Sudan University of Science and Technology

College of Graduate Studies

Diagnosis of Sickle Cell Anemia Using Laser Emission Spectroscopy

تشخيص الأنيميا المنجلية بإستخدام مطيافية الإنبعاث بالليزر

A thesis Submitted for Partial Fulfillment for Requirements of Master Degree in Laser Applications in Physics

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الآية

بسم الله الرحمن الرحيم

﴿وَأَن لَّيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَىٰ﴾

صدق الله العظيم

سورة النجم ﴿39﴾

DEDICATION

This work is dedicated to…

The Soul of my father

My mother

My brother

My friends

And all persons who support me

Acknowledgement

My great thanks to (**ALLAH**) who gives me the strength to complete this work.

I would like to express my special appreciation and thanks to my supervisor **Prof. Dr. Nafie A. Almuslet** for encouraging and advice me to grow as a researcher and for his guidance and supervision.

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Abstract

In this research, the laser emission spectroscopy was utilized to diagnose the sickle cell anemia among five samples of blood; one normal and four with sickle cell anemia. The five samples were irradiated by diode laser of 532 nm wavelength and 100 mW output power. The emission spectra of the five samples were recorded and compared.

The results showed that, two peaks in the emission spectrum of normal blood sample were appeared, the first one is attributed to hemoglobin A_2 while the second is due to hemoglobin A. Three peaks in the emission spectrum of sickle cell anemia samples were recorded, these peaks are attributed to hemoglobin A_2, F and S which appeared only in the sickle cell anemia samples. The three peaks are considered as characteristics of the sickle cell anemia.

The wavelength of the emission peak of hemoglobin S was red shifted with the increasing in the total hemoglobin, and the intensity of the peak of hemoglobin F was increased with decreasing the amount of total hemoglobin.

Also, the results showed that, the first peak in the emission spectrum of normal blood sample indicated the haemoglobin A_2 with low intensity, and the second peak is attributed to hemoglobin A with high intensity. The three peaks observed in the emission of sickle cell anemia, indicated the haemoglobin S as the major haemoglobin component with a little proportion of haemoglobin A_2 and a variable proportion of haemoglobin F.

This study concluded that laser emission spectroscopy succeeded in the diagnosis of sickle cell anemia compared with the healthy blood samples.

المستخلص

في هذا البحث تم إستخدام مطيافية الإنبعاث بالليزر لتشخيص الأنيميا المنجلية بإستخدام خمسة عينات دم ، عينة سليمة وأربعة عينات مشخصة على أنها مصابة بالأنيميا المنجلية، شععت العينات بإستخدام ليزر الدايود ذو الطول الموجي 532 نانوميتر وقدرة 100 ملي واط، ومن ثم تم تسجيل طيف الإنبعاث وقورنت النتائج.

قد أظهرت النتائج قمتين في طيف إنبعاث العينة السليمة أحدهما تعود للهموقلوبين A_2 والثانية للهموقلوبين A في حين ظهرت ثلاث قمم في طيف إنبعاث العينات المصابة بالأنيميا تعود للهموقلوبين A_2، F و S الذي ظهر فقط في العينات المصابة بالأنيميا.

وجد أنه كلما زادت القيمة الكلية للهموقلوبين إنزاح الطول الموجي لقمة هموقلوبين S باتجاه الأطوال الموجية الأطول ونقصت شدة القمة لهموقلوبين F.

بينت هذه الدراسة أنه في طيف إنبعاث العينة السليمة فإن القمة التي لها أعلى شدة هي للهموقلوبين A والقمة التي لها أقل شدة تعود للهموقلوبين A_2 .

ووجد أنه في طيف إنبعاث العينات المصابة بالأنيميا فإن القمة التي لها أعلى شدة تعود للهموقلوبين S والقمة التي لها أقل شدة تعود للهموقلوبين A_2 أما القمة الثالثة فأنها للهموقلوبين F.

وبذلك فإن الدراسة تستنتج أن مطيافية الإنبعاث بالليزر تمكنت من تشخيص الأنيميا المنجلية طيفيا مقارنة بعينات الدم السليمة.

Chapter One

Introduction and Basic Concepts

CHAPTER ONE

Introduction and Basic Concepts

1-1 Introduction:

Spectrometric techniques are the largest and most important single group of techniques used in analytical chemistry, and provide a wide range of quantitative and qualitative information. All spectrometric techniques depend on the emission or absorption of electromagnetic radiation characteristic of certain energy changes within an atomic or molecular system, the energy changes are associated with a complex series of discrete or quantized energy levels in which atoms and molecules are considered to exist. To understand how studies of such transitions between energy levels can yield information requires some knowledge of the properties of electromagnetic radiation and of the nature of atomic and molecular energy (Michael Hollas J, 2004).

When an examination is restricted to the identification of one or more constituents of a sample, it is known as qualitative analysis, while an examination to determine how much of a particular species is present constitutes a quantitative analysis, sometimes information concerning the spatial arrangement of atoms in a molecule or crystalline compound is required or confirmation of the presence or position of certain organic functional groups is sought. Such examinations are described as structural analysis, the set of energy levels associated with a particular substance is a unique characteristic of that substance and determines the frequencies at which electromagnetic radiation can be absorbed or emitted, qualitative information regarding the composition and structure of a sample is

obtained through a study of the positions and relative intensities of spectral lines or bands, quantitative analysis is possible because of the direct proportionality between the intensity of a particular line or band and the number of atoms or molecules undergoing the transition (Fifiled F.W, 2000).

1-2 The Study Objectives:

The objectives of this study are:

- Diagnostic of sickle cell anemia using laser emission spectroscopy with green laser of 532 nm wavelength and power of 100 mW.
- Analysis of the results.

1-3 Thesis Structure:

This thesis contains three chapters; chapter one contains an introduction about the subject, aims of work, and some basic concepts. Chapter two describes the experimental part. Chapter three contains the results, discussion, conclusions and recommendations.

1-4 General Picture about Spectroscopy:

Spectroscopy is defined as the physical processes happen inside the matter due to the interaction of electromagnetic radiation with this matter **(**Joseph, 2002).

All the information about the atoms, ions, molecules, complex matter structure can be collected via the study of the absorption or emission or what is called spectroscopy.

1-4-1 Types of spectroscopy:

1-4-1-1 Atomic spectroscopy:

Atomic spectroscopy involves the interaction of light with gaseous atoms. A device converts a sample (usually a solution) into gaseous atoms, based on the breakdown of a sample into atoms by expose sample to flame or high-temperature, followed by the measurement of the atom's absorption or emission of light, there are three basic types of atomic spectroscopy are: **(**Joseph, 2002).

Atomic emission.

Atomic absorption.

Atomic fluorescence.

While the three processes are related, they do offer three unique analytical techniques. In order to introduce these phenomena, we will initially consider an atom with only two electronic energy states, in which the ground (lowest energy) state is designated 0 and the excited state as 1, it can generally be assumed that under normal conditions the majority of atoms are in the ground state.

Atomic emission spectroscopy (AES) involves the transfer of energy, usually as heat, from the atom cell to the atom to promote a valence electron in the atom from the ground state to the excited state. The atom then may emit a photon, and deactivate to the ground state (emission). The energy of the photon is equal to the difference in energy between the states. This process is called an electronic transition, the setup is arranged in figure (1.1) **(**Joseph, 2002).

