

Utilizing laser-induced breakdown spectroscopy to investigate iron concentrations in the blood of individuals with iron deficiency anemia compared to healthy controls

Zainab H. Baqi

Department of Physics, College of Science, Mustansiriyah University, Baghdad, Iraq.

**Corresponding Author: Zainabmohee59@uomustansiriyah.edu.iq*

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Abstract: This study used Laser-Induced Breakdown Spectroscopy (LIBS) to detect iron deficiency in blood samples by comparing anemic to healthy samples. LIBS analyzed one healthy (H1) and two anemic (E1 and E2) blood samples. Iron spectral lines (Fe I, Fe II, and Fe III) were detected between 393 and 646 nanometers. The wavelengths were checked against the NIST database criteria to ensure accuracy. The relative technique was utilized to calculate the iron content in each spectral line. Divide the depth of each line by the intensity of the road with the highest spectrum depth. After that, the relative numbers' arithmetic suggestion was used to calculate each sample's average iron content. It became clear that the iron spectral strains were less strong inside the anemic samples (E1 and E2) than in the healthy samples (H1). The average iron degree in the healthy pattern (H1) became somewhat larger (zero.563) than in the anemic samples (0.456 for E1 and zero.486 for E2). Besides, iron content dropped more in the main anemic sample (E1) than in the second anemic sample (E2). This might be the result of the anemia within the samples not being as severe. This study shows that LIBS can detect iron shortages in blood samples accurately. This is so because the anemic samples contained substantially less iron and lower spectral strains than the healthy samples. These results indicate that LIBS might be a quick and painless way to locate human individuals with iron-deficiency anemia. Miles advised that further study be done with bigger sample sizes to make the impacts especially accurate and helpful.

Keywords: LIBS, anemia, iron deficiency, spectroscopy, relative method, spectral lines.

1. Introduction:

Iron is vital to the body. Many biological activities depend on it, including manufacturing hemoglobin, the protein that transports oxygen. Iron supports the body's defenses, neurons, and muscles, and numerous chemical processes. Unfortunately, many individuals don't receive enough iron, which is vital. Anemia can cause fatigue, weakness, dizziness, and inability to concentrate.[1, 2].

CKD, or chronic kidney disease, is harmful and alters how the body processes minerals and nutrients. Anemia from iron deficiency is common in CKD. Ineffective kidneys stop manufacturing erythropoietin (EPO), a hormone that signals bone marrow to create red blood cells. Chronic inflammation and iron deficiency worsen it. We need fast and precise techniques to measure CKD patients' blood iron levels.[3].

Biological sample chemicals may now be consistently and rapidly identified using

Laser-Induced Breakdown Spectroscopy (LIBS). A high-energy laser pulse illuminates the sample. For every element, it ionizes and creates plasma that emits a different pattern of light. Elements may be carefully measured using spectroscopy. Because of this, LIBS is a potential method for measuring blood iron levels without the need for chemicals or a lot of preparation. [4, 5].

There are one-of-a-kind approaches to doing evaluations that don't use lasers. Pump-probe spectroscopy is one of the most accurate laser-based ways to take a look at how atoms and molecules interaction with each other in real time. This technique is used to have a look at how molecules and nanomaterials change their properties. For greater facts, observe its use of [6-11]. Z-scanning can also be used to find out approximately the nonlinear optical properties of materials, which makes it useful for discovering the optical properties of thin films and nanoparticles. See research for more facts on how it is able to be used. [12].

Laser-Induced Breakdown Spectroscopy (LIBS), on the other hand, is one of the most hopeful ways to look at chemical elements in solid, liquid, and gaseous samples. LIBS is unique because it can study samples directly without prepping them first, and it can quickly and accurately find element ratios. Studies can tell you more about how it can be used.[13-19].

One of the most common ways to take pictures of nanoscale surfaces is with scan electron microscopy (SEM). This method

does not require lasers. Researchers can learn a lot about the size and composition of the material using scan electron microscopy (SEM), which takes pictures of samples with electron rays. Check the study to learn more about all the methods that can be used.[20-22]. Focused Ion Beam (FIB) generation can also be used to photograph and observe samples with the aid of the usage of a beam of ions. This makes it beneficial for duties like changing nanoscale samples and getting them geared up for microscopic evaluation. Studies can provide you with greater records on how it is able to be used. Earn approximately all the ways it can be used. FIB to photograph samples and take a look at their form and structure. See studies for greater records on how it could be used [23-30].

