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Assessment of ALT and AST Enzymes Via Optical sensing

A Thesis Submitted to the Institute of Laser for Postgraduate Studies, University of Baghdad in Partial Fulfilment of the Requirements for the Degree of Master of Science in Laser / Biology

By

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بسم الرحمن الرحيم

قالوا سبحانكَ لا علمَ لنا إلا ما عَلمتَنا إنكَ أنتَ العَزيزُ الحكيمْ

صَدقَ العَظيمْ

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Dedication

This work is in dedicated to my parents and my husband Emad, the reason of my success, the shine of my life, who gave me support and force in every step of the road. I would also like to dedicate this work to my son Hani, my daughter Lamar, my brother, my sisters and my friends.

Hadeel Salam

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Praise be to "ALLAH" Lord of the whole creation

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septemper 2019

Abstract

Background: Biosensors can be well-defined as analytical procedures which contain a combination of biological sensing elements like sensor system and a transducer they includes a biological elements with a suitable transducer to yield a measurable the signal related to the concentration of biochemical species in different types of samples. Biosensors demonstrate an excellent presentation value due to the progression of technology and the characteristics features of exact identification.

Aim of study: To detect the level of Alanine transaminase (ALT) and Aspartate aminotransferase (AST) liver enzymes and its concentration through the using of different set up of laser biosensors as a new method.

Materials and Methods: Twenty four blood samples were collected from patient's males and females, their ages ranged from (15-72) years admitted to Ibn -Alnafees hospital complaining from heart and liver diseases. From each patient taking (10 ml) of blood were taken labelled, centrifuged and divided into two parts, (5ml) used for detection the concentration of ALT and AST enzymes examined manually in the hospital using RANDOX kit and spectrophotometer in at a wavelength (546) nm. The other 5ml is used to detect the concentration of enzymes by using three setup of constructed laser biosensor device. According to the optical properties of ALT and AST enzymes , standard wavelength (530-550 nm) as listed in the RANDOX kit and the refractive index of ALT and AST standard ,1.3460 au, 1.3470 au respectively by using abbey refrectometer were usedto select the suitable laser green light diode (semiconductor) at wavelength 532 nm, Power <1000 mW. Three types of optical fibers are used for the construction of biosensors to detect the concentration of ALT and AST enzymes in serum samples:

1-Single mode fiber (SMF-28) is sensing in 531. 62 nm and 531.16 nm respectively.

2- Multimode fiber (MM) is sensing in 531.16 nm for both enzymes.

3- Photonic crystal fiber PCF Machzender- interferometer (LMA-10) sensing in 531.16 nm for both enzymes**.** After Constructing biosensors (SMF, MMF and PCF) they were connect with green diode laser 532 nm according to the optical properties of the standard of ALT and AST enzyme. On the prepared segment of fiber (2 cm) of all setup, (1 ml) of standard and all samples are placed, connect with an optical spectrum analyzer (ocean HR-2000) to show the signal of intensity.

Results: The absorption of laser light by highly concentrated samples are higher and inversely proportional to the intensity of light, this means that the intensity of light at the detector side were high when the concentration of enzymes are low. This phenomenon could be explained as, the higher absorption of light by samples (ALT and AST enzymes) due to the selection of suitable laser which depends on the absorption of the samples to the wavelength of the laser. Multimode fiber (MMF) biosensor considered the more effective type because the signal of intensity of multimode fiber have a large number of modes compared with single mode fiber and photonic crystal fiber which have limited number of modes due to their basic structure.

Conclusion: In construction of three types biosensor: single mode, multimode and photonic crystal using green light diode laser 532nm to detect the level of ALT and AST liver enzymes in blood samples, multimode laser biosensor considered the best biosensor for detection the concentration of both enzymes in the sample in addition it is highly sensitive in transmission of signal light intensity. Biosensor are most accurate, with a rapid diagnosis ,less costley method than the traditional method to avoid any biological changes in blood sample lead to changes in optical characterastic (refractive index and absorption) of blood sample.

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Abbreviations	Meanings
ALT	Alanine transaminase
AST	Aspartate transaminase
ALP	Alkaline phosphatase
C	The concentration of analytic in sample
CDs	Compact disc
E	Energy
GGT	Gamma-glutamyltransferase
H	Planks constant
I	Intensity
I°	Initial intensity
KW	Kilo watt
L	The length of the light path in glass sample cuvette
LASER	light amplification by stimulated emission of radiation
LD	L-lactate dehydrogenase
M	Meter
n	Refractive index
NAFLD	Non - alcoholic fatty liver disease
nm	Nano meter
Ɵ	Angel
Ɵ1	Incident angle
Ɵ2	Refractive angle
PT	Prothrombin time
SI	Intensity system
V	Velocity of light in matter
W	Watt
A	Absorption coefficient
λ	Wavelength
λ1	original wavelength
λ2	New wavelength
RI	Refractive Index
TIR	Total Internal Reflection
LOD	Limit Of Detection
EW	Electromagnetic Wave

Chapter One
Introduction and basic concepts

Chapter One

1.1 Introduction

This chapter focuses on liver and its enzymes, laser, fibers and biosensor. Where defined liver and its enzymes such as (ALT and AST) and its functions and disease. Then define laser, fibers, evanescent wave, in-Line PCF Mach-Zehnder Modal Interferometer, Biosensors and their classifications, applications and the optical biosensor explained briefly.

1.2 Liver

Liver is a part of the digestive system, second largest organ in the body and is located under rib cage on the right side.

and nutrients through the conversion of food and drinks, removal of harmful substances from the blood, detoxification, protein synthesis, and the production of chemicals that help in the digestion of food. [1, 2]

Shown in figure (1.1).