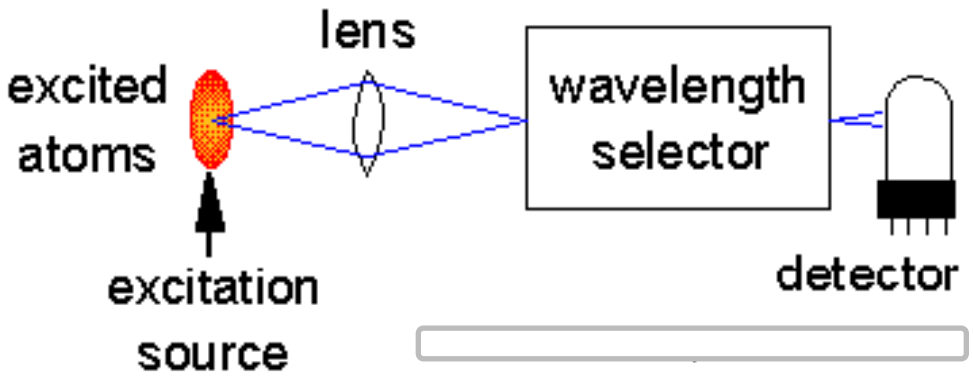

Figure 1.1: AES experiment setup

Atomic Absorption Spectroscopy (AAS) is an analytical technique used for the qualitative and quantitative determination of the elements present in different samples, this is done by reading the spectra produced when the sample is excited by radiation, the atoms absorb ultraviolet or visible light and make transitions to higher energy levels. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample. A detector measures the wavelengths of light transmitted by the sample, and compares them to the wavelengths which originally passed through the sample, a signal processor then integrates the changes in wavelength absorbed, which appear in the readout as peaks of energy absorption at discrete wavelengths. The energy required for an electron to leave an atom is known as ionization energy and is specific to each chemical element. The substances in a solution are suctioned into an excited phase where they undergo vaporization, and are broken down into small fragmented atoms by discharge, flame or plasma. When an electron moves from one energy level to another within the atom, a photon is emitted with energy E, atoms of an element emit a characteristic spectral line. Every atom has its own distinct pattern of wavelengths at which it will absorb energy, due to the unique configuration of electrons in its

outer shell. This enables the qualitative analysis of a sample, the concentration is calculated based on the Beer-Lambert law. Absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions (García R and Báez A.P, 2012).

Atomic fluorescence (AF) is a spectroscopic process which is based on absorption of radiation of specific wavelengths by an atomic vapour with subsequent detection of radiationally deactivated states via emission in a direction (typically) orthogonal to the excitation source. It involves the excitation of atoms from a lower energy state (usually the ground state) to a higher energy state by light, followed by the emission (fluorescence) of a photon to deactivate the atoms. Atomic spectra are characterized by their relative simplicity, typically consisting of narrow lines, which correspond to the limited number of possible energy levels. Each element has a unique set of energy levels and hence a unique spectrum. In most AFS systems wavelength selection is achieved using a filter located between the source and the detector. A number of excitation sources have been used in AFS, primarily spectral line sources and continuous sources. Since the intensity of the fluorescence radiation is proportional to the exciting radiation, excitation sources with a high intensity are required in order to achieve good sensitivity and wide linear dynamic range (Sanchez-Rodas D at. al, 2010).

1-4-1-2 Molecular spectroscopy:

Molecular spectroscopy is a means of probing molecules and most often involves the absorption of electromagnetic radiation. The absorbed electromagnetic radiation results in transitions between eigenstates of a molecule. The type of eigenstates involved in a transition depends on the energy of the radiation absorbed. Absorbed ultraviolet and visible

radiation generally results in transitions amongst electronic eigenstates. Figure (1.2) shows the experimental setup of molecular emission spectroscopy (Michael Mueller, 2002).

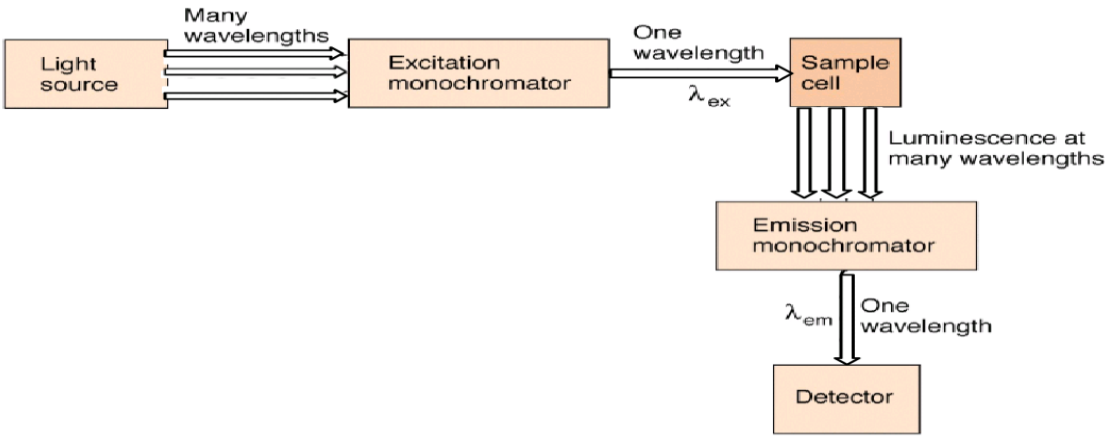

Figure 1.2: Molecular emission spectroscopy experiment setup.

Absorbed infrared radiation results in changes in vibrational and rotational eigenstates. Absorbed microwave radiation results in changes in rotational eigenstates. The specific wavelengths of radiation that are absorbed in each region of the electromagnetic spectrum depend on the energy difference between the eigenstates of a molecule. As an example, a diatomic molecule with a "stiff" bond will absorb at a higher energy photon (shorter wavelength) than another diatomic molecule with a less "stiff" bond. The absorbed radiation in a spectrum provides information on the energy differences amongst various eigenstates of a molecule; however, it does not provide any information on the actual eigenstates involved in the transitions. Figure (1.3) shows the experimental setup of absorption spectroscopy (Michael Mueller, 2002).

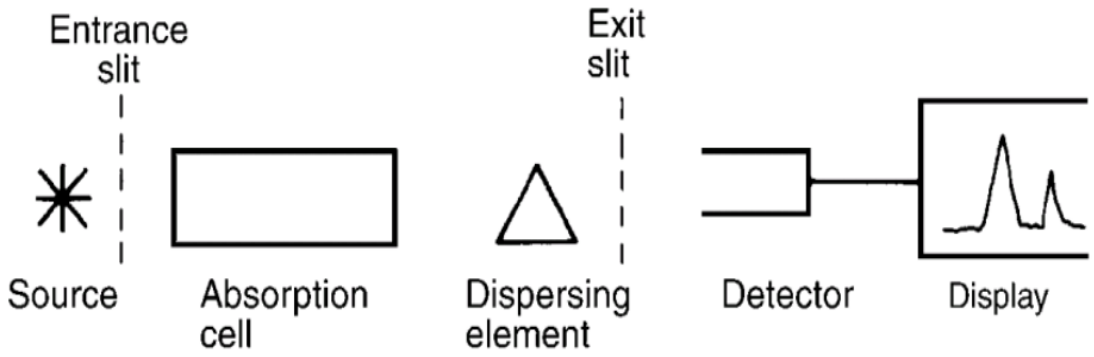

Figure 1.3: The components of a typical absorption experiment.

1-5 Laser Spectroscopy:

The increasing availability of intense, monochromatic laser sources provided a tremendous impetus to a wide range of spectroscopic investigations. Laser radiation is very much more intense, and the linewidth much smaller, than conventional source. As a result, weaker transitions can now be observed and higher resolution is obtainable (Michael Hollas J, 2004).

Laser Spectroscopy continues to develop and expand rapidly. There are firstly, the improvement of frequency-doubling techniques in external cavities, the realization of more reliable CW-parametric oscillators with large output power, and the development of tunable narrow-band UV source, which have expanded the possible applications of coherent light source in molecular spectroscopy. Furthermore, new sensitive detection techniques for the analysis of small molecular concentrations or for measurement of weak transitions, in addition time resolved spectroscopy that focus on the time evolution of emission, absorption, or scattering process to obtain information about the dynamics of chemical, physical, or

biological systems which use high resolution spectroscopic method. The most impressive progress has been achieved in the development of tunable femtosecond and subfemtosecond lasers, the development of ultra-short, powerful laser pulses has opened up an exciting area of potential applications.

The largest expansion of laser spectroscopy can be seen in its possible and already realized applications to chemical and biological problems and its use in medicine as a diagnostic tool and for therapy (Halina Abramczyk, 2005).

1-5-1 Laser in absorption spectroscopy:

One can compare between laser absorption spectroscopy and conventional absorption spectroscopy which uses incoherent radiation source:

In classical absorption spectroscopy, radiation sources with a broad emission continuum are preferred (e.g. high-pressure Hg arcs, Xe flash lamps, etc.). The radiation is collimated by the lens L1 and passes through the absorption cell. Behind a dispersing instrument for wavelength selection.

The spectral resolution is generally limited by the resolving power of the dispersing spectrometer.

