Sudan University of Science and Technology

College of Graduate Studies

Quality Assessment of Medical Heparin Using Laser Scattering Technique

تقييم جودة الهيبارين الطبي باستخدام تقنية التشتت بالليزر

A thesis submitted for partial fulfillment for requirements of master degree in laser application in physics

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بسم الله الرحمن الرحيم

Dedication

I dedicate my work to:

- *My loving father who his words of encouragement and push for tenacity ring in my ears.*
- *The spirit of my beloved mother.*
- *My step mother who supported me throughout this work.*
- *My sisters that have never left my side and motivated me.*
- *Also I dedicate this work with thanks to my friends for their invaluable help.*

Acknowledgment

I wish to thank my supervisor prof. Nafie A. Almuslet for his countless time of reflecting, reading, encouraging and most of all patience throughout the entire work.

Deep thanks to the family of Neelain University, especially to Dr. Abdulsakhi Suliman Mohammed who, with his help, make me able to accomplish this work. I thank him for great help and kind guidance.

Finally, I would like to thank the staff of the institute of laser in Sudan University of science and technology for their continued support.

Abstract

In this work, the goodness of the medical Heparin (concentration) was checked using the principle of laser scattering. A simple setup consists of diode laser with wavelength 532 nm and output power of 100 m W and USB spectrometer were used to record the laser scattered intensity at angles of ($45^{\circ}, 90^{\circ}$and 135°). First of all, the relation between the concentration of the standard sample (NaCl) and the scattered laser intensity in these angles was pointed. Then the relation between the heparin concentration and the intensity of the scattered laser light was deduced for the three angles. The results showed that the relation between the angular scattered intensity and the sample concentration is a linear relation.

This study proved that this setup was sensitive and efficient in discovering any change in the sample concentration and any manipulation in the concentration of Heparin, so the goodness of Heparin can be checked.

المستخلص

تم في هذا العمل فحص جودة المحلول الطبي هيبارين (من حيث التركيز) باستخدام تقانة تشتت الليزر حيث استخدمت منظومة بسيطة تتكون من ليزر الثنائي ذو الطول الموجي 532 نانومتر وبقدرة 100 ملي واط ومطياف لتسجيل شدة الليزر المشتتة بالزوايا $(45^{\circ},90^{\circ},135^{\circ})$. ثم تم إيجاد العلاقة لعينة عيارية هي الملح ثم العينة المستهدفة وهي الهيبارين و بتراكيز مختلفة. وقد بينت النتائج ان الشدة المشتتة بهذه الزوايا تتناسب طرديا مع تركيز العينات فعند تغير تركيز عينة الهيبرين بمقدار بسيط على سبيل المثال أعطت المنظومة تغير واضح في الشدة المشتتة في كل الزوايا.

أثبتت هذه المنظومة دقتها وفعاليتها في فحص جودة المحلول الطبي هيبارين من حيث التركيز واكتشاف أي تغير فيه.

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CHAPTER ONE

BASIC CONCEPTS

1 – 1 Introduction:

A laser is a device that emits electromagnetic radiation through a process of optical amplification based on the stimulated emission of photons. The wavelength of laser light is extremely pure when compared to other sources of light and all of the photons that make up the laser beam have a fixed phase relationship with respect to one another. Laser is a powerful source of light having extraordinary properties which are not found in the normal light sources like tungsten lamps and mercury lamps (Rao, 2013).

As a device, it is now used in medicine, astronomy, geodesy, metrology, chemistry, biology, spectroscopy, holography, power engineering, in various processes in engineering, as well as in communication technology, automation and remote control, in military technology, entertainment industry and art restorations. Industrial applications now include many new procedures, such as laser welding, drilling, cutting, annealing and sputtering (Rao, 2013).

The acronym LASER means **L**ight **A**mplification by **S**timulated **E**mission of **R**adiation. It developed to laser device for generation of coherent electromagnetic waves by stimulated emission of radiation (Renk, 2012).

1 – 2 The study objectives:

The objectives of this study are:

- Checking the quality of a certain type of medical solution (Heparin) using the angular scattering of laser light.
- Study the relation between the sample concentration and the laser intensity scattered at angles of $45^{\circ}, 90^{\circ}$ and 135°.

1 – 3 Thesis structure:

Chapter one presents the basic concepts of laser, laser properties, laser types, usage of lasers in spectroscopy, applications of lasers spectroscopy and lasers in scattering.

Chapter two covers the experimental part of this work, materials and the setup used to study the relation between the sample concentrations (Heparin and Sodium Chloride (NaCl) (Analytical regent) and scattered laser intensity at angles of $45^{\circ}, 90^{\circ}$and 135°.

Results and discussions are presented in chapter three, followed by conclusions and recommendations.

1 – 4 Energy Levels:

In 1915, Neils Bohr proposed a model of the atom that explained a wide variety of phenomena that were puzzling scientists in the late 19th century. This simple model became the basis for the field of quantum mechanics and, although not fully accurate by today's understanding, still is useful for demonstrating laser principles. Bohr's model atom is shown in figure (1 – 1).

When energy is absorbed by the atom, the energy goes to the electrons, these electrons move faster in its own orbits around nuclei from its ground state (lowest energy orbit) to a higher (excited) state, or it can decay from a higher state to a lower state, but it cannot remain between these states The Bohr atom has a limited number of fixed orbits that are available to the electrons.

The allowed energy states are called "quantum" states and are referred to by the principal "quantum numbers" 1, 2, 3, etc. The quantum states are represented by an energy-level diagram (Ewing et al., 2012).

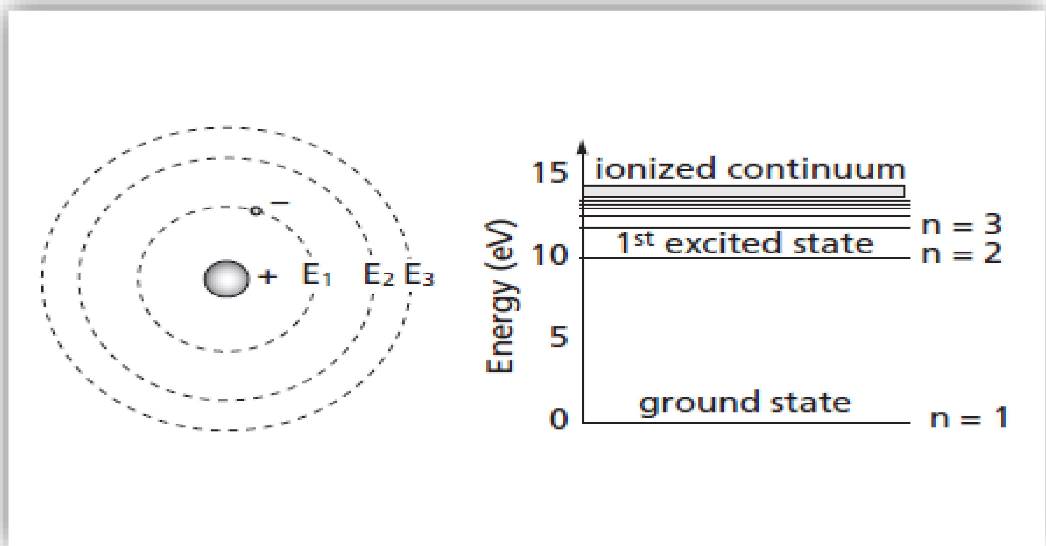


Fig (1 - 1): The Bohr atom and a simple energy-level diagram

There are a variety mechanisms can happen for an electron receive energy from the outside world to jump to a higher quantum state such as inelastic or elastic collisions with other atoms and absorption of energy in the form of electromagnetic radiation (e.g., light). Likewise, when an electron drops from a higher state to a lower state, the atom must give off energy, either as kinetic activity (nonradiative transitions) or as electromagnetic radiation (radiative transitions) (Ewing et al., 2012).