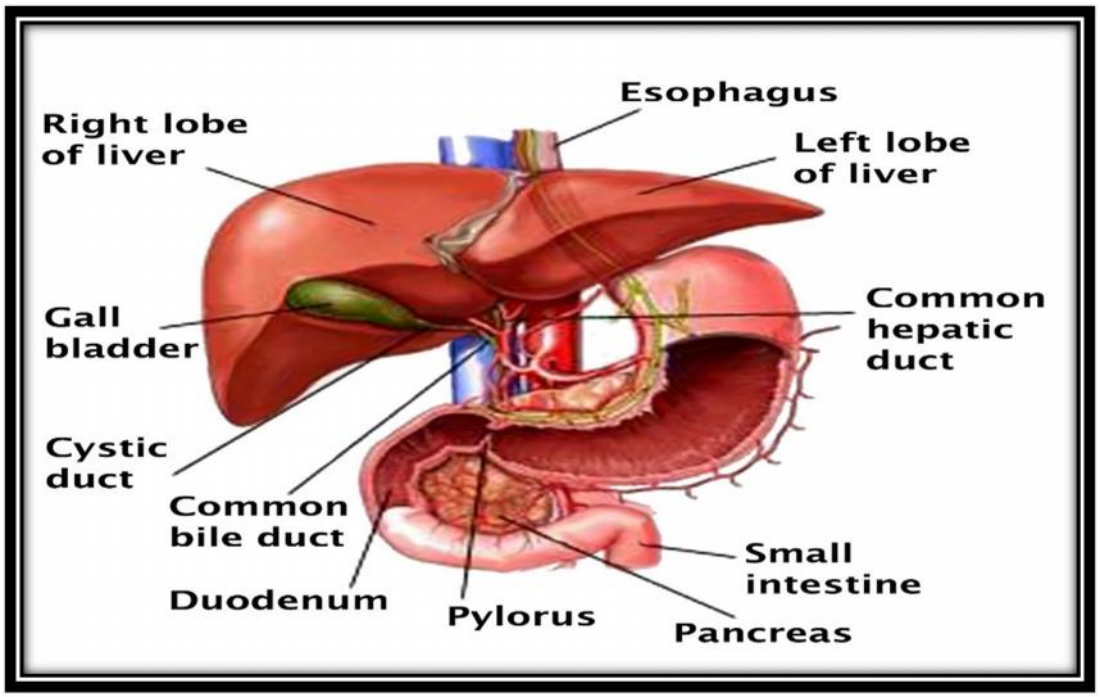


Figure (1.1) Anatomy of liver [3]

1.2.1 The major functions of the liver

The liver is classed as a gland and associated with many functions. It is difficult to give a precise number, as the organ is still being explored, but it is thought that the liver carries out 500 distinct roles:[4, 5] the major functions of the liver includes:

1-Fat metabolization: Bile breaks down fats and makes easier todigest.

2-Metabolizing of carbohydrates: Carbohydrates are stored in the liver, where they are broken down into glucose and siphoned into the bloodstream to maintain normal glucose levels. They are stored as glycogen and released whenever a quick burst of energy is needed.

3-Absorbing and metabolizing bilirubin: Bilirubin is formed by the breakdown of haemoglobin. The iron released from haemoglobin is stored in the liver or bone marrow and used to make the next generation of blood cells.

4-Vitamin and mineral storage: The liver stores vitamins A, D, E, K, and B12. It keeps significant amounts of these vitamins stored. In some cases, several years' worth of vitamins is held as a backup. The liver stores iron from haemoglobin in the form of ferritin, ready to make new red blood cells. The liver also stores and releases copper. [5]

5-Supporting blood clots: Vitamin K is necessary for the creation of certain coagulants that help to clot the blood. Bile is essential for vitamin K absorption and is created in the liver. If the liver does not produce enough bile, clotting factors cannot be produced.

6-Bile production : Bile helps the small intestine breaks down and absorbs fats, cholesterol, and some vitamins. Bile consists of bile salts, cholesterol, bilirubin, electrolytes, and water.

7-Production of albumin: Albumin is the most common protein in blood serum.It transports fatty acids and steroid hormones to help maintain the correct pressure and prevent the leaking of blood vessels.

8-Synthesis of angiotensinogen: This hormone raises blood pressure by narrowing the blood vessels when alerted by production of an enzyme called renin in the kidneys.

9- Metabolisem of proteins: Bile helps break down of proteins for digestion.

10-Filtration of blood: The liver filters and removes compounds from the body,including hormones, such as estrogen and aldosterone, and compounds from outside the body, including alcohol and other drugs. [6]

A. Tests of the liver's capacity to transport organic anions and to metabolize drugs- Serum bilirubin, urine bilirubin, urobilinogen etc

B. Tests that detect injury to hepatocytes (serum enzyme tests) Aminotransferases, alkaline phosphatase, ãglutamyl transpeptidase, 5 nucleotidase, leucine aminopeptidase etc.