The detection sensitivity of the experimental arrangement is defined by the minimum absorbed power that can still be detected. In most cases it is limited by the detector noise and by intensity fluctuations of the radiation source.

In laser absorption no monochromator is needed, since the absorption coefficient and its frequency dependence can be determined directly from the difference between the intensities of the reference beam and

transmitted beam. The spectral resolution is higher than in conventional spectroscopy.

The laser frequency may be stabilized onto the center of an absorption line. So it is possible to measure the wavelength of the laser with an absolute accuracy of 10^{-8} or better. This allows determination of the molecular absorption lines with the same accuracy.

It is possible to tune the laser wavelength very rapidly over a spectral region where molecular absorption lines have to be detected.

Figure (1.4) presents schematic diagrams for both methods (Demtröder W, 2003).

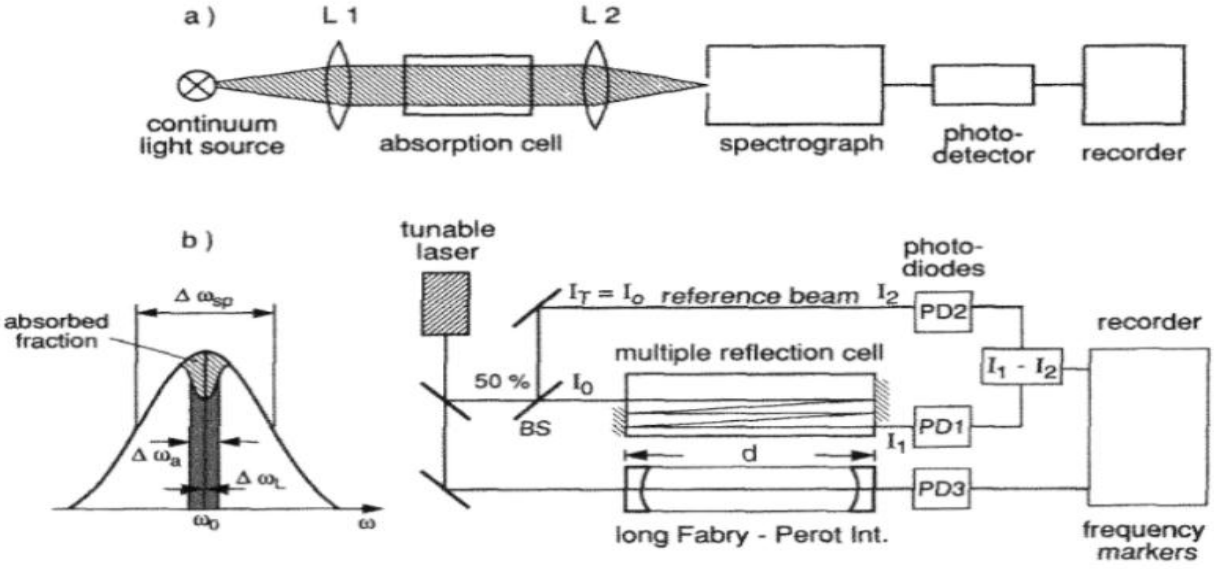

Figure 1.4: Comparison between absorption spectroscopy with a broadband incoherent source (a) and with a tunable single-mode laser(b).

The advantages of absorption and emission spectroscopy with laser are:

Because of the high spectral power density of many lasers, the detector noise is generally negligible.

The detection sensitivity increases with increasing spectral resolution.

Because of good collimation of the laser beam, long absorption paths can be realized by multiple reflection back and forth through the multiple-path absorption cell.

An important advantage of absorption spectroscopy with tunable single-mode lasers stems from their capabilities to measure line profiles of absorbing molecular transitions with high accuracy. In case of pressure broadening, the determination of line profiles allows one to drive information about the interaction potential of the collision partners.

In fluorescence spectroscopy and optical pumping experiments, the high intensity of lasers allows an appreciable population in selectively excited states to be achieved that may be comparable to that of the absorbing ground states.

The small laser linewidth favors the selectivity of optical excitation and results in favorable cases in exclusive population of form absorption and fluorescence spectroscopy of excited states and to transform spectroscopic methods, such as microwave or RF spectroscopy, which has until now been restricted to electronic ground states, also to excited states (Demtröder W, 2003).

1-5-2 Laser in emission spectroscopy:

Laser in emission spectroscopy include Laser-induced fluorescence spectroscopy (LIF), Laser induced breakdown spectroscopy (LIBS)

1-5-2-1 Laser-Induced Fluorescence:

Laser-induced fluorescence (LIF) spectroscopy is a versatile technique in which molecular species are irradiated with laser radiation in a specific wavelength range (in the visible and ultraviolet regions applications with a very high sensitivity can be achieved) that is in resonance with the differences in molecular energy levels. Such resonantly tuned radiation

has a fairly good probability of inducing a transition to excited state of the molecule, which may be followed by relaxation of the molecules to the ground electronic state by spontaneous emission of a photon whose energy corresponds to the separation in molecular energy levels. It is well suited to gain information on molecular states if the fluorescence spectrum excited by laser on a selected absorption transition is dispersed by monochromator. Another aspect of LIF concerns its application to the determination of the internal-state distribution in molecular reaction products of chemical reactions. Under certain conditions the intensity of LIF is direct measure of the population density in the absorbing level (Misra. P, 2005).

Figure (1.5) shows level scheme and experimental arrangement for laser fluorescence spectroscopy.

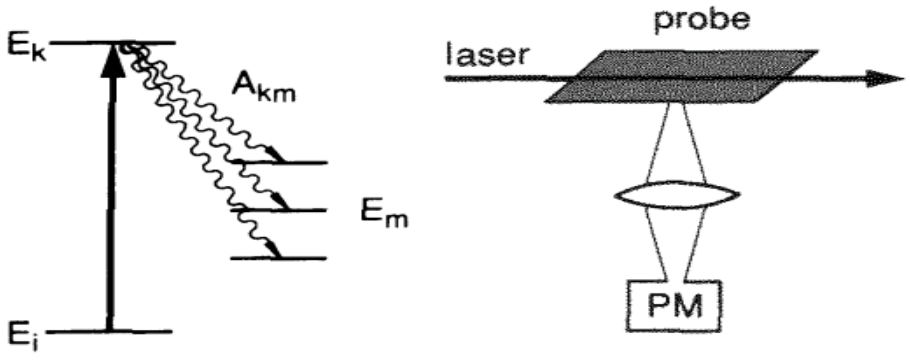

Figure 1.5: Level scheme and experimental arrangement for laser fluorescence spectroscopy.

1-5-2-2 Laser induced breakdown spectroscopy:

Laser induced breakdown spectroscopy (LIBS) is basically an emission spectroscopy technique where atoms and ions are primarily formed in their excited states as a result of interaction between a tightly focused laser beam and the material sample. The interaction between matter and high-

density photons generates a plasma plume, which evolves with time and may eventually acquire thermodynamic equilibrium. One of the important features of this technique is that it does not require any sample preparation, unlike conventional spectroscopic analytical techniques. Samples in the form of solids, liquids, gels, gases, plasma and biological materials (like teeth, leaf or blood) can be studied with almost equal ease. LIBS has rapidly developed into a major analytical technology with the capability of detecting all chemical elements in a sample, of real time response, and of close-contact or stand-off analysis of targets. Figure (1.6) shows the experimental setup of Laser induced breakdown spectroscopy (Jagdish singh, 2007).