In the 1600s and 1700s, early in the modern study of light, there was a great controversy about light's nature. Some thought that light was made up of particles, while others thought that it was made up of waves. Both concepts explained some of the behavior of light, but not all. It was finally determined that light is made up of particles called "photons" which exhibit both particle-like and wave-like properties. Each photon has an intrinsic energy determined by the equation

$$E = hv \qquad (1-1)$$

Where v is the frequency of the light and h is Planck's constant. Since, for a wave, the frequency and wavelength are related by the equation

$$\lambda v = c \qquad (1-2)$$

where λ is the wavelength of the light and c is the speed of light in a vacuum, equation (1 – 2) can be rewritten as (Nitish & Sudeep, 2011)

$$E = \frac{hc}{\lambda} \qquad (1-3)$$

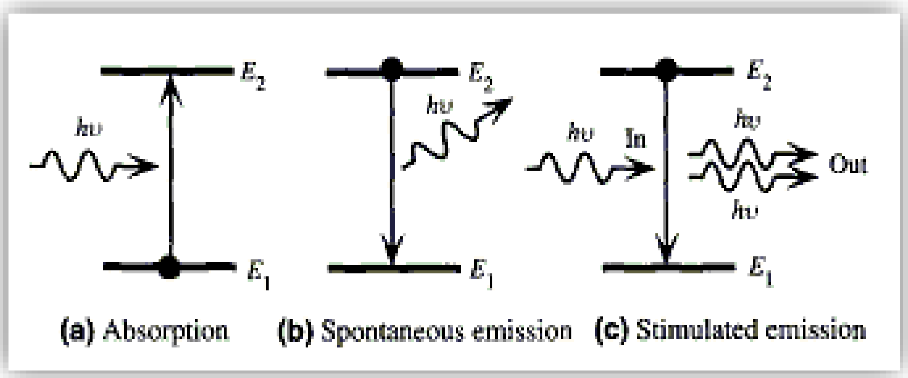
1 – 5 Spontaneous and stimulated emission, absorption of radiation:

In addition to transitions from the upper to the lower level due to **spontaneous emission**, there will also be:

• **absorption** of photons causing transitions from level 1 up to level 2.

• **stimulated emission** in which atoms in level 2 drop to level 1 induced by the incident radiation (Svelte, 2010).

The process of stimulated emission is a coherent quantum-mechanical effect. The photons emitted by stimulated emission are in phase with the photons that induce the transition. This is the fundamental as is of laser operation, as the name suggests: Light Amplified by Stimulated Emission of Radiation. Consider an atom irradiated by white light, with N_2 atoms in level 2 and N_1 atoms in level1. The part of spectrum at frequency v, where $hv = (E_2 - E_1)$, can induce absorption and stimulated emission transitions. The spectral energy density of the light can be write at frequency v as $u(v)$. The transitions that can occur are shown in Fig (1 – 2). In order to treat this situation, Einstein introduced his A and B coefficients.



Fig(1 – 2): Spontaneous and stimulated emission, absorption of radiation

The A coefficient determines the rates of spontaneous transitions. The introduction of the B coefficient extends the treatment to include absorption and stimulated emission. The transition rates for three processes are:

• Spontaneous emission (2 → 1):

$$\frac{dN_2}{dt} = -\frac{dN_1}{dt} = -A_{21}N_2 \qquad (1-4)$$

• Stimulated emission (2 → 1):

$$\frac{dN_2}{dt} = -\frac{dN_1}{dt} = -B_{21}N_2u(v) \qquad (1-5)$$

• Absorption (1→2):

$$\frac{dN_1}{dt} = -\frac{dN_2}{dt} = -B_{12}N_1u(v) \qquad (1-6)$$

These are effectively the definitions of the Einstein A and B coefficients (Svelte, 2010).

1 – 6 Properties of lasers:

Lasers, like any other light source, are governed by the same rules and principles, but laser light is unlike any other source found in nature. It has three special properties that lead to its usefulness in many applications: coherence, monochromaticity, and collimation (directionality) as shown in Figure (1 – 3).

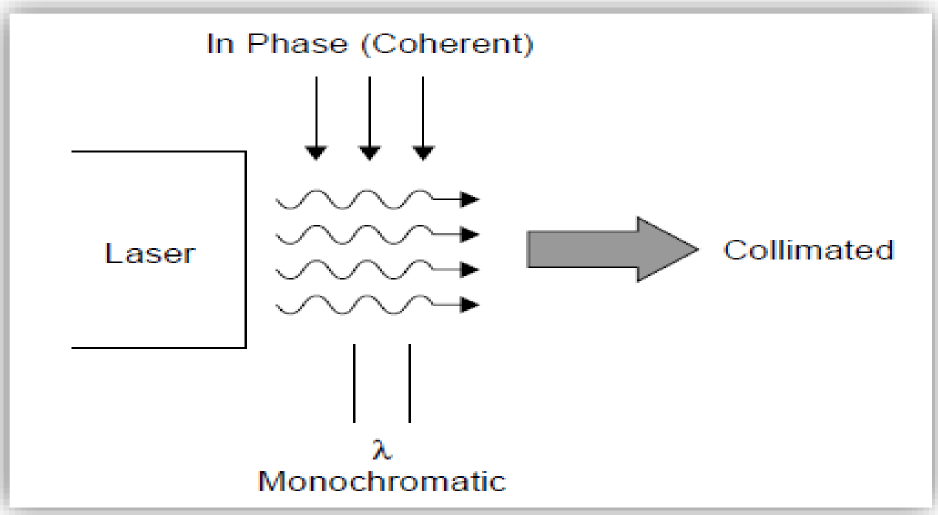


Fig (1 – 3): Properties of laser light

Coherence is the most interesting property of laser light. This property states that all photons emitted from a laser are at exactly the same phase; as waves they all "crest" and "valley" at the same time. Coherence is brought about by the mechanism of the laser itself (stimulated emission), in which photons are essentially copied.

Monochromaticity is the ability of the laser to produce light that is at one well-defined wavelength.

Collimation (directionality) is the property of laser light that allows it to stay as a tight, confined beam for large distances. It can be thought of as the spread in a beam of light (called divergence). This property of laser light makes it possible to use the laser as a level in construction or to pinpoint speeders on a highway (Csele, 2004).

1 – 7 Laser in spectroscopy:

1 – 7 – 1 Spectroscopy:

The word spectrum is often used in every day conversation. For example, the speaking of a spectrum of opinions, meaning a range or spread of opinions. This spectrum of opinions may be broad or narrow, depending on the group of people concerned. In a scientific context, the word spectrum also applies to a range or spread but, in physical chemistry and physics, it applies specifically to arrange or spread of wavelength or frequency. An everyday example of a spectrum of frequencies is apparent on our radios. An important example of a spectrum is that of visible light produced by a prism. In 1667, Sir Isaac Newton started his experiments on dispersion of white light (light from the sun) using a glass prism. He showed that a prism can separate, or disperse white light into its component colors that are classified as red, orange, yellow, green, blue and violet, although there are no sharp divisions between them. The simple prism separates the wavelengths only with no accurate, and it was not until 1859 that Bunsen and Kirchhoff invented spectroscope in order to analyse the light falling on the prism more accurately. They were then able to use this instrument for chemical analysis (Hollas, 2004).

Spectroscopy refers to different techniques that employ radiation in order to obtain data on the structure and properties of matter, which is used for solving a wide variety of analytical problems. The term is derived from a latin word "spectron" which means spirit or ghost, and a greek word "skopein" which means looking onto the world.

Most of our knowledge about the structure of atoms and molecules is based on spectroscopic investigations. Thus spectroscopy has made an outstanding contribution to the present state of atomic and molecular physics, to chemistry, and to molecular biology. Information on molecular structure and on interaction of molecules with their surroundings may be derived in various way from absorption or emission spectra generated when electromagnetic radiation interacts with matter.

General goals of spectroscopy are understanding how exactly light interact with matter and how that information can used to quantitatively understand certain sample (Demtroder, 2014).

1 – 7 – 2 The usage of lasers in spectroscopy:

From 1960 onwards, the increasing availability of intense, monochromatic laser sources provided a tremendous impetus to a wide range of spectroscopic investigations (Hollas, 2004).

The availability of laser sources has substantially changed the field of spectroscopy. Perhaps the most obvious form of laser-based spectroscopic measurement is simply to use a tunable laser, direct the beam through the sample to be measured, tune the laser wavelength, and measure the absorption spectrum of the sample. Thus, because of their high radiance and spectral purity, lasers have substantially advanced the capabilities of conventional spectroscopic techniques, like absorption spectroscopy. But lasers have been employed in many more innovative approaches to spectroscopic investigation. Many new classes of spectroscopic methods and equipments have been developed to take advantage of the unusual properties of laser light. The laser is far more than simply a bright new source of tunable light. Rather, it offers new and highly versatile capabilities for novel measurements of the structure and dynamics of molecules. The availability of the laser has led to a qualitative revolution in spectroscopic capabilities and techniques (Ready, 1997).

1 – 7 – 3 Types of laser spectroscopy:

A wide variety of configurations and methods for laser spectroscopy have been developed

1 – 7 – 3 – 1 Absorption spectroscopy:

The most obvious type of laser spectroscopy is absorption spectroscopy, which involves tuning the laser. Because of the narrow linewidth and high radiance of the laser, spectra with very high resolution may be obtained. The laser beam is transmitted through the sample the spectrum of which is desired. The intensity of the transmitted beam is monitored by a photo detector, and the wavelength of the laser light is tuned through the region of interest. There is no need for gratings, prisms, or any of the other dispersive elements used in conventional spectrometers.

The combination of narrow spectral linewidth, high radiance, and available with lasers has led to great improvement in resolution for absorption spectroscopy. The resolution of a tunable laser system is far better than that of the best conventional dispersive spectrometers (Ready, 1997).