C. Tests of the Liver's biosynthetic capacity- Serum proteins, albumin, prealbumin, serum ceruloplasmin, procollagen III peptide, a 1 antitrypsin, a feto protein, prothrombin time etc. [6]

1.3 Enzymes

Enzymes are cellular proteins that aid in speed up (catalyse) chemical reactions in the human body.They bind to molecules and alter them in specific ways,they are necessary for respiration, digesting food, muscle and nerve function, among thousands of other roles. Enzymes are built of proteins folded into complicated shapes; they are present throughout the body. The chemical reactions that keep us alive - our metabolism -rely on the work

That enzymes carry out. Enzymes speed up (catalyse)chemical reactions; in some cases, enzymes can make a chemical reaction millions of times faster than it would have bee with out it a **substrate** bindto the **active site** of an enzyme and is converted into **products**. Once the products leave the active site, the enzyme is ready to attach to a new substrate and repeat the process show in figure (1-2). [1]

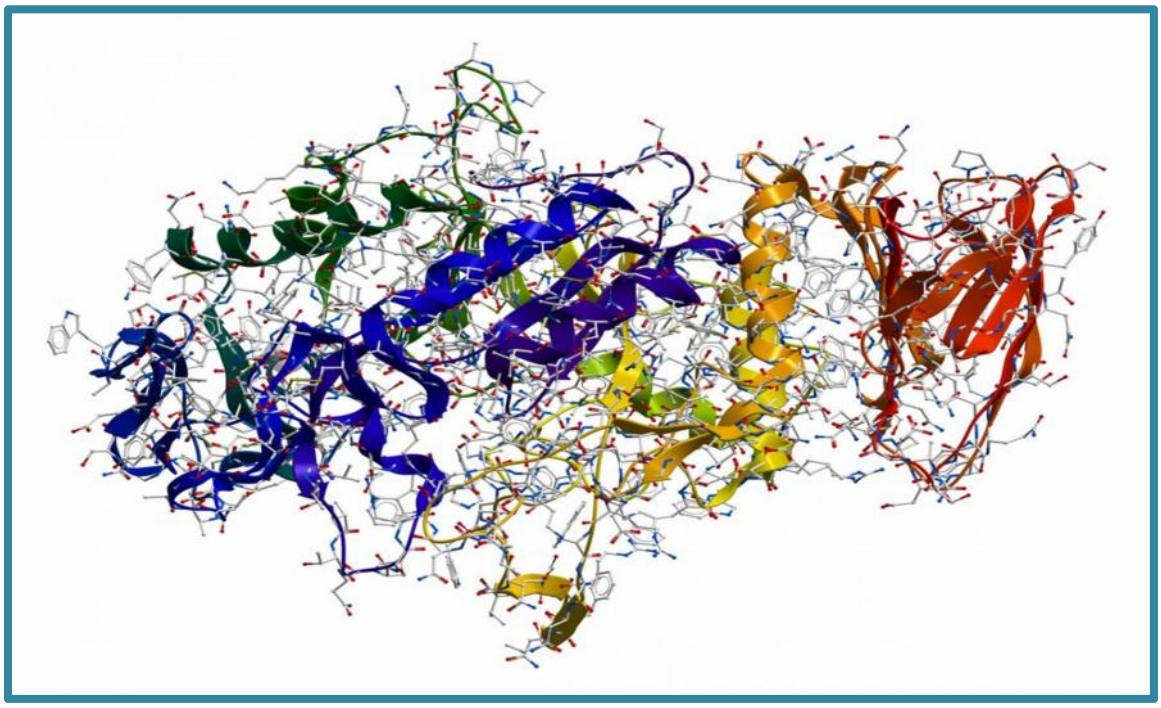


Figure (1- 2) The enzyme that amylase breaks down the starch. [1]

1.3.1 Mechanisms of enzymes

The "lock and key" model was first proposed in 1894. In this model an enzyme's active site is a specific shape, and only the substrate will fit into it, like a lock and key. This model has now been updated and is called the induced-fit model. In this model, the active site changes shape as it interacts with the substrate.Once the substrate is fully locked in and in the exact position, the catalysis can begin. The active site of an enzyme are bind to the substrate, later is changed into products. As the active site are leaved by the products as shown in figure (1-3), a new substrate

Are ready to attach by the enzyme and repeat the process. [1]