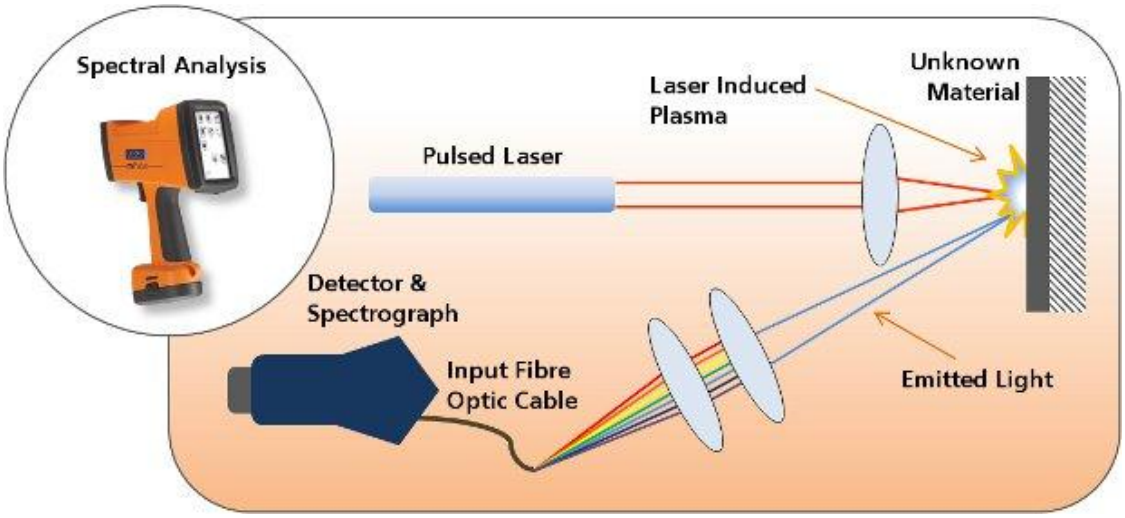

Figure 1.6: Laser induced breakdown spectroscopy setup.

1-5-3 Laser Raman spectroscopy:

This type of spectroscopy based on the scattering of the laser light after its interaction with the matter. The incident radiation should be highly monochromatic for the Raman effect to be observed clearly because Raman scattering is so weak, it should be very intense. The method used

is to pass the monochromatic radiation through the sample and detect the scattered radiation. Usually, this radiation is collected in directions normal to the incident radiation in order to avoid this incident radiation passing to the detector. Lasers are sources of intense, monochromatic radiation which are ideal for Raman spectroscopy and have entirely replaced atomic emission sources, they are more convenient to use, have higher intensity and are more highly monochromatic. For example, the linewidth at half-intensity of 632.8 nm (red) radiation from a helium–neon laser can be less than 0.05 cm^{-1}. Figure (1.7) shows a typical experimental arrangement for obtaining the Raman spectrum of a gaseous sample. Radiation from the laser source is focused by the lens L1 into a cell containing the sample gas. The mirror M1 reflects this radiation back into the cell to increase the amount of Raman scattering by the sample. Lens L2 collects the Raman scattering reaching it directly, and also that reflected by mirror M2; then directs and focuses it into a spectrometer where it is dispersed and detected (Michael Hollas J, 2004).

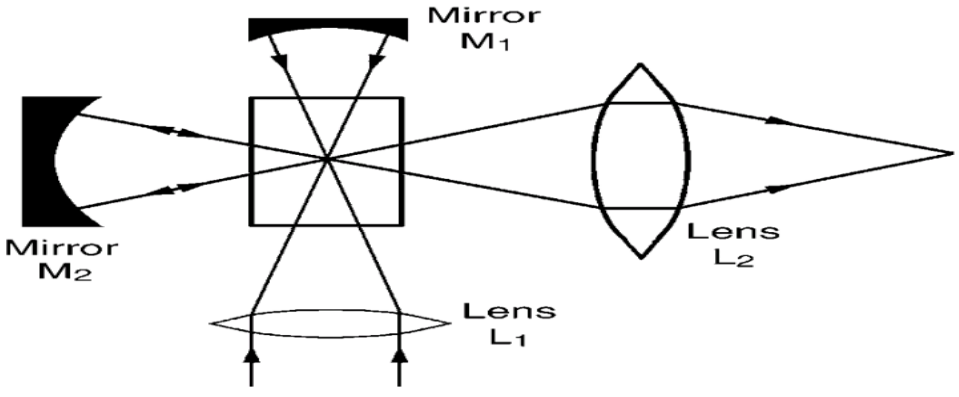

Figure 1.7: Experimental arrangement for gas phase Raman spectroscopy.

1-5-4 Applications of Laser Spectroscopy:

The relevance of laser spectroscopy for numerous applications in physics, chemistry, biology, and medicine, or to environmental studies and technical problems has rapidly gained enormous significance.

Applications in chemistry are:

In analytical chemistry, the first aspect of laser applications in analytical chemistry is the sensitive detection of small concentrations of impurity atoms or molecules. Another very sensitive detection is based on resonant two- or three-photon ionization of atoms and molecules in the gas phase.

Single molecule detection.

Laser-induced chemical reactions, the reaction is initiated by one- or multiphoton excitation of one or more of the reactants.

Laser femtosecond chemistry.

Isotope separation with lasers.

Environmental research with lasers include:

Absorption measurements.

Atmospheric measurements with LIDAR.

Spectroscopic detection of water pollution.

Applications in biology are:

Energy transfer in DNA complexes.

Laser microscope.

Medical Applications of laser spectroscopy include:

Most of these applications rely on high laser-output power, which can be focused into a small volume. The strong dependence of the absorption coefficient of living tissue on the wavelength allows selection of the penetration depth of laser beam by choosing the proper laser wavelength. For example, skin carcinoma or portwine marks should be treated at

wavelengths for a small penetration depth in order to protect the deeper layers of the epidermia from being damaged, while cutting of bones with lasers or treatment of subcutancous cancer must be performed at wavelengths with greater penetration depth. The most spectacular outcomes of laser applications in medicine have been achieved in laser surgery, dermatology, ophthalmology, and dentistry, there are, however, also very promising direct applications of laser spectroscopy for the solution of problems in medicine, they are based on new diagnostic techniques (Demtröder and Wolfgang, 2008).

1-6 Laser Spectroscopy in Medical Diagnostic:

Any discussion of applications of lasers in medicine must start with a thorough consideration of the absorption properties of the main constituents of tissue. Water has a very strong absorption at short UV wavelengths and again at wavelengths longer than 1.4 μ m, with particularly strong absorption peaks at 1.9 and 3.0 μ m, Proteins strongly absorb through the UV region below about 400 nm, while the melanin pigment in the skin has a broad absorption from the UV with successively weaker attenuation through the visible region. Of particular importance
is the chromophore hemoglobin, whose red color derives from the fact that it massively absorbs below 600 nm but is largely transparent to red color (wavelengths longer than 600 nm). The net effect of these major chromophores results in a region above 600 nm and below 1.4 μ m where the human body is relatively transparent, meaning that optical radiation can penetrate distances ranging from a millimeter to several centimeters. Depending on the exact wavelength, the spectral shapes are useful in identifying different substances in the tissue, apart from species identification, quantification of the constituents is of considerable interest.

Basically, the emission intensity is proportional to the number of molecules participating in the process, for absorption, the observed attenuation is related to the concentration through the Beer-Lambertian law, Light can emerge from tissue at wavelengths different from the incident one due to several spectroscopic interactions. The most important ones are fluorescence, laser- induced breakdown emission and Raman scattering (Helena Jelínková, 2013).

In diagnostics, lasers are advantageous if conventional incoherent light sources fail. One major diagnostic tool is confocal laser microscopy which allows the detection of early stages of retinal alterations. By this means, retinal detachment and also glaucoma1 can be recognized in time to increase the probability of successful treatment (Markolf H. Niemz, 2003).

1-7 Blood:

Blood flows through every organ of the body, providing effective communication between tissues. It is kept in continuous circulation by the pumping action of the heart, flowing through arteries which carry the oxygenated (bright red) blood from the heart to all parts of the body, and veins which carry the deoxygenated (dark red) blood from the different parts of the body back to the heart and to the lungs. The arteries divide into smaller vessels called capillaries forming the capillary, or peripheral, circulation which supplies oxygen to the tissues. The capillaries rejoin to form the veins, the same amount of blood that is pumped out of the heart returns to it. It carries oxygen and nutrients to the tissues and waste products to the lungs, liver and kidneys, where they can be removed from the body.

●Nutrients absorbed from the digestive tract, e.g. monosaccharides (especially glucose), amino acids, fatty acids, glycerol, and vitamins, are transported to the cells of the body for use or storage.

● Buffer systems in the plasma maintain the pH of the blood between pH 7.35–7.45 and the pH in body tissues within the physiological limits required for normal cellular activity.

● Proteins (particularly albumin) and salts (particularly sodium chloride) regulate plasma osmotic pressure, preventing excessive loss of fluid from the blood into tissues spaces (Monica Cheesbrough, 2006).

1-8 Hemoglobin:

The heme and globin portions of the hemoglobin molecule are linked together by chemical bonds.