Beer-Lambert law is the main law used in absorption spectroscopy. It composed of two laws derived by Beer and Lambert and given by

$$I = I_{\circ}\exp(-\alpha l) \qquad (1-7)$$

Where $I_{\circ}$ is the incident light intensity, I is the intensity after traversing a thickness l of medium and α is a constant known as absorption coefficient.

There are two commonly used methods of laser absorption spectroscopy :

- Direct transmission measurements.
- Acoustic measurements (Radzimeski, 1987).

In an optoacoustic measurement, the absorption introduces energy into individual molecules within a gas or liquid sample. Collisions cause energy transfer from the absorbing species into the bulk, which results in a local heating and thus a local increase in pressure. The wavelength dependence of magnitude of pressure changes resulting from sample heating after laser absorption is can be detected using a microphone. This the basis of optoacoustic (or photoacoustic) spectroscopy (Ashworth, 2002).

Laser absorption spectroscopy has been carried out in the visible, near infrared, and near ultraviolet regions of the spectrum for many years using tunable dye lasers (Radzimeski, 1987).

Many applications of laser absorption spectroscopy are available. They include as examples:

- Detection of trace components in automobile exhaust using far infrared tunable semiconductor diode lasers.
- Determination of relative populations of rotational and vibrational levels of molecules.
- Real-time measurements of the concentration and flow patterns of combustion products.
- Remote sensing of atmospheric pollutants (Ready, 1997).

1 –7 – 3 – 2 Emission spectroscopy:

The emission spectrum is the set of light frequencies emitted by substances after they have been excited with various forms of energy, most commonly heat or electrical. Since the frequency of light emitted under these conditions depends on the energies of the excited and ground states of electrons in the atoms, the spectrum serves as a very sensitive "fingerprint" of the atoms present. Although emission spectroscopy has many practical uses, it is equally interesting because it provided the first quantitative information about the energy levels in atoms, and allowed chemists to calculate values for the allowable energies of electrons in atoms.

1 –7 – 3 – 2 – 1 Laser Induced Fluorescence (LlF):

Fluorescence is now a dominant methodology used extensively in biotechnology, flow cytometry, medical diagnostics, DNA sequencing, forensics, and genetic analysis. A pair of electrons occupying the same electronic ground state have opposite spins and are said to be in a singlet spin state (Lakowicz, 2006).

When an electron absorbs an ultraviolet or visible photon, one of its valence electrons moves from the ground state to an excited state with a conservation of the electron's spin. Emission of a photon from the singlet excited state to the singlet ground state or between any two energy levels with the same spin is called fluorescence. The probability of fluorescence is very high and the average lifetime of an electron in the excited state is only (10^{-5}-10^{-8}) s. Fluorescence, therefore, decays rapidly once the source of excitation is removed. An absorption spectrum is obtained by measuring the excitation spectrum for creating fluorescing excited state molecules (Harvey, 2008).

Laser Induced Fluorescence (LlF) is a versatile technique in which molecules species are irradiated with laser radiation in a specific wavelength range that is in resonance with the difference in molecules energy levels. LlF finds widespread use in the determination of spatial and/or temporal concentration profiles of atomic (and some simple molecular) species in, for example, plasmas, flames and discharges environments in which there may already be a significant concentration of ions (Ashworth, 2002).

1 –7 – 3 – 2 – 2 Laser-Induced Breakdown Spectroscopy (LIBS):

Laser-induced breakdown spectroscopy (LIBS) is an atomic emission spectroscopy. Atoms are excited from the lower energy level to high energy level when they are in the high energy status. The conventional excitation energy source can be a hot flame, light or high temperature plasma. The excited energy that holds the atom at the higher energy level will be released and the atom returns to its ground state eventually. The released energy is well defined for the specific excited atom, and this characteristic process utilizes emission spectroscopy for the analytical method. LIBS employs the laser pulse to atomize the sample and leads to atomic emission. Compared to the conventional flame emission spectroscopy, LIBS atomizes only the small portion of the sample by the focused laser pulse, which makes a tiny spark on the sample. Because of the short-life of the spark emission, capturing the instant light is a major skill to collect sufficient intensity of the emitting species. Three major parts of the LIBS system are a pulse laser, sample, and spectrometer (Kim and Lin, 2012).

1 – 7 – 3 – 3 Lasers in scattering:

Laser light scattering spectroscopy is based on the evaluation of the frequency shift of laser light scattered by moving particles. This makes it particularly suitable for use in light guiding systems. There are a several types of laser scattering, as example Raman scattering.

1– 7 – 3 – 3 – 1 Raman scattering:

It was predicted in 1923 by Smekal and shown experimentally in 1928 by Raman and Krishnan that a small amount of radiation scattered by a gas, liquid or solid is of increased or decreased wavelength (or wavenumber). This is called the Raman effect. The Raman effect involves scattering of light by molecules of gases, liquids, or solids. The incident radiation should be highly monochromatic for the Raman effect to be observed clearly and, because Raman scattering is so weak, it should be very intense. Lasers are sources of intense, monochromatic radiation which are ideal for Raman spectroscopy and have entirely replaced atomic emission source (Hollas, 2004).

In Raman spectroscopy, sample is illuminated with a monochromatic laser beam which interacts with the molecules of sample and originates a scattered light. The scattered light having a frequency different from that of incident light (inelastic scattering) is used to construct a Raman spectrum. Raman spectra arise due to inelastic collision between incident monochromatic radiation and molecules of sample. When a monochromatic radiation strikes at sample, it scatters in all directions after its interaction with sample molecules. Much of this scattered radiation has a frequency which is equal to frequency of incident radiation and constitutes Rayleigh scattering. Only a small fraction of scattered radiation has a frequency different from the frequency of incident radiation and constitutes Raman scattering. When the frequency of incident radiation is higher than the frequency of scattered radiation, Stokes lines appear in Raman spectrum (Fig (1 – 4) transition B). But when the frequency of incident radiation is lower than the frequency of scattered radiation, anti-Stokes lines appear in Raman spectrum (Fig (1 – 4) transition C). Scattered radiation is usually measured at right angle to incident radiation (Bumbarh & Sharma, 2016).

Both Stokes and anti-Stokes Raman scattering increase greatly in strength if the incident light falls within a molecular absorption band (Fig (1 – 4) transition D), the scattering then is termed resonance Raman scattering (Parson, 2015).

Laser Raman spectroscopy has been used in a wide variety of applications, such as for identifying drugs, for detecting trace quantities of drugs in blood or urine samples, for detecting the metabolic by products of drugs, for determining the presence of impurities in products like medicines (Ready, 1997).

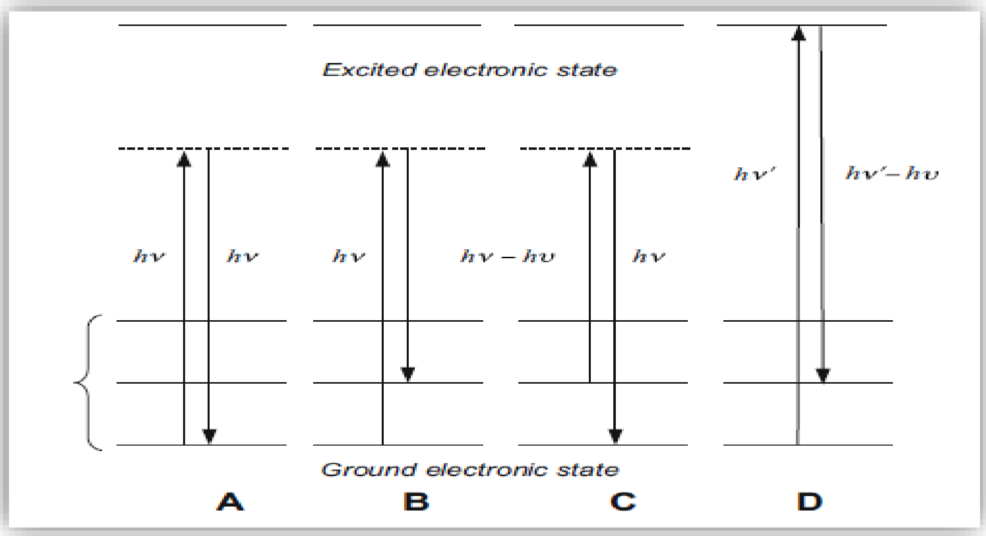


Fig (1 – 4): Types of light scattering. Rayleigh and Mie scattering (A) a molecule or particle absorbs a photon and emits a photon with the same energy. Raman scattering (B–C), (Stokes (B) and anti-Stokes (C) Raman scattering, respectively). Resonance Raman scattering (D).

1 – 7 – 4 Applications of laser spectroscopy:

The relevance of laser spectroscopy for numerous applications in physics, chemistry, industrial, and medicine, or to environment and technical problems has rapidly gained enormous significant (Demtroder, 2003).