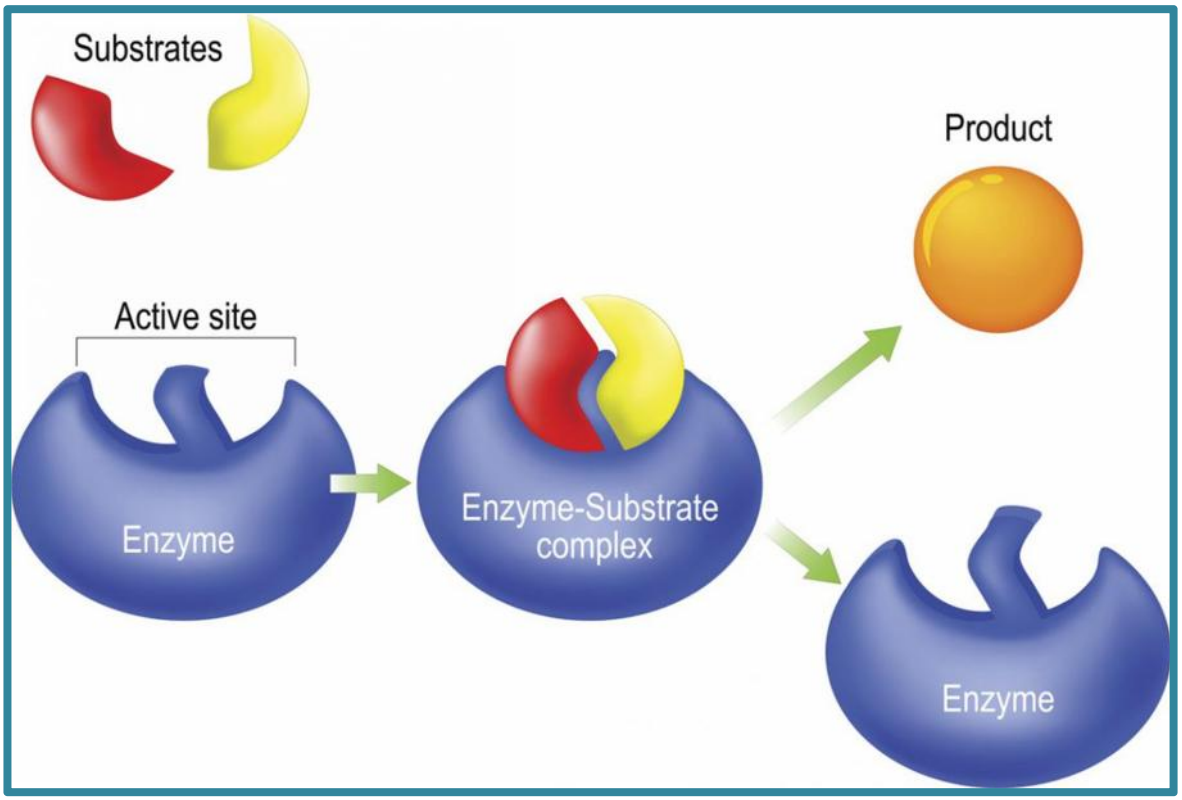


Figure (1-3): Enzyme lock and key model [1]

1.3.2 Liver enzymes

Examination of the levels of certain enzymes and proteins in the blood t hat are higher or lower than normal can indicate liver problems.

Liver function tests include:

1-Alanine transaminase (ALT). ALT is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and levels increase. [7]

2- Aspartate transaminase (AST). AST is an enzyme that helps metabolize amino acids. Like ALT, AST is normally present in blood at low levels. An increase in AST levels may indicate liver damage, disease or muscle damage.

3- Alkaline phosphatase (ALP). ALP is an enzyme found in the liver and bone and is important for breaking down proteins. Higher than normal levels of ALP may indicate liver damage or disease, such as a blocked bile duct, or certain bone diseases. [7]

4- Gamma - glutamyltransferase (GGT). GGT is an enzyme in the blood. Higher - than - normal levels may indicate liver or bile duct damage.

5- L - lactate dehydrogenase (LD). LD is an enzyme found in the liver. Elevated levels may indicate liver damage but can be elevated in many other disorders.

6- Prothrombin time (PT). PT is the time it takes by blood to clot. Increased PT may indicate liver damage. [7]

1.3.3 Liver diseases

An unhealthy, malfunctioning or diseaeased liver can be hazardous or even lethal. There is a variety of conditions that affect the liver. Examples of liver disease include:

1- **Fascioliasis:** caused by the two spp f.hepatica ,f.gigantica attack of a parasitic worm.

2- Cirrhosis: the liver cells exchanged in a process known as fibrosis, produced by an amount of factors, containing toxins, alcohol, and hepatitis. Fibrosis causes liver failure as the functionality of the liver cells is injured.

3- **Hepatitis:** Hepatitis is the term specified to a general infection of the liver, initiated by viruses, toxins, or an autoimmune response. It is characterized by inflammation of the liver, may cause liver failure in severe cases shown in figure (1-4), **NASH** (Non alcoholic state hepatitis) [8], [9].

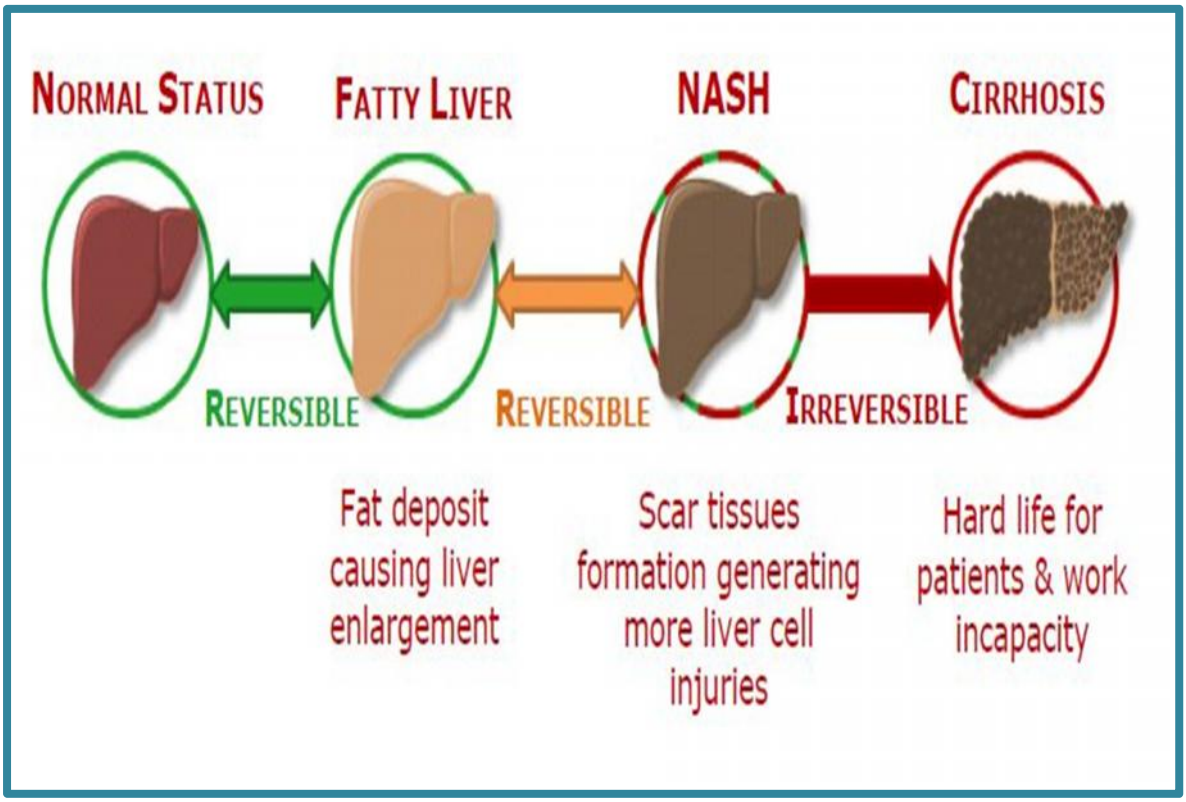


Figure (1-4) Steps of liver diseases [10]

1.3.4 Liver function tests

Liver function tests are important in the detect assistance of liver damage. The measurement of certain proteins and enzymes in blood had been done by these blood tests.