• Heme portion, this structure involves four iron atoms in the ferrous state (Fe^{2+} because iron in the ferric state, Fe^{3+}, cannot bind oxygen) surrounded by protoporphyrin IX, or the porphyrin ring, a structure formed in the nucleated red cells. Protoporphyrin IX is the final product in the synthesis of the heme molecule. Defects in any of the intermediate products can impair hemoglobin function.

• Globin portion, these consist of amino acids linked together to form a polypeptide chain, a bracelet of amino acids.

• An additional structure that supports the hemoglobin molecule is 2,3 diphosphoglycerate (2,3-DPG), a substance produced via the Embden-Meyerhof pathway during anaerobic glycolysis.1.

Each heme molecule consists of four heme structures with iron at the center and two pairs of globin chains as shown in figure (1.8). The heme structure sits lodged in the pocket of the globin chains (Betty Ciesla, 2007).

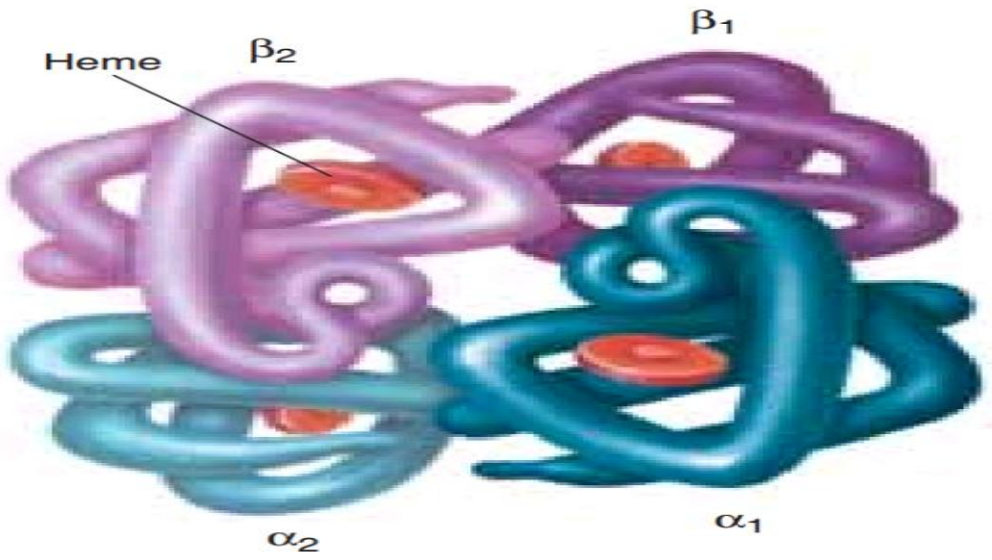

Figure 1.8: The hemoglobin molecules: note four heme molecules tucked inside globin chains.

1-8-1 Types of hemoglobin:

There are three types of hemoglobin that are synthesized: embryonic hemoglobins, fetal hemoglobin (HbF), and the adult hemoglobins (HbA). Each of these types of hemoglobins has a specific arrangement of globin chains and each globin chain is under the influence of a specific chromosome.

Amino acids are an essential component of each of the globin chains. The unique position of amino acids in each chain, as well as the specificity of the amino acid itself, is essential to the normal function of the hemoglobin molecule. Synthetic or structural abnormalities of the protein chains may lead to hemoglobin defects (Betty Ciesla, 2007).

Each molecule of haemoglobin contains four linked polypeptide (globin) chains which in an adult consist of two alpha (α) chains containing 141 amino acids and two beta (β) chains containing 146 amino acids.

96–98% of normal adult haemoglobin is HbA, having 2 alpha chains and 2 beta chains (α α /ββ). Up to 3.5% is HbA2 consisting of 2 alpha chains and 2 delta chains (α α /δδ). Less than 1% is HbF (fetal) composed of 2 alpha chains and 2 gamma chains (α α /γγ). HbF is the predominant haemoglobin in a fetus and for the first 3–6 months of life. Each polypeptide chain is combined with an iron containing porphyrin pigment called haem which is the oxygen carrying part of the haemoglobin molecule (Monica Cheesbrough, 2006).

1-9 Anemia:

Broadly defined, when red cells are no longer able to supply oxygen to the body's tissues, the individual becomes anemic (Betty Ciesla, 2007).

1-9-1 Variations in Red Cell Shape:

1-9-1-1 Sickle Cells:

Sickle cells are a highly recognizable red cell morphology, with their crescent shape and pointed projections at one of the terminal ends of the red cells. Sickle hemoglobin, hemoglobin S, is an abnormal hemoglobin. When red cells containing hemoglobin S try to maneuver through the spleen and the kidney, the hemoglobin lines up in stiff bundles. This makes the red cell less elastic and unable to squeeze through the microcirculation of the spleen. The cell deforms, takes the sickle shape, and is permanently harmed (Betty Ciesla, 2007).

Individuals with sickle cell anaemia have haemoglobin S as the major haemoglobin component with a small proportion of haemoglobin A2 and a variable proportion of haemoglobin F. As there is no synthesis of normal β chain, there is a total absence of haemoglobin A. Red cells sickle due to polymerization of haemoglobin S under conditions of low oxygen tension,

This process is cyclical, but eventually membrane damage leads to the red cell becoming irreversibly sickled. The irreversibly sickled cell has an increased calcium content, which triggers calcium dependent potassium transport and loss of potassium and water. Potassium/chloride (K^+/Cl^-) cotransport is also increased. The dehydrated cell becomes even more rigid.

The clinicopathological features of sickle cell anaemia result directly or indirectly from vascular obstruction by sickled red cells, with consequent tissue infarction. In addition to the shape change, erythrocytes show increased adhesion to endothelium, which contributes to vascular occlusion. As the synthesis of haemoglobin F decreases and that of haemoglobin S increases. Figure (1.9) shows sickle cells anemia (Barbara J, 2006).

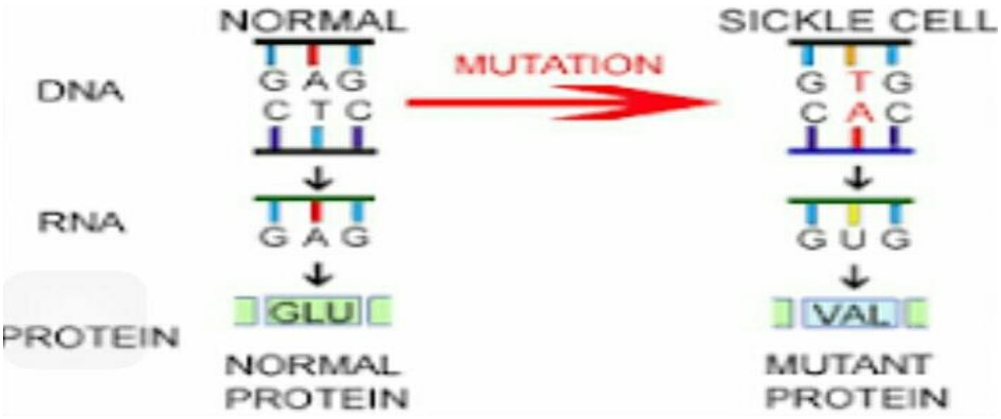

Figure 1.9 : Sickle cells anemia.

1-10 Literature Review:

In June 2008 Thomas H et. al. studied full blood count and haemozoin-containing leukocytes in children with malaria. Compared to standard

light microscopy of Giemsa-stained thick films, diagnosis by platelet count only, by malaria pigment-containing monocytes (PCM) only, or by pigment-containing granulocytes (PCN) only yielded sensitivities/specificities of 92%/93%; 96%/96%; and 85%/96%, respectively. The platelet count was significantly lower in children with malaria compared to those without ($p < 0.001$), and values showed little overlap between groups. Compared to microscopy, scatter flow cytometry as applied in the Cell-Dyn 3000 instrument detected significantly more patients with PCL ($p < 0.01$). Both PCM and PCN numbers were higher in severe versus non-severe malaria yet reached statistical significance only for PCN ($p < 0.0001$; PCM: $p = 0.14$). Of note was the presence of another, so far ill-defined pigment-containing group of phagocytic cells, identified by laser-flow cytometry as lymphocyte-like gated events, and predominantly found in children with malaria-associated anaemia. It was found that in the age group examined in the Lambaréné area, platelets are an excellent adjuvant tool to diagnose malaria. Pigment-containing leukocytes (PCL) are more readily detected by automated laser flow cytometry than by microscopy (Hänscheid T et. al, 2008).