1 – 7 – 4 – 1 Applications in chemistry:

For many fields of chemistry, lasers have become in dispensable tools. They are employed in analytical chemistry for ultrasensitive detection of small concentration of pollution, trace element, or short - lived intermediate species reaction. Important analytical applications are represented by measurement of the internal – state distribution of reaction with LIF and spectroscopic investigation of collision – induce energy – transfer processes. One of the laser application in analytical chemistry is the sensitive detection of small concentration of impurity atoms or molecules. With laser spectroscopy techniques, detection limits down into the parts-per-billion rang can be achieved for molecules which corresponds to a relative concentration of (10^{-9}). Another laser application in analytical chemistry is laser induced chemical reactions. The reaction can be initiated by one or more multi-photon excitation of one or more of the reactants. The excitation can be performed before the reactants collide or during the collisions. Another example is the femtosecond chemistry: Chemical reactions are based on atomic or molecular collision. These collisions which may bring about chemical-bond formation or bond breaking occur on a time scale of (10^{-11} to 10^{-13}) s. In the past, the event that happened in transition state between reagents and reaction could be investigated. The study of chemical dynamics concerned with ultrashort time interval when a chemical bond is formed or broken may be called real-time femtochemistry. It relies on ultrafast laser techniques with femtosecond time resolution (Demtroder, 2003).

1 – 7 – 4 – 2 Applications in environment research:

Since in densely populated industrial areas air and water pollution has become a serious problem, the study of pollution and their reaction with natural components of our environment is urgently needed. Various techniques of laser spectroscopy have been successfully employed in atmospheric and environment research such as direct absorption measurements, laser – induce fluorescence techniques, photoacoustic detection, spontaneous Raman scattering. For examples of laser applications in this field are:

Absorption measurement: the concentration of atomic or molecules pollutants in the lower part of our atmosphere just above the ground can be determined by measurement of direct absorption of laser beam propagation through the atmosphere.

Detection the water pollution: unfortunately, the pollution of water by oil, gasoline, or other pollutants is increasing. Several spectroscopic techniques have been developed to measure the concentration of specific pollutants. These techniques are not only helpful in tracing the polluting source but they can also be used to initiate measures against the pollution (Demtroder, 2003).

1 – 7 – 4 – 3 Applications in technical problems:

Although the main application range of laser spectroscopy is related to basic research in various fields of physics, chemistry, biology, and medicine, there are quite few interesting technical problems where laser spectroscopy offers elegant solution. Examples include investigation and optimization of flames and combustion processes in fossile power stations, in car engines, or in steel plants, analytical spectroscopy of surface or liquid alloys for the production of high – purity solids or measurements of flow velocity and turbulence in aerodynamic and hydrodynamic problem (Demtroder, 2003).

1 –7 – 4 – 4 Applications in medicine:

Most of these applications rely on the 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 the laser beam by choosing the proper laser wavelength. For example, skin carcinoma or portwine marks should be treated at wavelengths for 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 subcutaneous cancer must be performed at wavelengths with greater penetration depth. The spectacular outcomes of laser applications in medicine have been achieved in laser surgery, dermatology, ophthalmology, and dentistry (Demtroder, 2003).

1 –7 – 4 – 5 Industrial applications:

High power lasers have long been used for cutting and welding materials. Today the frames of automobiles are assembled using laser welding robots, complex cardboard boxes are made with laser-cut dies, and lasers are routinely used to engrave numbers and codes on a wide variety of products. Some less well-known applications include photolithography, marking and scribing. In photolithography, lasers are used throughout the manufacture of semiconductor devices, but nowhere are they more important than in exposing photoresist through the masks used for creating the circuits themselves. Approximately ten years ago, manufactures started to switch to ultraviolet lasers operating at approximately 300 nm to expose the photoresist. Manufacturers are now using wavelengths as short as 193 nm to get the resolution needed for today's semiconductor integrated circuit applications. In marking and scribing, lasers are used extensively in production to apply indelible, human and machine-readable marks and codes to a wide variety of products and packaging. Typical applications include marking semiconductor wafers for identification and lot control, removing the black overlay on numeric display pads, engraving gift items, and scribing solar cells and semiconductor wafers. Laser scribing is similar to laser marking, except that the scan pattern is typically rectilinear, and the goal is to create microscoring along the scan lines so that the substrate can be easily broken part. A wide variety of materials, including metal, wood, glass, silicon, and rubber, are amenable to laser marking and scribing (Griot, 2009).

1 – 8 Introduction to laser scattering theory:

When light "hits" a small object (a particle or a molecule), and thereby changes its direction, the thing that happens is called light scattering. Laser like all other kinds of electromagnetic radiation (radio waves, micro waves, heat radiation, ultraviolet radiation, X-rays, gamma radiation) interacts with matter in two ways:

1. Absorption: the photons (the light) disappear.
2. Scattering: the photons change their direction (Qgendal, 2016).

depending on wavelength of light and optical properties of the material. The net result of the absorption and scattering causes by material is known as the extinction of incident light,

$$\text{Extinction} = \text{absorption} + \text{scattering}$$

The scattering is only observed when a material is itself heterogeneous, either due to local density fluctuation in a pure material or due to the optical heterogeneity for dispersed particle in medium.

Used as a general term, "light scattering" can be encountered in several branches of the physical science, such as optics, physical chemistry, material science, fluid dynamics and solid physics. There are many ways to use light scattering phenomena to study various properties of material (Xu, 2002).

1 – 9 The mechanics of light scattering:

The scattering of light may be thought of as the redirection of light that takes place when an electromagnetic (EM) wave (i.e. an incident light ray) encounters an obstacle or nonhomogeneity, in our case the scattering particle. As the EM wave interacts with the discrete particle, the electron orbits within the particle's constituent molecules are perturbed periodically with the same frequency (v_0) as the electric field of the incident wave. The oscillation or perturbation of the electron cloud results in a periodic separation of charge within the molecule, which is called an induced dipole moment. When the oscillating induced dipole moment is manifest as a source of EM radiation, scattered light is result. Fig (1 – 5) shows the light scattering by an induced dipole moment due to an incident EM wave (Homyak et al., 2009).

The amplitude of the scattered light at different angles (the scattering pattern) depends not only on concentration and particle size, but also on the ratio of the refractive indices of the particles to the medium in which the particle exists. The more the particles differ from the medium (i.e. the more their refractive indices differ), the more light will be scattered by the particles. At the other extreme, if there is no difference in refractive indices, no light will be scattered (Webb, 2000).

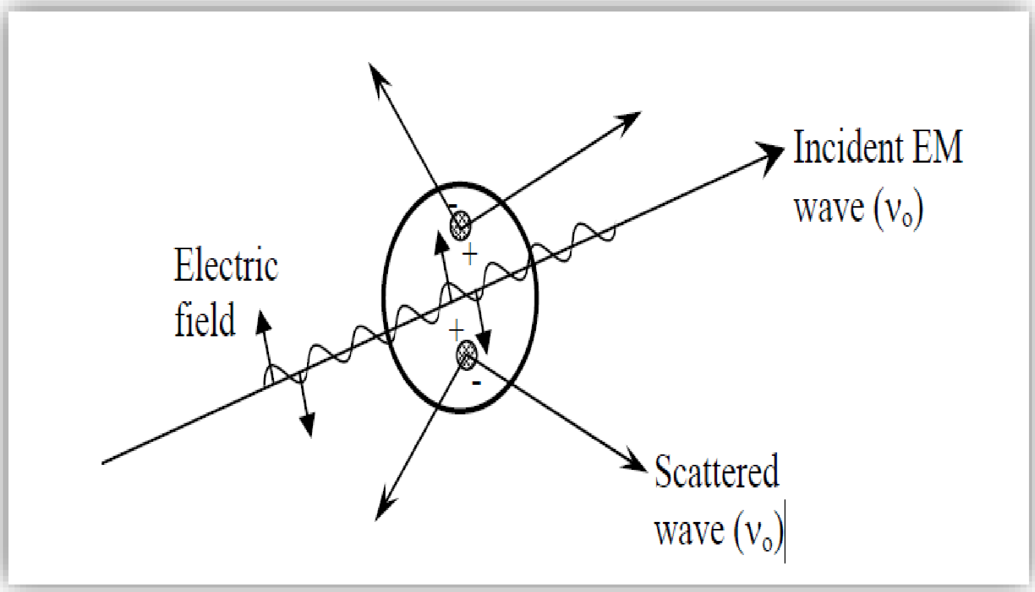


Fig (1 – 5): Light scattering by an induced dipole moment due to an incident EM wave.

1 – 10 Types of light scattering:

Scattering involves both the properties of light radiation and those of the encountered matter. Therefore, we have to deal with aspects such as wavelength and polarization of the light, and depending on the size and structure of the matter, with diffraction, refraction and reflection, or absorption and re-emission of (light) energy by an oscillator (matter).