Liver function tests remain important for the followings:

1- Monitor the infections of liver, such as hepatitis.

2- Observe the development of a disease, like alcoholic, viral hepatitis, and control the treatment.

3- Give notice about the progression of a disease, mainly scarring (cirrhosis).

4- Screen medications side effect mainly with warfarin (blood-thinning drugs). [11]

1.3.5 Alanine transaminase (ALT)

This enzyme established in the liver, kidneys, heart, and muscles in high concentrations, mainly consist of 496 amino acids and are determined by a gene situated in the long arm of chromosome 8.

The transamination reaction are catalyzed by enzyme, elevation the rises in ALT level appeared with any kind of liver damage. Liver damage are caused by liver inflammation (hepatitis), injury due to toxin and ischemic liver disease leads to increasing the level of ALT enzyme, in Hepatitis C the level of ALT enzyme increases more than in Hepatitis A and B. the rises in ALT level mainly linked to fat accumulation in liver during childhood obesity, inflammation due to fatty liver disease, Alcoholic and Non-alcoholic fatty liver disease .

Metabolic syndrome (Bright liver syndrome) is suggestive when there is increasing in level of ALT enzyme. [9]

The reaction it catalyzed and transfer the amino group from L-alanine to $\propto -$ ketoglutarate , the product of this reaction are pyruvate + L- glutamate.

1.3.6 Aspartate transaminase (AST)

Highest concentration level of AST enzyme are found in the liver, heart, muscle, kidney, brain, pancreas, and lungs. Increasing the level of AST contained in certain organs are a moderately less specific indicator of liver damage as related to ALT level. Tissue necrosis happened in myocardial infarction and chronic liver diseases, liver cirrhosis indicate a highly suggestive of increasing the level of mitochondrial AST in bloods.

The normal range for two enzymes ALT and AST are:

In Males from (8-35) U/L.

In Females from (6-25) U/L.

1.3.7 AST: ALT ratio

The ratio of AST to ALT is of use in Wilson disease, CLD and alcoholic liver disease and a ratio of more than 2 is usually observed. The lack of ALT rise is probably due to pyridoxine deficiency. In NASH the ratio is less than one in the absence of fibrosis on liver biopsy. [12]

In viral hepatitis the ratio is usually less than one. The ratio invariably rises to more than one as cirrhosis develops possibly because of reduced plasma clearance of AST secondary to impaired function of sinusoidal cells. [13]

ALT exceeds AST in toxic hepatitis, viral hepatitis, chronic active hepatitis and cholestatic hepatitis, if the AST: ALT ratio > 2 that means occurs cirrhosis. [14]

1.3.8 Mitochondrial AST: Total AST ratio:

This ratio is characteristically elevated in alcoholic liver disease. Abstinence from alcohol improves this ratio. It is also seen to be high in Wilson's disease. [12]

1.4 LASER

LASER (Light Amplification by Stimulated Emission of Radiation)

Laser is a light that have the high intensity and highly directional light. [15] Charles Hard Townes is an American scientist and two Soviet scientists, Alexander Mikhailovich Prokhorov and Nikolai Gennediyevich Basov, they desirable Nobel Prize (1964) and basic concepts for laser [16]. Scientist who was firstly demonstrated laser by experiment through light flashing determined ruby crystal is TH Maiman from Research Laboratory Hughes, California, in 1960. [17, 18]

Laser is a light through a method called stimulated emission of radiation which intensifies or increases the intensity of light. Some lasers yield visible light but others produce ultraviolet which arein visible. The energy of the emitted photon

When electron displays from the level of higher energy to the level of a lower energy, it releases light or photon show in figure (1-5). [19, 20]
This is equal to the energy variance between the energy levels. Laser is diverse from the conventional light. Laser light has extra-ordinary properties which are not existing in the conventional light bases like sun and incandescent lamp.Laser produce greatly directional, monochromatic, coherent and polarized light beam. There are many uses of laser mainly in welding and Cutting, surveying, in medical applications, laser nuclear fusion, garment industry, treatment, barcode. [21, 22]

The energy emitted from the orbit of electron is bigger for orbits additional after the nucleus of atom as seen in classical view. Therefore, originated electrons are now at exact level of energy of an atom, are illustrated in figure (1-6).