In March 2007 Bayden R.Wood et. al. studied the resonance Raman spectroscopy of red blood cells using near-infrared laser excitation. Resonance Raman spectra of oxygenated and deoxygenated functional erythrocytes recorded using 785 nm laser excitation are presented. The high-quality spectra show amixture of enhanced A_{1g}, A_{2g}, B_{1g}, B_{2g}, E_u and viny1 modes. The high sensitivity of the Raman system enhanced spectra from four oxygenation and deoxygenation cycles to be recorded with only 18 mW of power at the sample over 60-minute period. The large

database consisting of 210 spectra from the four cycles was analyzed. The PC1 loadings plot provided with the oxygenated and deoxygenated states. The enhancement of a band at 567 Cm^{-1}, observed in the spectra of oxygenated cells and the corresponding PC1 loading plot, was assigned to the Fe-O_2 stretching mode, while a band appearing at 419 Cm^{-1} was assigned to the Fe-O-O bending mode. For deoxygenated cells, the enhancement of B_{1g} modes at 785 nm. In the case of oxygenated cells, the enhancement of iron-axial out-of-plane modes and non-totally symmetric modes is consistent with enhancement into the y, z polarized transition. This study provides new insights into the vibrational dynamics, electronic structure and resonant enhancement of heme moieties within functional erythrocytes at near-IR excitation wavelengths (Wood BR et. al, 2007).

Chapter Two

The Experimental Part

CHAPTER TWO

The Experimental Part

2-1 Introduction:

In this work, sickle cell anemia was diagnosed using laser emission spectroscopy with green laser of 532 nm and power of 100 m W. This chapter presents the materials, equipments, tools and setup that were used in this work, followed by the experimental procedure.

2-2 The Setup:

The experimental setup that was used in this work consists of green laser, sample cell, USB spectrometer and PC. Figure (2.1) shows a schematic diagram of the setup arrangement.

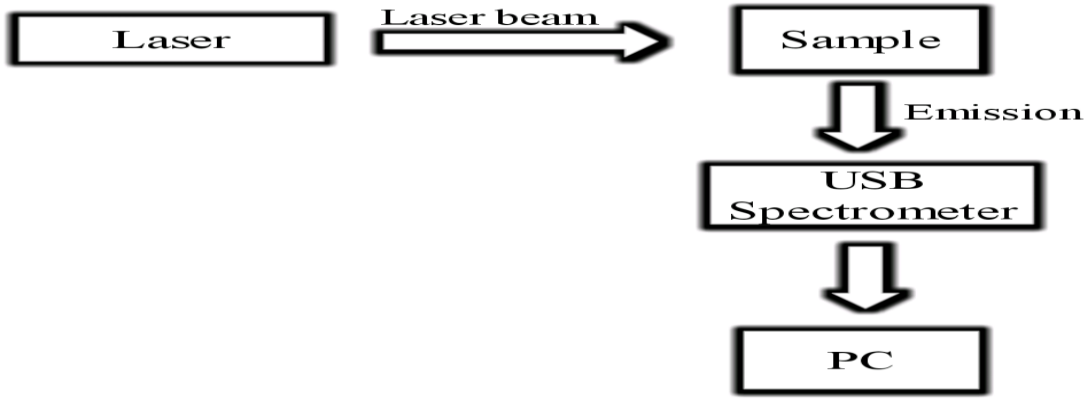

Figure 2.1: Laser emission Spectroscopy setup used in this work.

The sample was excited by laser. After the excitation of the molecules, they relaxed to lower energy levels, the emitted radiation corresponding to the energy differences, $\Delta E = h\nu = h\, c/\lambda$, between the various energy

levels of the sample. After that, the emitted radiation is dispersed and detected by the USB Spectrometer then entered the PC for analysis.

2.3 The Diode Laser:

Diode laser (continuous wave) with wavelength of 532 nm, and output power of 100 mW was used to excite the blood samples. Figure (2.2) shows a photo of this laser.

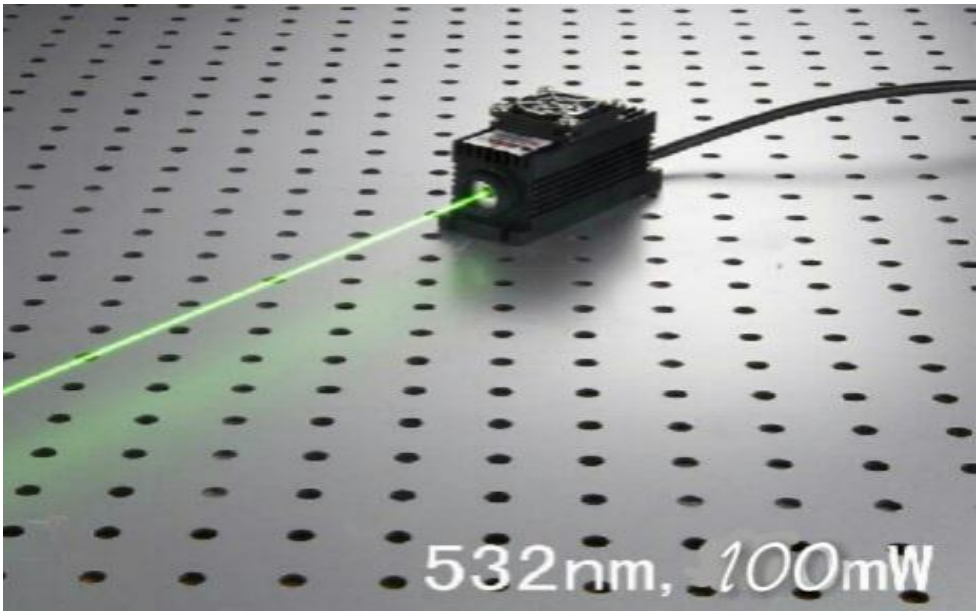

Figure 2.2: The diode laser (532 nm, 100 mW).

2-4 The Spectrometer:

A spectrometer model USB4000 manifactured by Ocean optics- USA was used in this work.

Data programmed into a memory chip on USB4000. The spectrometer operating software simply reads these values from the spectrometer, a feature that enables hot swapping of spectrometers among computers.

The USB4000 Spectrometer was connected to a computer via the USB port or serial port. The USB4000, can be controlled by Ocean View

software, a Java-based spectroscopy software platform that operates on Windows, Macintosh and Linux operating systems.

Figure (2.3) shows a photo of Ocean Optics USB4000 Fiber Optic Spectrometer.

Figure 2.3: Ocean Optics USB4000 Fiber Optic Spectrometer.

2-5 Materials:

The materials used in this work were

2-5-1 Normal blood:

Normal blood sample (with value of total hemoglobin = 11.8 g/dL was recorded by the Sysmex devise in the laboratory) was used as standard sample.

2-5-2 Sickle cell anemia:

Patients with sickle cell anemia are usually diagnosed through neonatal screening programs or between 6 months and 2 years of age. Prior to this time, red cells are protected from sickling with high levels of hemoglobin

F, because the switch from the production of hemoglobin F to hemoglobin A occurs between 3 and 6 months of age.

In Hb S, valine is substituted for glutamic acid in the 6th amino acid of the β chain. When in the deoxy form, Hb S forms polymers that damage the RBC membrane. Distorted, inflexible RBCs adhere to vascular endothelium and plug small arterioles and capillaries, which leads to infarction. Hb F cannot participate in polymer formation and its presence retards sickling. (Betty Ciesla, 2007).

In this work four samples of sickle cell anemia (with value of total hemoglobin = 11.8 g/dL was recorded by the Sysmex devise in the laboratory) were investigated.

2-6 Procedure:

The experimental procedure was done as follows:

The sample of normal blood was excited by green laser with wavelength 532 nm and power 100 m W, and the USB4000 spectrometer was used to record the emission from the sample after excitation.

The OOIBase32 operating software of the USB4000 spectrometer was operated to perform the emission of normal sample as standard emission spectrum.