Lasers are used in spectroscopic evaluation, which makes them stand out from the others. LIBS also has some advantages that make it extraordinary from different techniques. First, LIBS would not want to put together samples, which makes it short and smooth. It can also check numerous exceptional forms of facts (strong, liquid, and gaseous) without having to alternate the settings. Third, LIBS gives short and actual results, which makes it best to be used in business and medicine. LIBS is a sturdy and particular tool within the area of spectroscopic research due to these sturdy factors [5, 31, 32].

Using Laser-Induced Breakdown Spectroscopy (LIBS), this looks at targets to discover how a good deal of iron is in blood samples from humans with anemia and examines them to samples from a healthy

individual. The predominant objective is to discover how well LIBS can detect iron deficiencies, which is a major cause of anemia. In addition, the look at desires to looks into the link between anemia and iron deficiency. This should assist medical doctors in figuring out better methods to diagnose and deal with humans with this sickness.

2. Theoretical Part:

2.1 Introduction to LIBS:

LIBS stands for "Disintegration Caused by Laser Spectroscopy." It is a new way to study substances by using high-energy laser bursts to create plasma on the surface of the thing being studied. This way of doing things is quick and can test parts in different sample states (like liquid, solid, or gaseous) without needing a lot of preparation. The sample goes through a rapid heating process when a high-energy laser wave is applied, which causes plasma to form and atoms to become ionized. When the plasma cools, the ionized atoms will return to lower energy states. This will cause light to be emitted at wavelengths that match the elements in the sample. A spectrometer looks at light to precisely figure out the parts and amounts of those parts.[34 ,33].

2.2 Principles of Spectroscopic Analysis Utilizing LIBS:

LIBS studies plasma spectral lines to determine element concentration. Because each element has its own set of colors, spectral lines can be thought of as a "fingerprint" for that element. The concentration of the element in the sample

can be found by measuring how strong these spectral lines are. If there is more of an element in the sample, its spectral line will be stronger.[35] .

2.3 Ratio Method:

One popular way to use LIBS to find out the concentration of elements is the Ratio Method. Using the relationship between intensity and concentration to figure out how much of the element is present, this method compares the levels of two spectral lines that come from the same element. The Boltzmann Equation shows this connection. It considers things like the likelihood of a shift, the statistical weights of energy levels, and the wavelengths of the spectral lines. [36].

$$\frac{I_1}{I_2} = \frac{A_1 \cdot g_1 \cdot \lambda_2}{A_2 \cdot g_2 \cdot \lambda_1} \cdot e^{\frac{E_1 - E_2}{kT}} \quad (1)$$

The values (I_1, I_2) Show how bright the two spectral lines are, (A_1, A_2) Show how likely it is that the spectral lines will change, (g_1, g_2) Show the statistical weights of the energy states, (λ_1, λ_2) Show the wavelengths of the spectral lines, (E_1, E_2) Show the energy levels that correspond to the spectral lines, (K) is the Boltzmann constant, and (T) is the plasma's temperature.

It is possible to simplify the equation to the following form when the energy differences between the two spectral lines are very small: [37]:

$$R.C = \frac{I_{element}}{I_{Max}} \quad (2)$$

In this case, (R.C) Is the relative intensity ratio used to set the concentration, and ($I_{element}, I_{Max}$.) Is the intensity of the two spectral lines that were used to make the measurement?

The arithmetic average of all relative values of spectral lines is used to find the average total concentration of the element in the sample. This is done first by detecting the relative concentration of each spectral line. The following equation can display this:

$$\begin{aligned} &\text{Average Total Concentration} \\ &= \frac{\sum_{i=1}^n C_{R,i}}{n} \quad (3) \end{aligned}$$

Where: (n) Number of spectral lines for the element in the sample, and (R) is the relative concentration of spectral line i.

3. Experimental Part:

3.1 Sample Preparation:

Three blood samples were taken from volunteers. The first and second samples came from people who had been labeled with anemia. The third sample came from a healthy person who didn't have any blood disorders and was used as a standard. Donors' blood was drawn using clean needles and normal medical methods to make sure the samples were safe and the results were correct. After the samples were collected, they were put in the centrifuge in the right tubes (see Figure 1). The centrifuge was run at the right speed to sort the different parts of the blood. The serum, which is the liquid part of the blood that has dissolved chemicals like iron, was collected after spinning. The red blood cells and other

parts of the blood fell to the bottom of the tube.



Fig. (1): shows the centrifuge.

The blood from each sample was placed in clean tubes to avoid contamination and skew the assay. Drying the serum on a stand prepared the samples for LIBS analysis. This created an analysis-ready flat surface. Figure 2 shows how to prepare serum for laser spectroscopy. These steps are necessary to make sure that the results of the research are correct and reliable. It separates the serum from the solid parts of the blood, making it possible to get an exact reading of the iron concentration. This helps researchers figure out how iron levels vary between healthy people and people who have anemia.