There are several possibilities of classifying the various scattering processes. Here the distinction between elastic and inelastic scattering is made (Mayinger & Feldmann, 2001).

1 – 10 – 1 Elastic scattering:

An elastic scattering refers to an interaction without a permanent exchange of energy between the light and the matter. This restriction of energy exchange does not prohibit a change in direction, but it does prohibit a change in frequency (wavelength, etc.) (Mayinger & Feldmann, 2001).

1 – 10 – 1 – 1 Rayleigh scattering:

In Rayleigh scattering there is no net transfer of energy between the molecule and the radiation field: the incident and emitted photons have the same energy (Fig (1 – 4), transition A) (Parson, 2015).

If light is scattered by particles that are smaller than the wavelength of the light, the process is called Rayleigh scattering. Unlike Mie scattering, the size and structure of the particle are not important and the laws of reflection, diffraction and refraction are not applicable in this case. Typical scattering particles are molecules, as those in the example of the color of the sky, or very small particles. Rayleigh scattering can effectively be applied to obtain density or temperature measurements in gases and gaseous mixtures. An example of a simple Rayleigh scattering setup is shown in fig (1 – 6) (Mayinger & Feldmann, 2001).

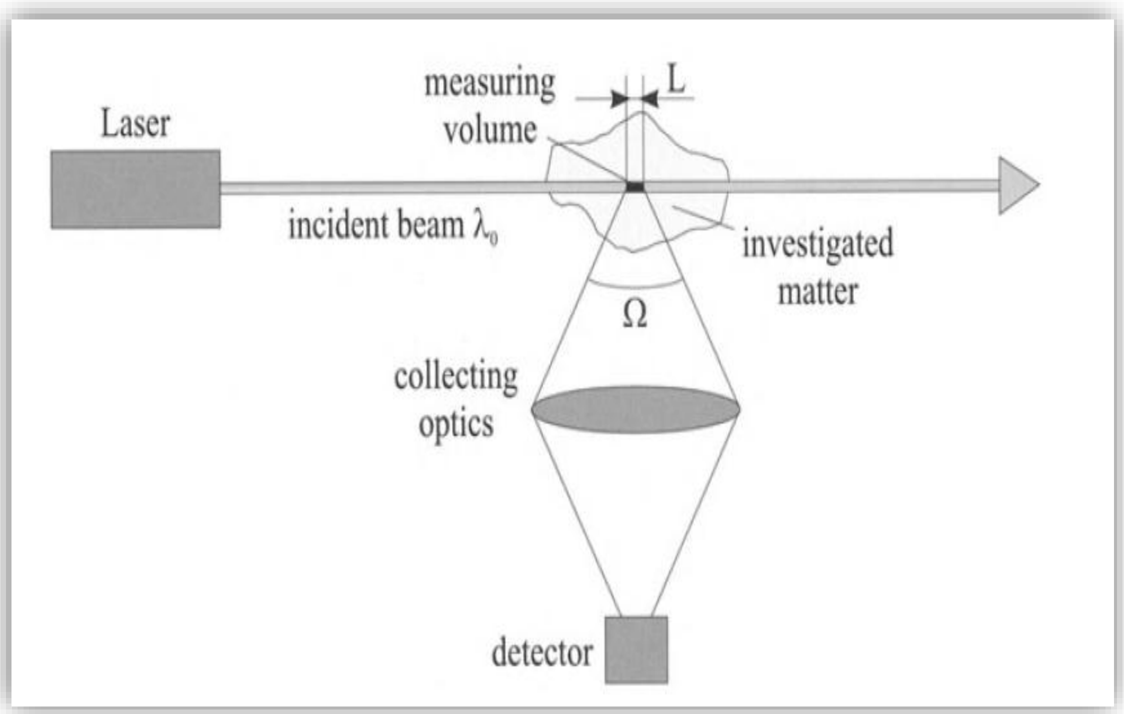


Fig (1 – 6): Schematic of a typical setup for temperature and density measurements with Rayleigh scattering

1 – 10 – 1 – 2 Mie scattering:

Mie scattering appears if the scattering particles are large compared to the wavelength. Typical scattering particles for Mie scattering with visible light are soot or dust particles with diameters between 1 pm and 10 pm.

The scattering process is based on a change of the electrical and magnetic properties in the vicinity of the scattered light. Due to the large size of the particles, reflection and diffraction have to be considered beside the actual scattering by the particle. The resulting effects on the emitted light compared to the incident light are changes in phase, amplitude and polarization. In addition, the light waves coming from different spots of the particle interfere and a characteristic field of radiation is formed (Mayinger & Feldmann, 2001).

1 – 10 – 2 Inelastic scattering:

An inelastic process, on the other hand, leads to a permanent energy exchange. The energy content of the matter after the interaction is either higher or lower than that in the original state. Due to the conservation of total energy, the energy of the emitted radiation is changed as well, resulting in a change of frequency (wavelength, etc.) (Mayinger & Feldmann, 2001).

1 – 10 – 2 – 1 Brillouin scattering:

It is another type of light scattering that involve transfer of different forms of energy between the molecule and the radiation field. In Brillouin scattering, the energy difference between the absorbed and emitted photons creates acoustical waves in the sample; in quasielastic or dynamic light scattering, the energy goes into small changes in velocity or rotation. In two-photons absorption, the second photon is absorbed rather than emitted, leaving the molecule in a excited electronic state whose energy is the sum of the energies of the two photons (Parson, 2015).

1 – 11 Angular scattering:

The characteristic variation in intensity of light versus angle is called angular scattering. The amount of light that scattered depend on number of factors including size, shape of particles, and their concentrations (Glenross et al., 2011).

Mie theory of scattering is used to provide new information on how changes in particle volume, with no change in dry weight, should influence light scattering for various scattering angles and particle sizes. Small volume changes can cause very large observable changes in large angle scattering if the sample particles are uniform in size; however, the natural particle size heterogeneity of most samples would mask this effect. For heterogeneous samples of most particle size ranges, particle shrinkage is found to increase large angle scattering (Latimer & Pyle, 1972).

The intensity of the scattered light is a function of the wavelength λ, the scattering angle θ, the particle size d, and the relative index of refraction n of the particle and the medium. Symbolically, then (Wepp, 2000)

$$I_{sc} = I_{in}(\theta, \lambda, d, n) \qquad (1-12)$$

1 – 12 Scattering measurement techniques:

The two main techniques used in light scattering are: static and dynamic scattering. By the static light scattering technique, particle size information is extracted from intensity characteristics of the scattering pattern at various angles. With dynamic light scattering, particle size is determined by correlating variations in light intensity to the Brownian movement of the particles. Values obtained by the latter technique vary widely depending on the concentration and condition of the sample, as well as environmental factors. With both techniques, however, the scattered light has undergone no alteration in wavelength (Wepp, 2000).

1 – 12 – 1 Static light scattering measurement:

Static **L**ight **S**cattering (**SLS**) is a non-invasive technique used to characterize the molecules in a solution. The particles in a sample are illuminated by a light source such as a laser, with the particles scattering the light in all directions. But, instead of measuring the time-dependent fluctuations in the scattering intensity, static light scattering makes use of the time-averaged intensity of scattered light instead (Merkus, 2009).

The angular dependency of the time-mean-intensity of laser light scattered by the particles is measured. The course of the scattered intensity as a function of the detector angle depends on size and structure of the particles as shown in Fig (1 – 7). Rayleigh developed a theory that predicts the angular intensity distribution of light scattered by particles much smaller than the wavelength of light ($R < \lambda/20$). In this case the intensity of the scattered light depends only on the size of the particles and not on their structure or the concentration (Bussche et al., 2004).