The emission spectra of the four anemia's samples were recorded and compared with the spectrum of the normal sample, and from the comparison major differences were indicated.

These difference represent the finger print of the sickle cell anemia which indicated the diagnosis of this disease.

Chapter Three

Results and Discussion

CHAPTER THREE

Results and Discussion

3-1 Introduction:

In this chapter the results obtained from the experimental work are presented, in addition to the discussion of these results. Emission of the sickle cell anemia blood was considered as a diagnostic of this disease. Conclusions and recommendations are presented in the end of this chapter.

3-2 Emission Spectrum of the Normal Blood Sample:

Figure (3.1) shows the emission intensity versue wavelength for normal blood sample excited by green laser with wavelength (532nm) and with power of 100mW. It can be observed from this figure that two peaks are recorded in the visible and NIR region. The peak with higher intensity (λ = 762.57 nm, I = 27.16) represents the hemoglobin A because 96–98% of normal adult haemoglobin is HbA, and the peak with lower intensity (λ = 685.13 nm, I = 22.64) indicates the hemoglobin A_2 for the reason of up to 3.5% of normal haemoglobin is HbA2.

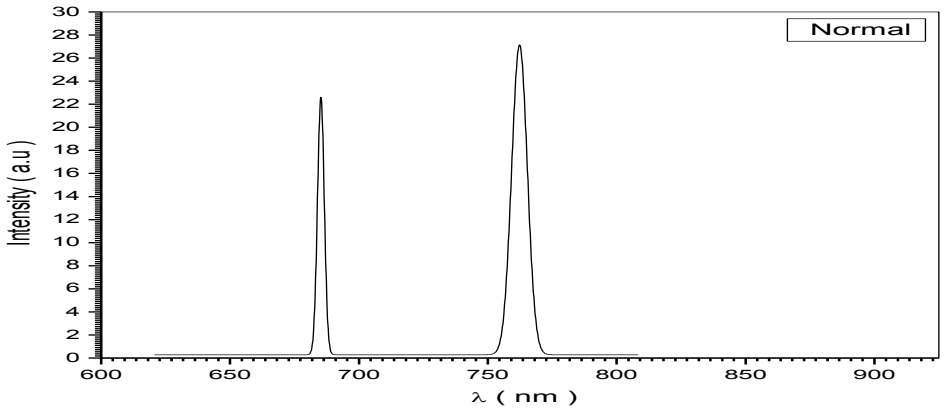

Figure 3.1: The emission spectrum of normal blood sample.

3-3 Emission Spectra of Sickle Cell Anemia:

1) Sample one:

Figure (3.2) displays the emission spectrum of sample one of Sickle Cell anemia excited by green laser with wavelength (532nm) and with power of 100mW. Table (3.1) lists the details of the analysis of this spectrum. Three peaks can be observed in figure (3.2) and table (3.1). The peak with higher intensity (wavelength = 639.29 nm with intensity = 36.00) signifies the hemoglobin S because hemoglobin S represent the largest amount in the total hemoglobin of sickle cell anemia. The peak with lower intensity (wavelength = 831.58 nm, and intensity = 30.10) denotes the hemoglobin A_2, likewise the 3rd peak (wavelength = 726.76 nm, with intensity = 35.47) characterizes the hemoglobin F because individuals with sickle cell anemia have a little amount of haemoglobin A_2 and a variable proportion of haemoglobin F.

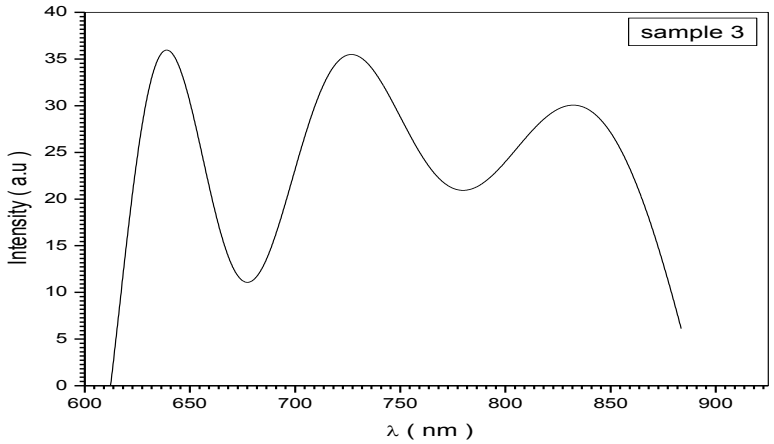

Figure 3.2: The emission spectrum of sample one of Sickle Cell anemia.

Table 3.1: Analysis of the emission spectrum of sample one.

Wavelength (nm)	**Intensity (a.u)**
639.29	36.00
726.76	35.47
831.58	30.10

2) Sample two:

Figure (3.3) denotes the emission spectrum of sample two of Sickle Cell anemia excited by green laser with wavelength (532nm) and with power of 100 m W. Table (3.2) illustrates the analysis of this emission spectrum. Three peaks can be observed in figure (3-3) and table (3-2). The peak with higher intensity (wavelength = 672.47 nm and intensity = 29.71) indicates the hemoglobin S because hemoglobin S has a largest amount in the total hemoglobin of sickle cell anemia. The peak with lower intensity (wavelength = 797.22 nm, and with intensity = 16.08) represents the

hemoglobin A_2, while the 3rd peak (wavelength = 867.39 nm, with intensity = 27.58) represents the hemoglobin F for the reason of less than 1% of normal adult haemoglobin is HbF, because individual with sickle cell anemia has a small proportion of haemoglobin A_2 and a variable proportion of haemoglobin F.

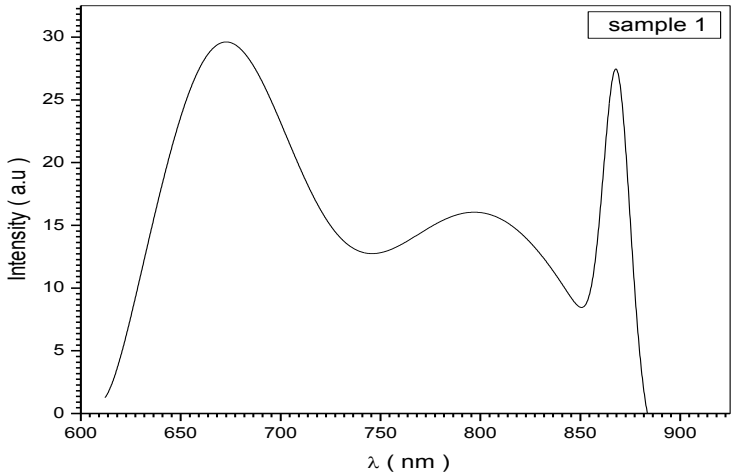

Figure 3.3 : The emission spectrum of sample two of Sickle Cell anemia.

Table 3.2: Analysis of the emission spectrum of sample two.

Wavelength (nm)	**Intensity (a.u)**
672.47	29.71
797.22	16.08
867.39	27.58

3) Sample three:

Figure (3.4) represents the emission spectrum of sample three of Sickle Cell anemia excited by green laser with wavelength (532nm) and power of

100 m W. The analysis of this emission spectrum is illustrated in Table (3.3). Three peaks can be observed in figure (3.4). The peak with higher intensity has wavelength = 806.87 nm and intensity = 40.94 denotes the hemoglobin S for the reason that hemoglobin S has a largest amount in the total hemoglobin of sickle cell anemia. Individuals with sickle cell anemia have a little amount of haemoglobin A_2 and a variable proportion of haemoglobin F. For this reason the peak with lower intensity (wavelength = 751.99 nm, with intensity = 16.89) defines the hemoglobin A_2, Besides the 3rd peak (wavelength = 614.78 nm, and intensity = 20.28) represents the hemoglobin F.