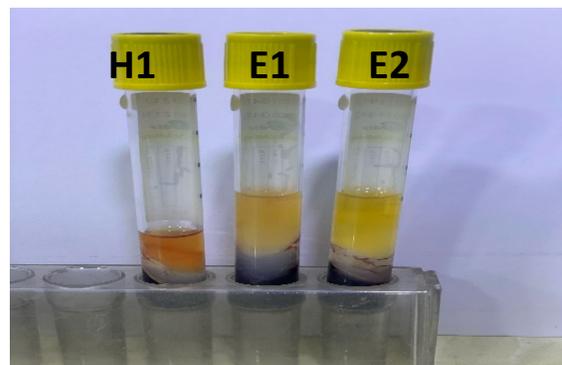


Fig.(2) displays the serum for the three samples.

3.2 Instrument for Laser-Induced Breakdown Spectroscopy (LIBS):

A laser source sends out high-energy pulses, focusing lenses aim the laser beam precisely, a sample holder holds the dried serum, and a light collection system gathers the emitted spectrum. It sends it to a spectrometer for wavelength analysis, and a CCD detector saves the data and sends it to the control and analysis unit, which has a computer and special software. Figure 3 shows the LIBS instrument's main parts.

First, the serum is spun to sort the samples. Droplets of serum are put on a plate and dried so that they stay stable enough to be measured. When the laser waves hit the sample, plasma forms, which makes a recorded and studied light spectrum that can be used to find iron levels. These steps are done several times to achieve accurate readings, and the spectra are compared between samples to see how anemia affects blood iron levels. LIBS is a good method for clinical analysis because it can quickly look at changes in mineral levels in the body without having to prepare samples in a lot of detail.

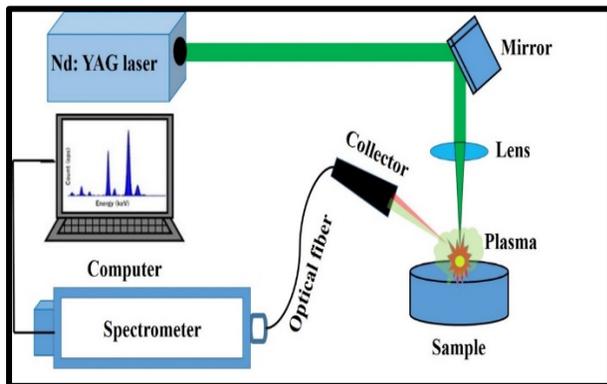


Fig.(3): Schematic Representation of the LIBS Technique

4. Results

4.1 Matching with NIST and Analysis of Spectral Lines:

LIBS was employed to get the three spectra of the samples (H1, E1, and E2). The iron spectral lines (Fe I, Fe II, and Fe III) were documented within the 393–646 nanometer wavelength spectrum. Figures 4–6 display these spectra alongside the line intensity spectrum for each sample. The spectral lines in the healthy sample (H1) were clearer and stronger than those in the anemic samples (E1 and E2). This is because people with anemia have lower iron levels.

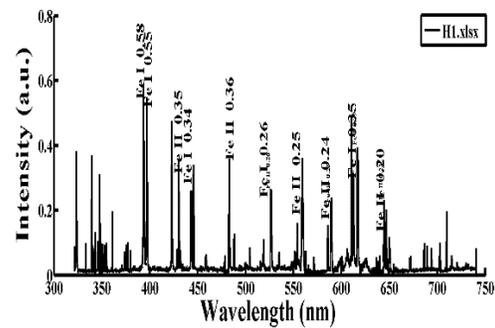


Fig.(4) illustrates the analytical spectrum of the healthy sample H1.

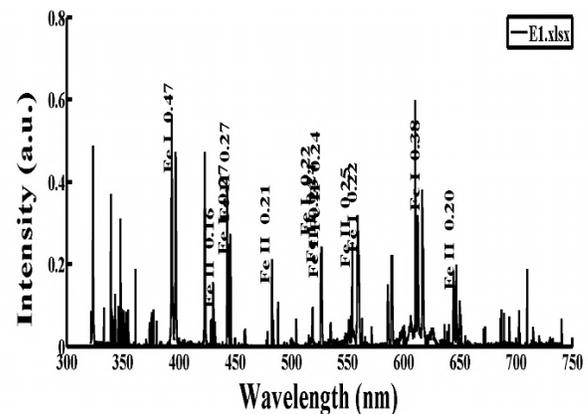


Fig.(5): Illustrates the analytical spectrum of the anemic sample E1.