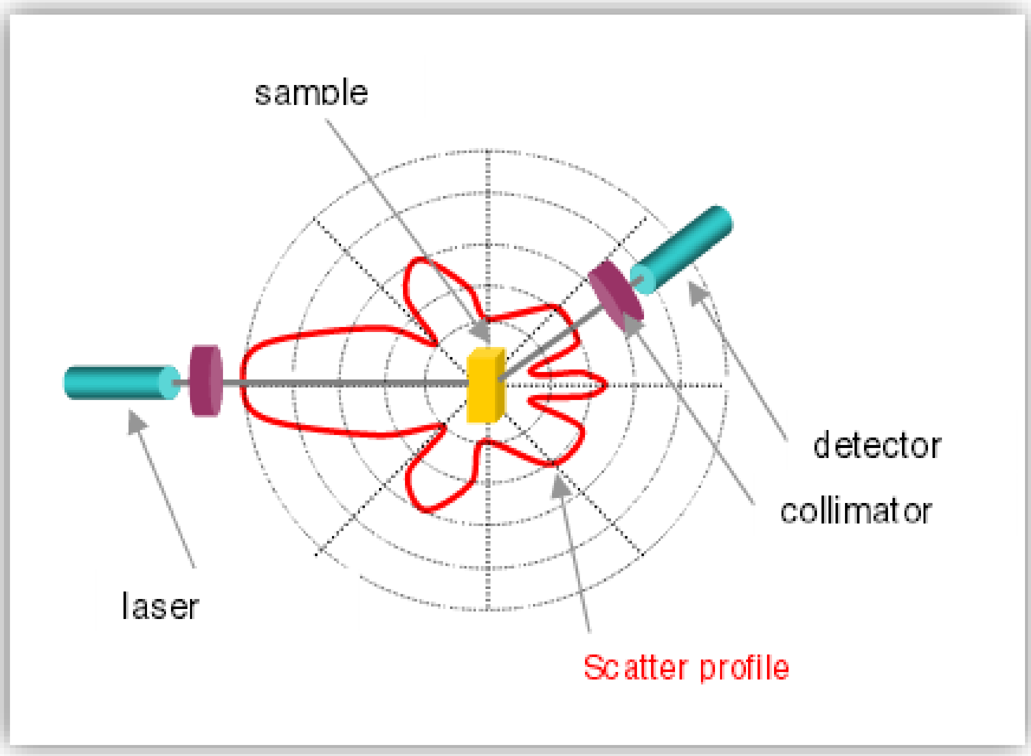


Fig (1 – 7): Basic principle and setup of static light scattering measurements

The intensity of light scattered over a period of time, is accumulated for a number of concentrations of the sample. This time averaging removes the inherent fluctuations in the signal, hence the term "Static Light Scattering".

The intensity of scattered light that a particle produces is proportional to the concentration of the particle. The Intensity of the scattered light is differ of various concentrations (C) of sample at one angle. The graphical representation of this is called a Debye plot (Zhelev & Barudov, 2005).

1 – 12 – 2 Dynamic light scattering (DLS):

Dynamic light scattering (DLS) is the name that covers different techniques for measurement of particle dispersion or molecular solution such as particle size, molecular weight. In the DLS technique, the intensity of the scattered light by an ensemble of particles is measured at a given angle as a function of time. The Brownian motion of the dispersed particles determines the rate of change of the scattered light intensity (Merkus, 2009).

1 – 13 Literature review:

In 2001 Marie Carolin Michalski, Valerie Briand and Francois Michel studied the optical parameters of milk fat globules (refractive index and absorption coefficient) by using laser light scattering at two different wavelengths, a $k_a = 10^{-5}$ was obtained for liquid milk fat. The n_i of milk fat globules at 20 °C was measured at 589 nm and was calculated to be 1.470 at 466 nm and 1.460 at 633 nm.

In 2010 Wafaa Soliman, Noriharu Takada, and Koichi Sasaki applied laser-light scattering for investigating the growth processes of nanoparticles in liquid-phase laser ablation. They observed the growth of nanoparticles inside the cavitation bubble. This means that particles ejected from the target are transported into the cavitation bubble, and they condense into nanoparticles inside it. The production of nanoparticles was efficient until 3μs after the irradiation of the laser pulse for ablation, indicating the fast growth of nanoparticles. A part of nanoparticles was transported from the cavitation bubble toward the water, but the great portion of nanoparticles was stored in the cavitation bubble until the collapse.

In 2007 Rasha Y. Ibrahim investigated the concentration goodness for Benzylpenicillin Sodium, Metronidazole Intravenous Infusion, Actrapid HM (Insulin Human) by using laser angular scattering.

In 2006 Hongping Ye. using multiangle laser light scattering as a technique to investigate the aggregation and degradation of glycosylated and nonglycosylated proteins and antibodies under various conditions such as addition detergent, change in Ph, and variation of protein concentration.

In 2017 Xiaoyn Yu. et al used the laser scattering principle to detect the dust concentration and the detection basis of Mie scattering theory. Through simulation, the influence of the incident laser wavelength, dust particle diameter, and refractive index of dust particles on the scattered light intensity distribution were obtained for determining the scattered light intensity curves of single suspended dust particles under different characteristic parameters. A genetic algorithm was used to study the inverse particle size distribution, and the reliability of the measurement system design is proven theoretically. The dust concentration detection system, which includes a laser system, computer circuitry, air flow system, and control system, was then implemented according to the parameters obtained from the theoretical analysis.

In 2015 Chanyang Wang. et al detected the surface roughness of optical element by using total integrated scattering method. At the same time, they established the relational model between surface roughness and subsurface damage to get the depth of subsurface damage rapidly and accurately after they measured surface roughness so as to achieve the goal of detection of optical element's surface microstructure.

Chapter Two

The Experimental Part

CHAPTER TWO

THE EXPERIMENTAL PART

2 – 1 Introduction:

This chapter describes the experimental setup and the samples (medical solution (Heparin) and Sodium Chloride (NaCl) (Analytical reagent)) which were used. The setup make use of the angular distribution of the scattered laser intensity in different scattering angles for different samples concentrations.

2 – 2 The Experimental setup:

The main devices of the experimental setup are: diode laser, sample chamber, USB spectrometer, and P.C. Fig (2 – 1) shows a diagram of the experimental setup.

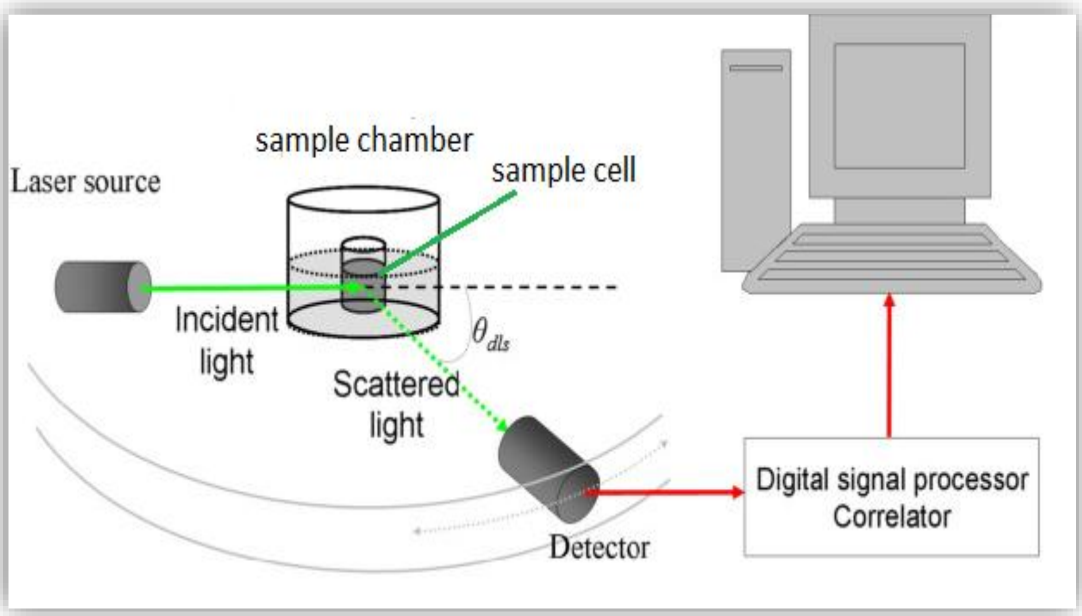


Fig (2 – 1): The experimental setup

2 – 2 – 1 The diode laser:

Laser diodes, are unique when compared to other types of lasers. They are very small, they operate with low power input and they are very efficient. The laser diode is similar to light emitting diode (LED), it is comprised of a junction between two semiconductors (one positive and one negative) known as a p-n junction. These semiconductors are incredibly small, made of very thin slices of semiconducting materials, and they are very carefully manufactured so as to create a perfect p-n junction. The n-type has an excess of electrons and the p-type has a deficit of electrons or an excess of holes (missing electrons). When forward bias voltage across the junction, the electrons are forced into region from n-type and holes are forced into junction from p-typ. These electrons (negative charges) and holes (positive charges) are attracted to each other and when they collide they neutralize each other. This process emit recombination radiation and the energy will be released. The lose energy is in the form of photons, which are light quanta. These photons are all produced simultaneously at same frequency and direction. Because all the electrons jumped down to fill holes in a certain semiconducting material, this causes a laser beam to form and come out through the lens of the laser diode (Silfvast, 2004).

The diode laser used in this work is green diode laser with wavelength of 532 nm and output power of 100 mW. Fig (2 – 2) shows a photo of this diode laser.