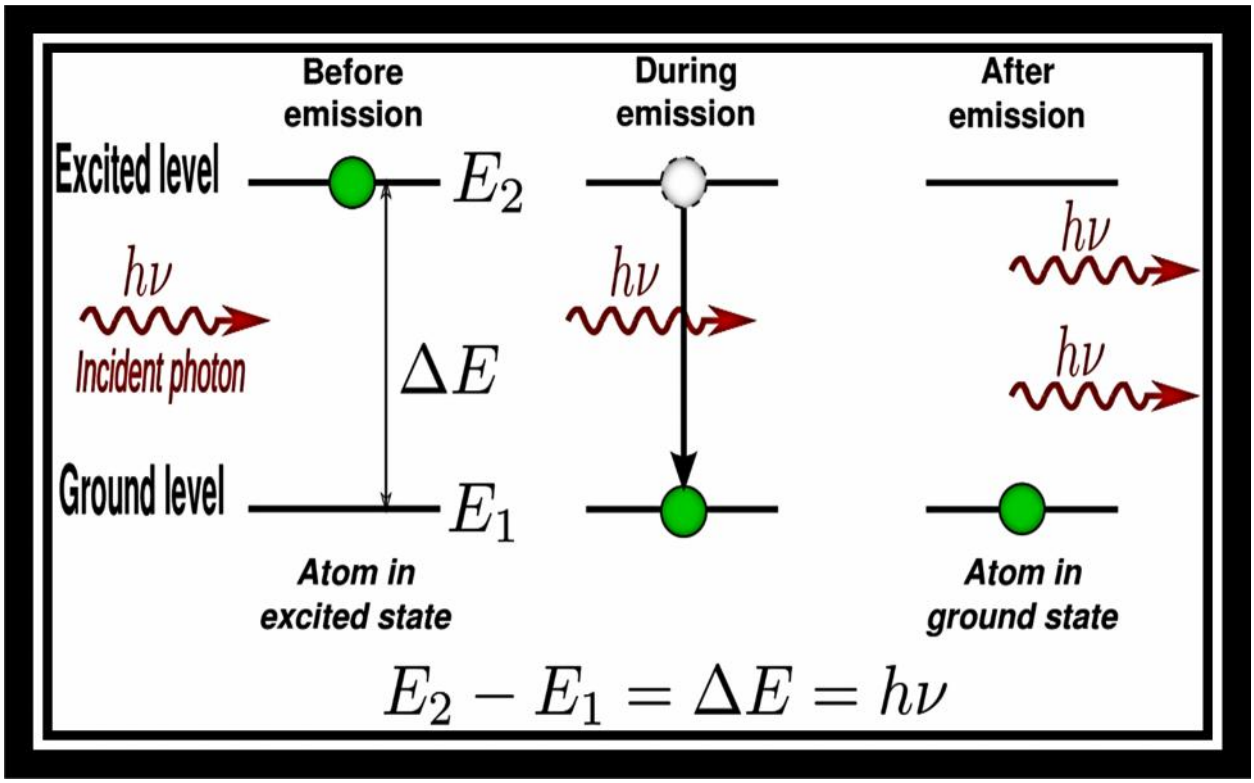


Figure (1-5): excitation state [23]

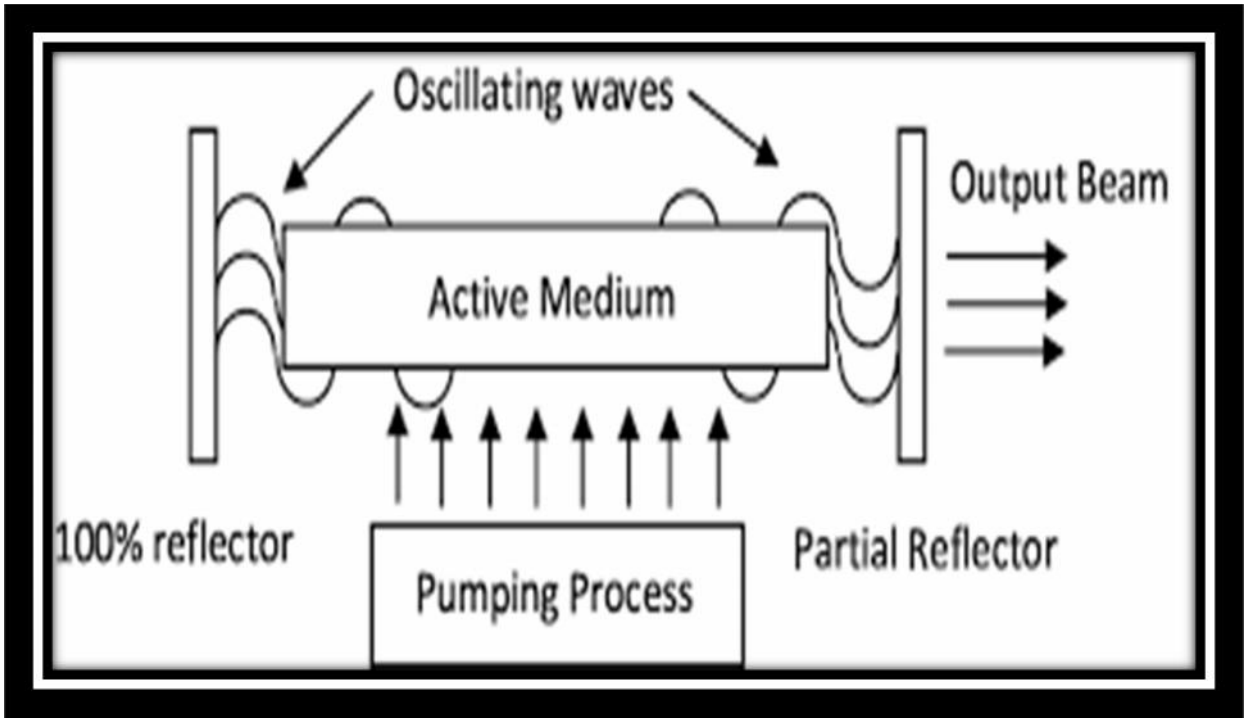


Figure (1-6): laser principle design [23]