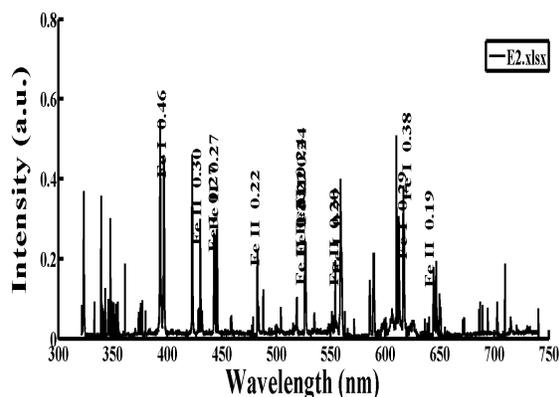


Fig.(6): Illustrates the analytical spectrum of the anemic sample E2.

To make sure everything was right, LIBS spectral lines were compared to numbers in the NIST database. There was a good match between the observed and standard wavelengths. The healthy sample (H1) had an iron (Fe I) line at 393.49191 nm that was the same as the NIST standard value of 393.53065 nm (see Table 1). This matching makes the results more reliable and shows that LIBS chemical analysis is right.

Tables 1–3 contain nine principal columns of the study results. The name of the element and its atomic symbol (Fe I, Fe II, Fe III) are shown in the first column. The second column shows the spectral line wavelength recorded by LIBS. The NIST standard range is located in the third column. The measured intensity of the spectral line is found in the fourth column. The fifth column from NIST denotes the element's transition probability. The sixth column denotes the element's minimal energy level, whereas the seventh column delineates its highest energy level. The statistical weight of the spectral line is presented in the eighth column. Equation 2 is employed to get the relative magnitude of the spectral line in the ninth column by dividing its intensity by the maximum intensity of the spectrum.

Table 1 :Shows the iron spectral lines in sample H1, which is healthy.

Element	Wavelength Peak	Wavelength NIST	Intensity	Transition Probability	Lower_Level Energy	Upper_Level Energy	Statistical Weight	Concentration
Fe I	393.4919	393.5307	0.58	2,200,000	2.845043	5.994711	3	1
Fe I	396,937	396.9257	0.54	22600000	1.484864	4.607593	7	0.93842
Fe II	430.2814	430.3168	0.35	220000	2.704349	5.584768	4	0.60515
Fe I	445.6299	445.6325	0.33	206000	3.046869	5.828295	11	0.58030
Fe II	482.7306	482.6683	0.35	7500000	10.28805	12.85606	12	0.61077
Fe II	526.3629	526.3491	0.26	6,700,000	10.46729	12.82219	4	0.4522
Fe I	526.9418	526.9537	0.26	1270000	0.858996	3.211189	9	0.44919
Fe II	558,787	558.8033	0.25	4900000	10.60007	12.8182	8	0.43583

Fe III	588.9728	588.977	0.22	2720000	25.0486	27.15312	9	0.388745
Fe II	589.5595	589.5339	0.23	610000	10.75078	12.85329	4	0.409247
Fe I	616.4993	616.536	0.39	654000	4.142649	6.153073	9	0.673238
Fe I	617.3248	617.3335	0.35	231000	2.222712	4.23054	1	0.605458
Fe II	644.2685	644.2958	0.23	43000	5.548766	7.472571	8	0.395038
Fe II	646.6371	646.5676	0.20	210000	9.735914	11.65296	8	0.345229
Average Relative Concentration								0.563488535

Table 2: Illustrates the iron spectral lines in the anemic sample E1.

Element	Wavelength Peak	Wavelength NIST	Intensity	Transition Probability	Lower Level Energy	Upper Level Energy	Statistical Weight	Concentration
Fe I	396.8258	396.7961	0.47	6090000	3.240969	6.364718	9	0.788478
Fe III	397.0482	397.0511	0.45	4600000	20.88156	24.00331	3	0.755174
Fe II	430.2814	430.3168	0.15	220000	2.704349	5.584768	4	0.2601456
Fe I	445.5168	445.5027	0.27	4100000	3.881604	6.663841	7	0.455546
Fe II	445.7431	445.7946	0.27	0.318	0.047708	2.828122	8	0.454342
Fe II	482.6161	482.6137	0.21	2600000	10.32891	12.8972	8	0.355586
Fe II	526.2471	526.2313	0.22	1720000	10.54469	12.90011	6	0.38053761
Fe II	526.4786	526.4801	0.24	35000	3.230459	5.584768	4	0.406828
Fe II	526.826	526.84	0.24	4400000	10.49963	12.85233	6	0.402397
Fe I	527.0576	527.0356	0.21	3670000	1.607896	3.959724	3	0.361069
Fe II	554.1238	554.1001	0.25	50000	10.51942	12.75638	8	0.42251272
Fe I	558.6704	558.6756	0.22	2190000	3.368256	5.586893	7	0.375731
Fe I	616.3814	616.3544	0.38	8440	2.197866	4.208883	5	0.635437
Fe II	646.6371	646.5676	0.19	210000	9.735914	11.65296	8	0.332163
Average Relative Concentration								0.456137837

Table 3: Illustrates the iron spectral lines in the anemic sample E2.