Fig (2 – 2): The diode laser

2 – 2 – 2 The Sample chamber:

The sample chamber is a sphere made of poly tetra fluoroethne, completely opaque and have negligible internal reflection. Inside the sphere there is a cylinder of internal radius 9 cm, external radius 11.2 cm, height at 7 cm and a removable top cover for introducing the sample into the sample cell. At the base of the chamber there is 1 cm^2 ditch engraved where the sample cell fit in place that could not move during the experimental measurement. At one side of the chamber a 0.6 cm aperture was drilled to pass the incident laser light to inside the chamber. At same plane a 0.2 cm, and in 0 angle, aperture was drilled to pass the transmitted light to the detecting device, and at angles of $45^{\circ}, 90^{\circ}$and 135° there are apertures drilled with 2 cm to pass the scattered laser light to the detecting device. Fig (2 – 3) shows a photo of the sample chamber.

Fig (2 – 3): The photo of sample chamber.

2 – 2 – 2 – 1 The Sample cell:

In this work sample cell from plastic (polytstren) was used as cuvette. This cuvette is designed to be used in the visible spectral range. Fig (2 – 4) shows the sample cell.

Fig (2 – 4): The sample cell

2 – 2 – 3 The USB Spectrometer:

The USB 2000 spectrometer used in this work is a versatile, general-purpose UV – VS – NIR spectrometer for absorption, transmition, reflection, emission, color and other applications. As spectrometer have become smaller, faster and more powerful, application once considered impractical outside laboratory are now feasible. USB 2000 spectrometer is connected via free-space or via fiber to light source and sampling accessories to measure liquid, solid, and other samples.

The USB 2000 spectrometer is a simple optical instrument based on a diffraction grating and a one-dimensional CCD detector array. The spectrometer box is shown in Figure (2 – 5). This instrument achieves a spectral resolution of about 0.5 nm between wavelengths of 370 to 680 nm (Graham, 2009).

Table (2 – 1) lists the specifications of this spectrometer

Fig(2 – 5): USB 2000 Fiber Optic Spectrometer

Table (2 – 1): The USB 2000 Spectrometer Specifications

Dimensions	89.1 mm x 63.3 mm x 34.4 mm
Weight	190 g (without cable)
Power consumption	90 mA - 5 V DC
Detector range	200 -1100 nm
Resolution	~ 0.5nm
Sensitivity (estimate)	400 nm – 90 photons/count; 600 nm – 41 photons/count; 800 nm – 203 photons/count

2 – 3 The samples:

In this work some drugs were used as samples such as :

2 – 3 – 1 Sample no (1): Sodium Chloride (NaCl) (Analytical reagent):

The sample of (NaCl) was used as standard sample to calibrate the measurement of the angular distribution of the scattered intensity.

2 – 3 – 2 Sample no (2): Heparin:

Heparin is medication which is used as a blood thinner and it is an injectable anticoagulant that is used to prevent the formation of blood clots in the vessels. It is also used to manage and treat blood clots that may occur in the heart, legs and lungs (Robertson, 2014). The chemical structure of Heparine is shown in fig (2 – 6), its composed of: (1) α-L-iduronic acid 2-sulfate, (2) 2-deoxy-2-sulfamino-α-D-glucose-6-sulfate, (3) β-D-glucuronic acid, (4) 2-acetamido-2-deoxy-α-D-glucose, and (5) α-L-iduronic acid. These sugars are present in decreasing amounts, usually in the order (2) > (1) > (4) > (3) > (5), and are joined by glycosidic linkages, forming polymers of varying sizes (Nachtmann et al., 1990).

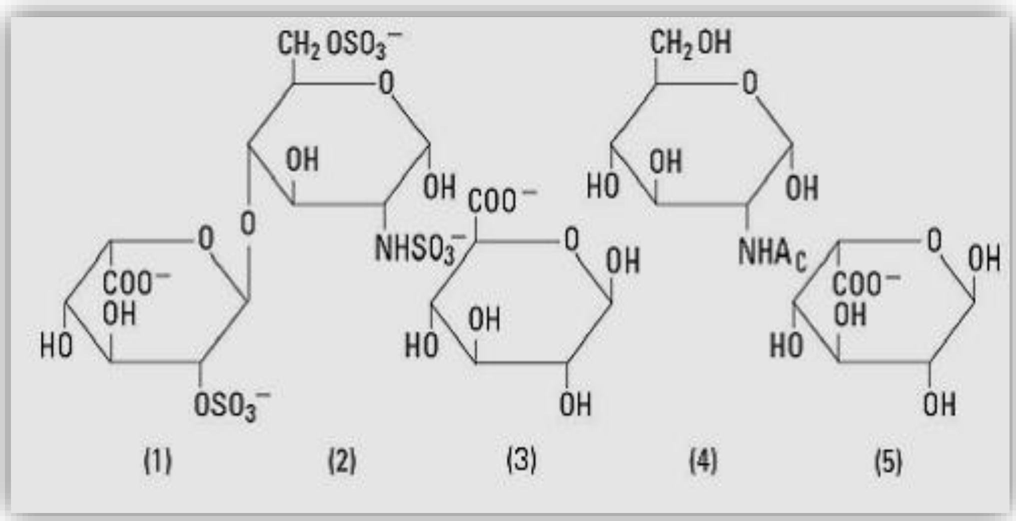


Fig (2 – 6): Chemical structure of Heparin

2 – 4 Preparation of the samples:

2 – 4 – 1 Apparatus:

Beaker (100 ml), pipette (5 ml), sensitive balance (4 digit), glass rode.

2 – 4 – 2 Sodium Chloride (NaCl) (Analytical reagent):

This sample was prepared as follows :

By beaker (100 ml) of distilled water as zero concentration was taken, and then the (2 g) of NaCl was weight by using sensitive balance, after that the concentration was increased gradually by adding (2 g) of NaCl to the distilled water and mixed together by glass rode each time until (14 g) of NaCl was dissolved in (100 ml) of water.

2 – 4 – 3 Heparin:

This sample was prepared as follows:

By pipette (5 ml) of pure Heparin as standard sample was taken, and then the concentration of Heparin was decreased gradually by adding (0.5 ml) of distilled water that taken by pipette to Heparin each time until (5 ml) of Heparin was mixed with (5 ml) of water.

2 – 5 The Experimental procedure:

The laser source, detector, P.C, and all the connecting wires were covered by aluminum foil to minimize any environmental noise.

The experimental procedure was done as follows :

- The angular distribution of the scattered laser light was measured by the setup described in previous section and illustrated in Fig (2 – 1).
- The laser was turn on for (10 – 15) min to insure the stability of the laser light before any measurement.
- After that, the distilled water, as a standard concentration, was introduced into the cell in the sample chamber and then the upper led was put in place. The concentration was increased by added NaCl gradually to distilled water in each time. The scattered intensity at angles of ($45^\circ, 90^\circ$ and 135°) were recorded by the USB spectrometer.
- The pure solution (standard concentration) of the drug sample (Heparin) was introduced into the cell in the sample chamber, and then the upper led was put in place. The intensity of the scattered laser intensity at angles of ($45^\circ, 90^\circ$ and 135°) were recorded by the USB spectrometer.
- The results were plotted in graphical representation showed the scattered laser intensity as a function of the concentration for each scattering angle.

Chapter Three

Results and Discussion

CHAPTER THREE

RESULTS AND DISCUSSION

3 – 1 Introduction:

In this chapter, the values of the intensity of the scattered laser light in the different scattering angles for each sample concentration are presented.

The relation between the scattered laser intensity with different concentrations of each sample was plotted and studied. The results are discussed in this chapter. Finally, conclusions and recommendations are pointed.

3 – 2 The Results:

3 –2 – 1 Results of Sodium chloride (NaCl):

Table (3 – 1) illustrates the concentrations of Sodium chloride (NaCl) in (g/ml) and the scattered laser intensity at angles of ($45^{\circ}, 90^{\circ}$ and 135°) using the diode laser with wavelength of 532 nm and output power of 100 mW.

Table (3 – 1) NaCl concentration and scattered laser intensity

Sample No	Concentration (g/ml)	scattered intensity (a.u) with $\theta = 45^{\circ}$	scattered intensity (a.u) with $\theta = 90^{\circ}$	scattered intensity (a.u) with $\theta = 135^{\circ}$
1	0.00	350.39	243.33	258.63
2	0.02	370.63	243.37	263.37
3	0.04	390.87	243.42	268.88
4	0.06	411.52	243.46	274.01
5	0.08	431.97	243.50	279.14
6	0.10	452.41	243.54	284.26
7	0.12	472.86	243.58	289.39
8	0.14	493.30	243.63	294.47

Fig (3 – 1) shows the relation between the scattered laser intensity at angles of ($45^{\circ}, 90^{\circ}$ and 135°) and the concentration of Sodium chloride.