1.4.1 Absorption

A simple phenomenon can be used in bio-sensing, Absorption is A process in which all or part of the energy of the photon is converted into Other types of energy, like heat. The absorbance can be measured by directing a beam of radiation at the sample and detecting the intensity of the radiation (emission) that comes across it according to beer lambert law. [24]

Beer-Lambert law

- Light Absorbance: $(A) = \log (I_0 / I) = \varepsilon LC$
- Light Transmission $(T) = I/I_0 = 10^{-\varepsilon CL}$

- I_0: Light Intensity entering a sample
- I: Light Intensity exiting a sample
- C: The concentration of analyte in sample
- L: The length of the light path in glass sample cuvette
- ε: a constant for a particular solution and wave length

Some techniques of absorption spectroscopy have been advanced to estimate the absorption as a function of wavelength, such as ultraviolet-visible, infrared and X-ray absorption spectroscopy. [25]

1.4.2 The intensity of light

Intensity is the influence transported in the area, anywhere the area is distinguished continuously the flat vertical to the energy direction. Using SI system, it has parts watts per square meter (W/m^2). It is used most regularly with waves (example - sound or light), in which case the normal power transfer over one period of the wave is used. **Intensity** can be useful to other conditions where energy is transported. [26]

The intensity of modulated light can be measured by a light receiving component and transformed into an electrical signal. The electrical signal change is proportional to the change of the measured. Different sensors, like Mass and refractive index sensors have been implemented based on this modulation technique. [23]

1.4 .3 Light-matter interaction.

Some effects are produced once the light incidence with matter with no disturbance toward the electron levels of atoms and so, variations not present in the light wavelength or energy. So, the simulated of light occurred, absorbed, dispersed, and directed with the original wavelength (λ1). When the levels of electrons are changed, some molecules are absorbed light, causing original luminescence (light emission), through greater wavelength (λ2) .Altogether of these occurrences are illustrated in Figure (1-7). [27]

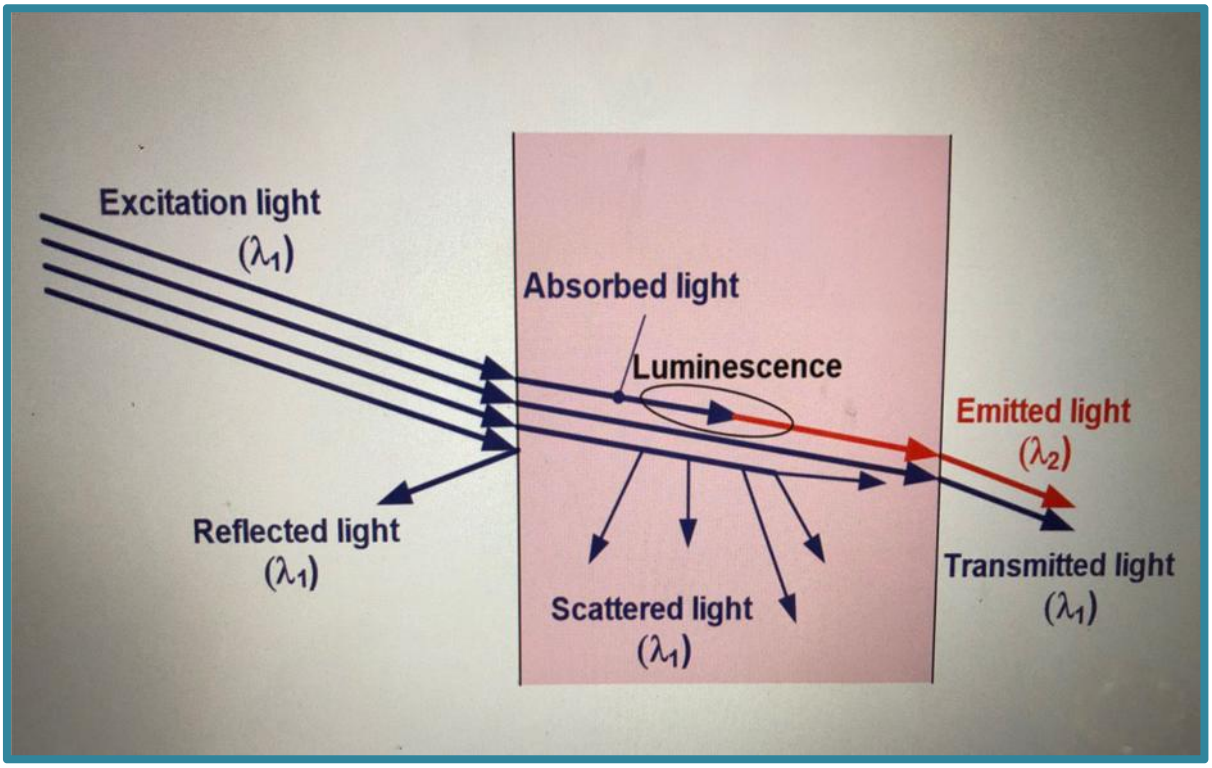


Figure (1-7) some occurrences produced by the light-matter interaction [27]

Additional variations container can perform, like polarization of light or alteration in light polarization angle. Therefore the substance characteristics are changed (direction, intensity, wavelength and polarization) of matter. [28]

1.4.4 Laser tissue interaction (propagation of laser light in matter)

Beam of laser that meetings media superficial can be imitated, transmitted, absorbed or may be scattered at different level. When the laser beam drops on the media, it is not consider as a light but as a nonstop or pulsed is trains of photons. Photon as a particle can only intermingle by substance through removing energy amount. Thus, photons that are absorbed only can yield effect in the tissue. Achromophore is requisite for absorption of photon Inside the tissue. The photon absorption are increased by reducing its reflectance, scattering and transmission. [29]

1.4.5 Types of lasers

Lasers may be classified according to several criteria

1-Based on the mode of operations:

- ❖ Pulsed system (single pulsed or repetitively pulsed).
- ❖ Continuous wave system.
- ❖ Single pulsed Q-switched system.
- ❖ Mode locked system.