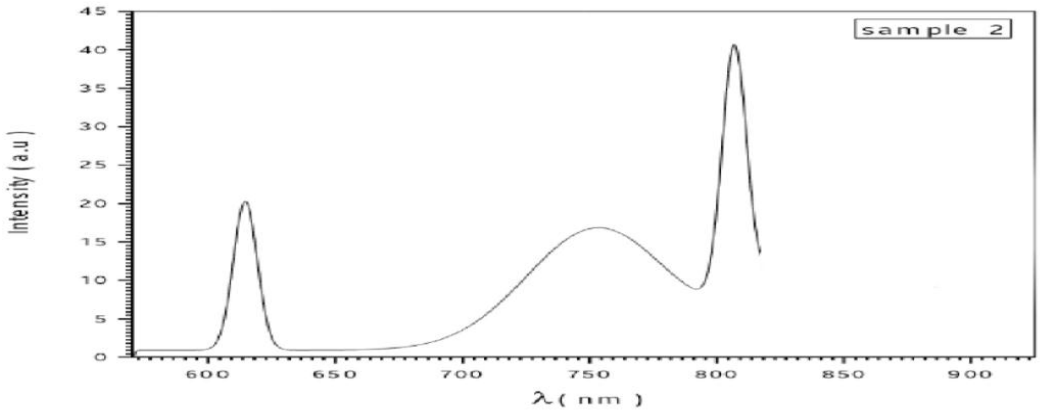

Figure 3.4: The emission spectrum of sample three of Sickle Cell anemia.

Table 3.3: Analysis of the emission spectrum of sample three.

Wavelength (nm)	**Intensity (a.u)**
614.78	20.28
751.99	16.89
806.87	40.94

4) Sample four

Figure (3.5) demonstrates the emission spectrum of sample four of Sickle Cell anemia excited by green laser with wavelength (532nm) and with power of 100mW. Table (3.4) illustrates the analysis of this emission spectrum. Three peaks can be observed in figure (3.5) and table (3.4). The peak with higher intensity (wavelength = 803.35 nm and with intensity = 32.5) indicates the hemoglobin S because hemoglobin S has a largest amount in the total hemoglobin of sickle cell anemia and a small proportion of haemoglobin A_2 and a variable proportion of haemoglobin F for this reason the peak with lower intensity (wavelength = 754.54 nm, and with intensity = 18.95) represents the hemoglobin A_2, whereas the 3rd peak (wavelength = 847.44 nm, and intensity = 19.26) defines the hemoglobin F.

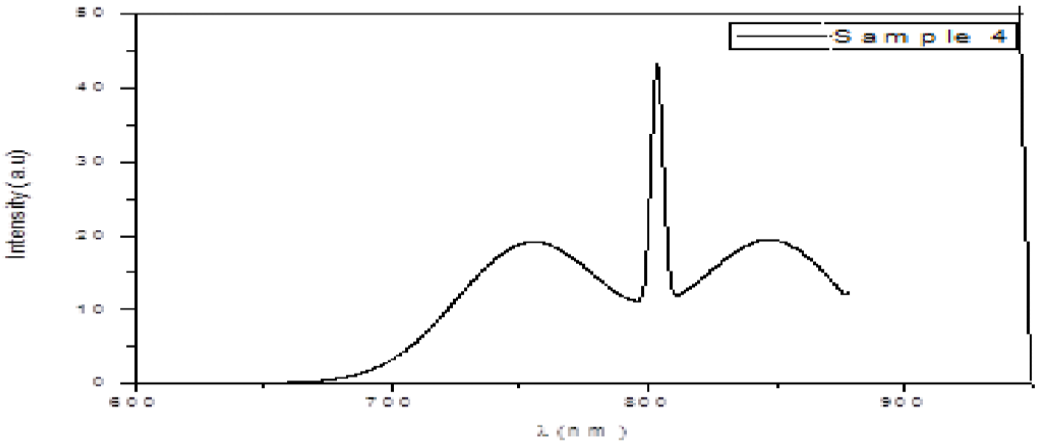

Figure 3.5: The emission spectrum of sample four of Sickle Cell anemia.

Table 3.4: Analysis of the emission spectrum of sample four.

Wavelength (nm)	Intensity (a.u)
754.54	18.95
803.35	32.5
847.44	19.26

3-4 The Discussion:

As can be observed in figure (3.1), the emission spectrum of normal blood sample has two peaks indicate the hemoglobin A and hemoglobin A_2, because, 96–98% of normal adult haemoglobin is HbA, having 2 alpha chains and 2 beta chains (α α /ββ). Up to 3.5% is HbA2 consisting of 2 alpha chains and 2 delta chains (α α /δδ) (Monica Cheesbrough, 2006).

The normal β chain has no emission but after the change in position 6 and transfer to abnormal protein it can be observed in the emission spectrum of sickle cell anemia because hemoglobin S is an inherited variant of normal adult hemoglobin (hemoglobin A). It results from a substitution of valine for glutamic acid in the sixth position of the β globin chain (Barbara J, 2006).

For this reason we observed two emission peaks in normal blood emission spectrum, whereas in sickle cells anemia, three peaks were recorded. As it can observed in the spectra of the sickle cell anemia, individuals with sickle cell anaemia have haemoglobin S as the major haemoglobin component with a small proportion of haemoglobin A_2 and a variable

proportion of haemoglobin F. As there is no synthesis of normal β chain, there is a total absence of haemoglobin A (Barbara J, 2006).

In table (3.5) one can see that there is a red shift in the wavelength of the peak of hemoglobin S with increasing of the total hemoglobin, as recorded in samples 1 and 3. The samples with low amount of total hemoglobin have a wavelength of hemoglobin S in the range of 600 nm (672.47 nm _ 639.29), respectively. When the amount of total hemoglobin is increased, as in samples 2 and 4, the wavelength of the emission of hemoglobin S is in the range of 800 nm (806.87 nm _ 803.35 nm).

As hemoglobin goes through the loading and unloading process, changes appear in the molecule, these changes are termed allosteric changes, a term that relates to the action of salt bridges between the globin structures. The amount of hemoglobin S is insignificant because the purpose is to detect the presence of hemoglobin S in the test sample. The end point is easy to read as a turbid solution in the presence of hemoglobin S and a clear solution if hemoglobin S is not present (Betty Ciesla, 2007).

All samples of sickle cell anemia have a considerable amount of hemoglobin A_2 emitted at wavelength between 750 nm to 850 nm, as in sample 1 at (797.22 nm), sample 2 at (751.99 nm), sample 3 at (831.58 nm), and sample 4 at (754.54 nm).

The emitted wavelength of the hemoglobin F is in the range from 614.78 nm to 867.39 nm, and the intensity of hemoglobin F increased when the amount of total hemoglobin is decreased. The sample with higher intensity of hemoglobin F (wavelength = 726.76 nm and intensity = 35.47) means that this sample is little sickling, whereas the sample with low intensity of

hemoglobin F (wavelength = 847.44 nm with intensity = 19.26) implying that this sample is more sickling, due to the presence of hemoglobin F retards sickling. (Betty Ciesla, 2007).

Table (3.5): Characteristics of emission of the investigated samples.

Samples	Hb (g/dL)	Wavelength (nm)				Intensity (a.u)			
		λ_A	λ_S	λ_{A2}	λ_F	I_A	I_S	I_{A2}	I_F
Normal Sample	11.8	762.57		685.13		27.16		22.64	
Sample 1	5.8		639.29	831.58	726.76		36.00	30.10	35.47
Sample 2	6.1		672.47	797.22	867.39		29.71	16.08	27.58
Sample 3	7		806.7	751.99	614.78		40.94	16.89	20.28
Sample 4	7		803.35	754.54	847.44		32.5	18.95	19.26

3-5 Conclusions:

From the results obtained in this work, the followings can be concluded:

Three peaks were observed from the emission spectra of different samples of sickle cell anemia, indicated haemoglobin S as the major haemoglobin component with a small proportion of haemoglobin A_2 and a variable proportion of haemoglobin F.

The wavelength of the emission peak of hemoglobin S has a red shift with increasing the total hemoglobin.

The intensity of hemoglobin F is increased with decreasing the amount of total hemoglobin.

The sample with higher intensity of hemoglobin F (wavelength = 726.76 nm and intensity = 35.47) means that the sample is little sickling, whereas the sample with low intensity of hemoglobin F (wavelength = 847.44 nm wit intensity = 19.26) implying that the sample is more sickling.

The laser emission spectroscopy proved to be suitable for the diagnosis of sickle cell anemia.

3-6 Recommendations:

The followings can be recommended as future work:

-Usage of diode laser 532nm for diagnostic of other blood diseases.

-Usage of other types of laser (in the UV range because hemoglobin absorbs at UV also) for blood disease diagnostic.

-Comparison between the method of laser emission and the scattering method.

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