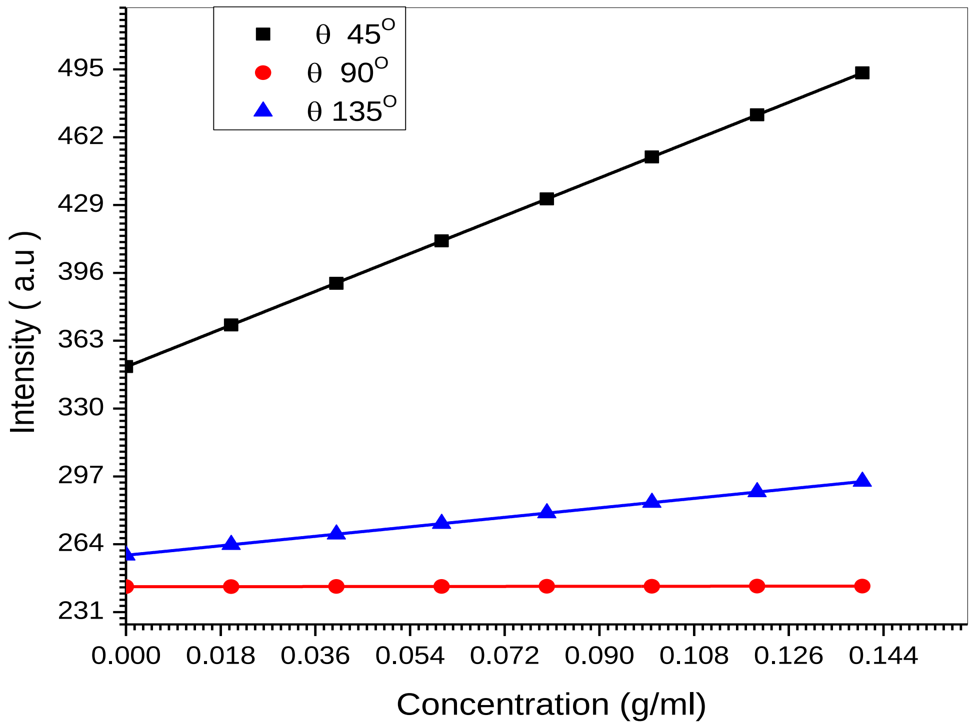


Fig (3 – 1): Laser intensity scattered at angles of (45°, 90°, and 135°) as a function of NaCl concentrations.

Fig (3 – 1) shows that the scattered intensity at different angles increased with any simple increasing in concentration

3 – 2 – 2 Results of Heparin:

Table (3 – 2) illustrates the dilution values of Heparin samples and the scattered laser light intensities at angles of ($45^{\circ}, 90^{\circ}$ and 135°) using the same setup.

Table (3 – 2) Heparin dilution ratio and scattered laser intensity

Sample No	Dilution (ml/ml)	scattered intensity (a.u) with $\theta = 45^{\circ}$	scattered intensity (a.u) with $\theta = 90^{\circ}$	scattered intensity (a.u) with $\theta = 135^{\circ}$
1	0.0	260.51	222.09	245.94
2	0.1	254.60	219.90	238.55
3	0.2	249.18	216.37	232.30
4	0.3	243.40	213.09	225.87
5	0.4	237.70	206.37	217.88
6	0.5	231.60	202.65	210.65
7	0.6	226.05	199.96	203.45
8	0.7	219.26	196.24	198.13

Fig (3 – 2) shows the relation between the scattered laser intensity at angles of ($45^{\circ}, 90^{\circ}$and 135°) and dilution ratio of Heparin.

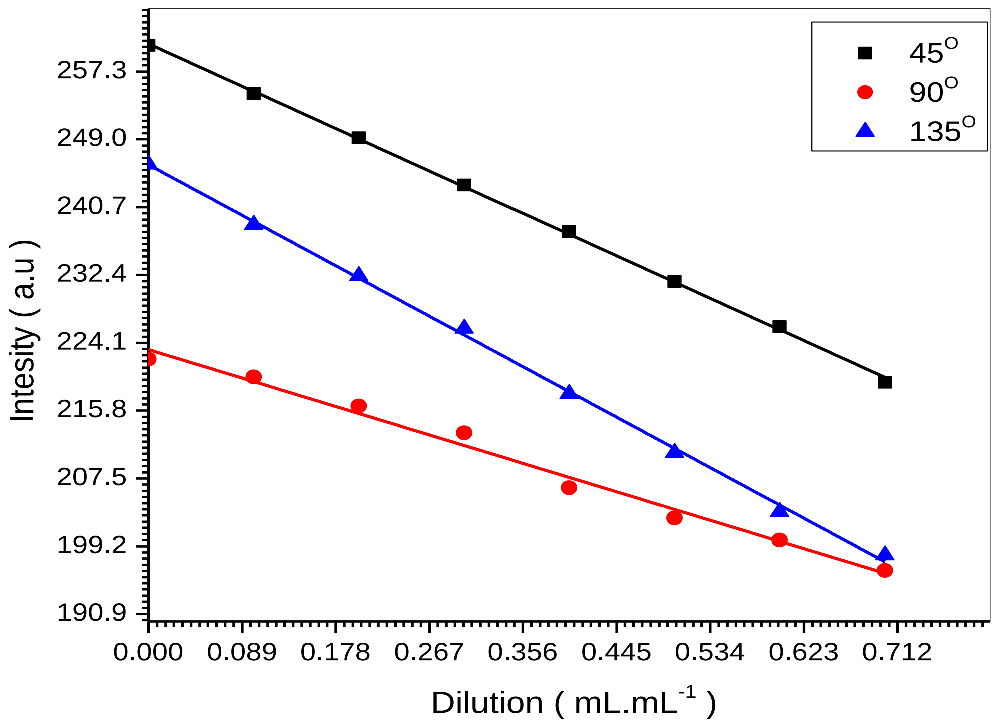


Fig (3 – 2): Scattered laser intensity at angles of ($45^{\circ}, 90^{\circ}$, and 135°) as a function of Heparin dilution ratio

3 –3 The discussion:

Based on the principles of laser scattering, the results that obtained are plotted in fig (3 – 1) and fig (3 – 2). Fig (3 – 1) which showed that the relation between the scattered laser intensity and the concentration of NaCl is linear relation and from fig (3 – 2) it can be observed that when the dilution values of Heparin increased the scattered laser intensity at angles of ($45^\circ, 90^\circ$ and 135°) decreased.

When the light hits the particles or molecules in solution of NaCl and Heparin, it causes perturbation or oscillation in the periodic separation of charge within the molecules called induced dipole moment which result scattering of light at all directions (different angles). The scattered light intensity depend on wavelength, concentration and properties of samples. In the static light scattering used in this work, the laser was used because of its high intensity, monochromaticty and collimation beam compared with other light sources. Increasing the concentration means increasing the number of molecules which lead to increase the collisions between the laser and the Sodium Chloride. Also one can note that the scattered laser intensity at 45° of the zero concentration was (350.39) and that for the maximum concentration it was (493.30) and the scattered laser intensity at 90° of the zero concentration was (243.33) and for the maximum concentration it was (243.63) which was less than that of 45°. This proved that the scattered intensity at 90° is less than the scattered intensity at 45°. The scattered laser intensity at 135° of the zero concentration it was (258.63) while at maximum concentration it was (294.47) which were in middle way between the results of 45° and 90°. One can see that the intensity at 45° and 135° was higher intensity than 90° and also the intensity at 135° was lower than 45° ($I_{45} > I_{135} > I_{90}$). This is can explained as follows:

The scattered light at angle of 45° has higher intensity than others because the light has to pass longer path and this causes more collisions in the propagation direction. The angle of 90° has lowest scattered intensity than other angles because it has to pass shorter path and causes less collisions through the propagation direction. The angle of 135° has higher scattered intensity than 90° and less than 45°.

The setup and the detection system were very sensitive for any little change in the concentration of Heparin even of 1%.

It was found that the angular distribution of scattered intensity afford advantage, the laser scattering intensity at angles of ($45^{\circ}, 90^{\circ}$ and 135°) allow to study the goodness of the medical solution with any low amount of change in the concentration. It means that the setup was efficient for checking the quality of medical solutions.

3 – 4 Conclusions:

From the obtained results and analysis one can conclude that:

1- The laser scattered intensity at ($45^{\circ}, 90^{\circ}$ and 135°) increased with increasing the concentration.

2- The scattered light at 45° and 135° has higher intensity than 90° and the intensity at 135° was lower than 45° ($I_{45} > I_{135} > I_{90}$).

3- The angular distribution of the scattered laser light is an efficient technique for checking the quality of medical solutions from the point of concentration.

3 – 5 Recommendations:

Different suggestions can be recommended for future work like :

1- It is recommended to study the scattered intensity change in time, in addition to the angular distribution of the scattered intensity.

2- It is recommended to apply this technique for other medical solutions.
3- It is recommended to study the scattered intensity at other angles, in addition to angles of (45°, 90° and 135°).
4- It is recommended to use another laser sources like, HeNe laser 632.8 nm.
5- Using this technique to determine the molecular weight of the samples.

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