Element	Wavelength_Peak	Wavelength_NIST	Intensity	Transition_Probability	Lower_Level_Energy	Upper_Level_Energy	Statistical_Weight	Concentration
Fe I	396.8258	396.7961	0.45908	6090000	3.240969	6.364718	9	0.835831
Fe II	430.2814	430.3168	0.29976	220000	2.704349	5.584768	4	0.545762
Fe I	445.5168	445.5027	0.27456	4100000	3.881604	6.663841	7	0.499882
Fe II	445.7431	445.7946	0.27391	0.318	0.047708	2.828122	8	0.498698
Fe II	482.7306	482.6683	0.22281	7500000	10.28805	12.85606	12	0.405662
Fe II	526.2471	526.2313	0.22809	17200000	10.54469	12.90011	6	0.415275
Fe II	526.4786	526.4801	0.24302	35000	3.230459	5.584768	4	0.442458
Fe II	526.826	526.84	0.24043	4400000	10.49963	12.85233	6	0.437742
Fe I	527.0576	527.0356	0.21684	3670000	1.607896	3.959724	3	0.394793
Fe II	554.1238	554.1001	0.19587	50000	10.51942	12.75638	8	0.356614
Fe I	558.6703	558.6756	0.21878	21900000	3.368256	5.586893	7	0.398325
Fe I	616.4993	616.536	0.38225	654000	4.142649	6.153073	9	0.695949
Fe I	617.3248	617.3335	0.28691	231000	2.222712	4.23054	1	0.522367
Fe II	646.6371	646.5676	0.19418	210000	9.735914	11.65296	8	0.353537

5. Discussion

Tables 1-3 indicate that healthy (H1) samples had less strong iron spectral lines than anemic (E1 and E2). The healthy sample (H1) showed the highest Fe I line spectral intensity (0.58483) at 393.49191 nm. The samples that were anemic (E1 and E2) had much lower Fe I line values at 396.82578 nm (0.47162 and 0.45908, respectively) (see Tables 1-3). Anemic persons have less iron in their blood, which is how iron-deficiency anemia impacts the body.

We found that the healthy sample (H1) had the highest relative concentration of iron (1.0) for the Fe I line when we looked at the relative concentration of iron. On the other hand, the concentration of iron was reduced to 0.788 and 0.836 in the anemic samples (E1 and E2), respectively. Since the concentration of the second anemic sample (E2) was slightly higher than that of the first anemic sample (E1), this drop shows that patients definitely have lower iron levels than healthy people. This may imply that there are differences in the degree of anemia between the two samples.

In the healthy sample (H1), the total iron content was found to be 0.563 on average, but in the anemic samples (E1 and E2), the total iron concentration was determined to be 0.456 and 0.486, respectively. With the second anemic sample (E2) showing a little greater concentration than the first anemic sample (E1), this substantial difference demonstrates that patients have a shortage in iron levels. This variation may be due to variances in the severity of the anemia (see Tables 1-3).

Anemic samples (E1 and E2) have lower iron levels than healthy samples (H1), supporting the clinical diagnosis of iron-deficiency anemia. The first anemic sample (E1) had a larger iron reduction than the second (E2), which may indicate patient severity.

LIBS can distinguish iron levels between samples. Measurements using this method match NIST reference values, improving reliability. The wavelengths' excellent match showed LIBS's elemental analysis accuracy.

Increase the quantity of samples and include zinc and copper, which may be linked to anemia, to boost results. LIBS can also be used with Atomic Absorption Spectroscopy (AAS) for more complete and accurate analysis.

5. Conclusions

Because the anemic samples had a clear decrease in spectral line intensity and average total iron concentration compared to the healthy sample, the results suggest that LIBS can correctly identify blood samples that are low in iron. Because of these results, LIBS shows more promise as a quick and painless way to find out if someone has iron-deficiency anemia. For the purpose of generalizing the findings and improving their accuracy, it is recommended that more research be conducted using larger sample sizes.

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