light collection without the need for a fibre optic cable near the sample.

15. LIBS is minimally destructive because the amount of sample consumed is very small (nanograms) depend- ing on the laser pulse energy. Thus it is suitable where only small amounts of material are available.

3.2 LIBS has certain limitations:

1. Difficult to get suitable matrix-matched standards, which makes the technique qualitative or at best semiquantitative.

2. Detection limits are generally not as good as those of the conventional techniques.

3. Precision is poor as compared to conventional techniques

4. Safety measures are required to avoid ocular damage by the high-energy laser pulses.

3.3 studies of LIBS applications in biomedicine

The unique advantage of LIBS in allowing the study of a broad variety of samples without sample preparation is attractive for the analysis of biological samples. Therefore, the LIBS technique is being utilized promisingly for the analysis of biomaterials. We discuss here the use of LIBS for the study of biological samples particularly in the field of biomedical science

3.4 Tissue analysis

Sun et al. [18] reported the use of LIBS for the detection of the trace element Zn in human stratum corneum. The authors found that zinc is absorbed through the skin and its concentration decreases exponentially with depth in the skin. The authors concluded that LIBS is a useful tool for trace element analysis in human skin. De Souza et al. [18] used LIBS to investigate the relative elemental composition of chick myocardium tissue. The analysis showed the presence of elements including Na, K, Ca, and H which were identified separately and compared with the common elements present in tissues. They found that in the extracellular matrix Na predominates and in the intracellular space K predominates together with Ca and Mg. Finally, they concluded that the measurement of the relative atomic composition by means of the laser ablation might lead to a technique for the discrimination of different materials or tissues.

Kumar et al. [18] reported the first experiments to explore the possibility of using LIBS for cancer detection. They analysed malignant and normal tissue from a canine haemangiosarcoma. Canine haemangiosarcoma, which is a model for human angiosarcoma, may be valuable to define and analyse these types of tumours and suggest potential means of improving their classification in humans [19].

3.5 Analysis of stones in different organs of the human body

Lasers have been used to breakdown urinary and kidney calculi since 1987 [20]. The laser shock-wave disintegrates the calculus into tiny fragments. Fang et al. [21] used LIBS for the quantification of the elemental contents Ca, Mg, Na, Sr, K and Pb in urinary calculi, and they concluded that LIBS offers the possibility to accurately measure trace elements in such stones without the need for any elaborate sample preparation. Recently, Singh et al. [22,23] characterized qualitatively and quantitatively the different types of gallbladder stone (cholesterol stones, pigment stones, mixed stones). They analysed different parts of the gallstones and found higher levels of metal elements in the centre than in the shell and surface of the gallstones. Singh et al. [24] also reported the use of LIBS for the in situ quantitative estimation of the elemental constituents in different parts of kidney stones (centre, shell and surface parts) obtained during surgery. They estimated the quantities of Cu, Zn, Mg and Sr in the stones using calibration curves. They also used the ratios of the intensities of the different elemental lines to determine the spatial distribution of different elements inside [25]

3.6 Analysis of minerals in the human body

Discussed the utility of the LIBS technique for the analysis of minerals and potentially toxic elements present in calcified tissues including bones and teeth to study the influence of environmental exposure and other biomedical factors. They investigated the multidimensional profiles of the elements present in the

teeth and bone samples. Recently, the use of LIBS for the rapid identification of teeth affected by caries has been demonstrated by Singh and Rai .
They were able to detect a broad range of elements including Ca, Mg, Cu, Zn, Sr, Ti, C, P, H, O, Na and K. They found that the caries-affected part of the teeth contained lower amounts of Ca and P than the healthy part, but higher amounts of Mg, Cu, Zn, Sr, C, Na, K, H and O. They explained the presence of the different metal elements present in the teeth and also discussed their role in the formation of caries. [26].

3.7 Analysis of biological aerosols and nonaerosolized biological materials

In recent years, the analysis of microscopic particles, cells, aerosols, and especially bioaerosols (bacteria, fungi, viruses, pollen) has received increasing interest because of biological threats to public and defence security. Minute amounts of inhaled bioaerosols can cause disease, toxicity and allergic reactions. Thus, the detection and identification of biological aerosols and agents is an urgent civil and military requirement which will be useful in environmental monitoring.morel et al. used time-resolved LIBS for analysing biological matter for the detection of biological hazards[27].

3.8 Molecular detection using LIBS

Traditionally, LIBS is considered as a physical diagnostic tool based on elemental analysis because information about the chemical composition of the materials is lost as the temperature of the laser-induced plasma is usually greater than 10,000 K. However, much effort has been made to provide the capability to identify the molecular species in pure materials. The identification of explosive materials is based on ratios of the intensity of two spectral lines to determine relative molecular concentrations. De Lucia et al. recorded the LIBS spectra of several explosives including pentaerythritol tetranitrate, cyclotetramethylene tetranitramine, cyclotrimethylene trinitramine, and trinitrotoluene [66]. Such spectra are the start of a LIBS database for molecular identification schemes aimed at identifying parent materials. Methods to distinguish between spectra usually involve either analysing the whole LIBS spectrum or focusing on selected atomic lines; for organic materials these atomic lines mainly include C, H, N, and O, which are ubiquitous in nature which complicates the analysis of organic species.

Portnov et al. [28] have shown that molecular emission may also be used to infer material composition. They applied LIBS to nitroaromatic and polycyclic aromatic hydrocarbon samples to characterize the resultant emission in ambient air. The emission consisted of spectral features related mostly to CN and C2 molecular fragments and to C, H, N, and O atomic fragments.

They found that the Pathak et al. recorded the LIBS spectra of gallstone samples and used PCA to classify them (cholesterol type, mixed type and pigmented type). They prepared a LIBS library (a set of LIBS spectra of training samples of each category) and PCA was used to differentiate the gallstone samples. Their results clearly demonstrate the ability of PCA based on LIBS spectra to classify various types of gallstones. They also utilized the point detection ability of LIBS to study the spatial distribution of the major and trace elements including Mg, Mn and Ca etc. in the different parts (centre, shell and surface) of the mixed gallstone.

The most recent publications indicate that due to its unique capabilities, LIBS can be used to detect biological hazardous materials, and LIBS spectra provide an ample amount of useful information pertaining to the measurement of the molecular and cellular moieties. In such applications LIBS might presently play a supporting and leading diagnostic role. Improvements in LIBS sensitivity provided by new dedicated LIBS hardware, and in statistical methods (e.g. chemometrics, PCA analysis, and PLS-DA etc.) are expected to lead to improvements in LIBS-based discrimination of chemical and biological samples in the future[29].

4.0 Summary and future prospects

In this review we present the most recent developments in LIBS in the field of biomedicine. In the past decade there has been a burst of research activity in the use of LIBS for the analysis of trace elements in biomedicine matrices. As noted at the beginning of this review, LIBS is an effective technology with a wide range of potential applications in the detection and monitoring of major and trace elements in the human body, and LIBS technology has great potential for clinical practice. Many of these applications cannot be addressed using conventional analytical methods such as AAS, ICP, and XRF, but can be solved using LIBS. For the quantitative analysis of biomaterials where CRMs are available to prepare a calibration curve, the utility of CF-LIBS for determining the concentrations of the major and minor elements present in biological samples has been proven. Improving instrumentation, understanding the laser plasma, and data analysis are currently active areas of LIBS research.

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