2-Based on mechanism in which population inversion:

- ❖ Three level system.
- ❖ Four level system.

3-Based on active medium:

- ❖ Gas laser.
- ❖ Solid laser.
- ❖ Semiconductor laser.
- ❖ Tunable dye laser.

4-Based on characteristics of the laser radiation:

- ❖ Fixed frequency.
- ❖ Tunable.

5- Based on the excitation mechanism (pumping) method of the active medium:

- ❖ Optical pumping.
- ❖ Electrical pumping.
- ❖ Laser pumping.

6-Bsed on the laser output power:

- ❖ Low.
- ❖ Medium.
- ❖ High. [30]

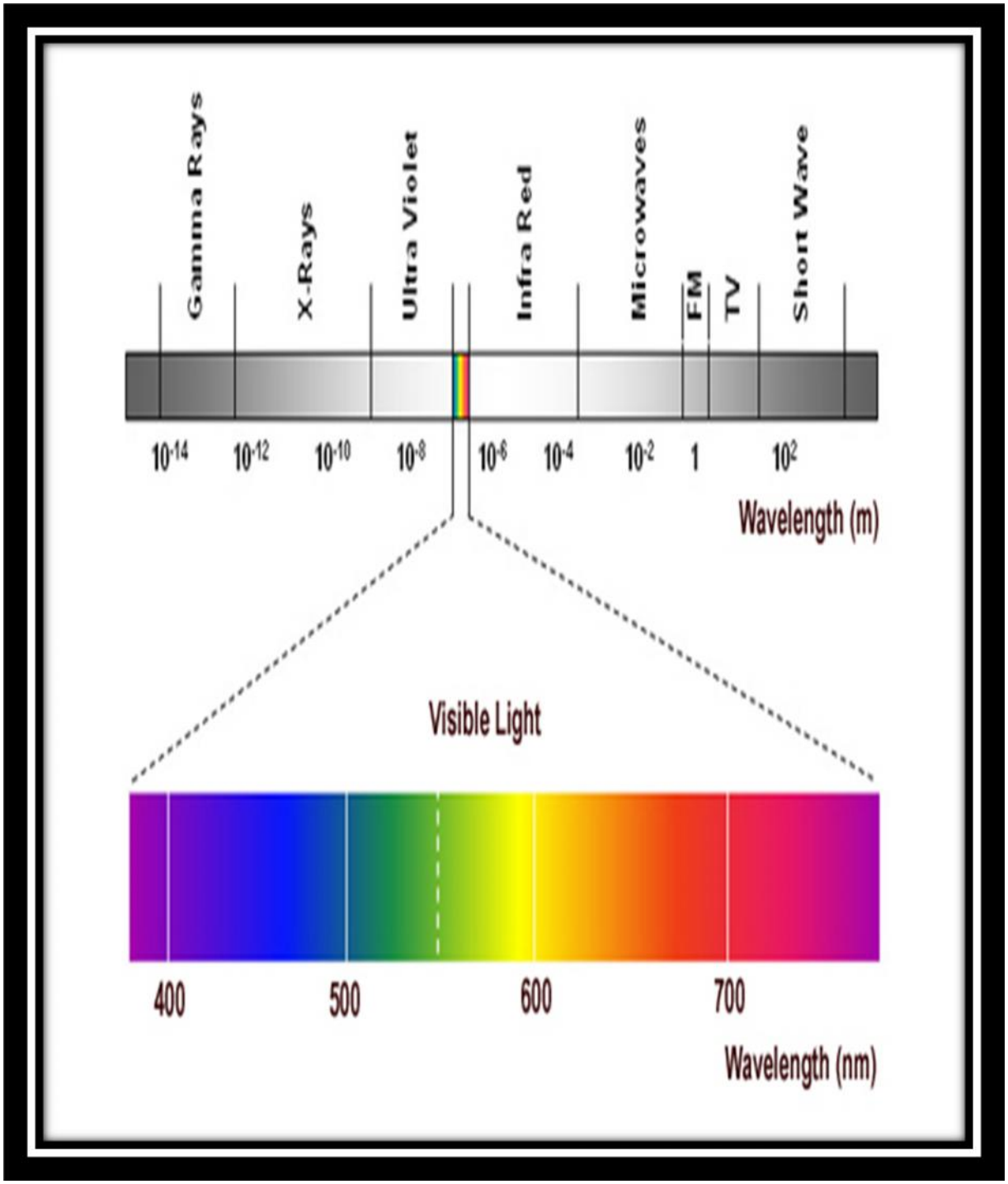


Figure (1-8) wavelength of commercially available lasers [31]

1.5 FIBERS

Optical fibers are used to transfer of light and find different application in fiber optic transportations, optical documentation.
Fiber transmission above lengthier spaces and having higher bandwidths more than present in the wire cables [32]. Optical fibers are used a instead of metal wires because amounts of signals damage are minor in amounts that moves along the fibers. They are resistant to interference occurred electromagnetically, easy after which wires metal in type wounded extremely. Optical fibers are also appliedin illumination, enclosed in packages (bundles) for this they can be used in images transportation.
Therefore permitting watching limited spaces, like in a fiberscope.
. Exactly directed fibers have a variety of application, like fiber lasers and fiber optic sensors presented in figure (1-9). [33]

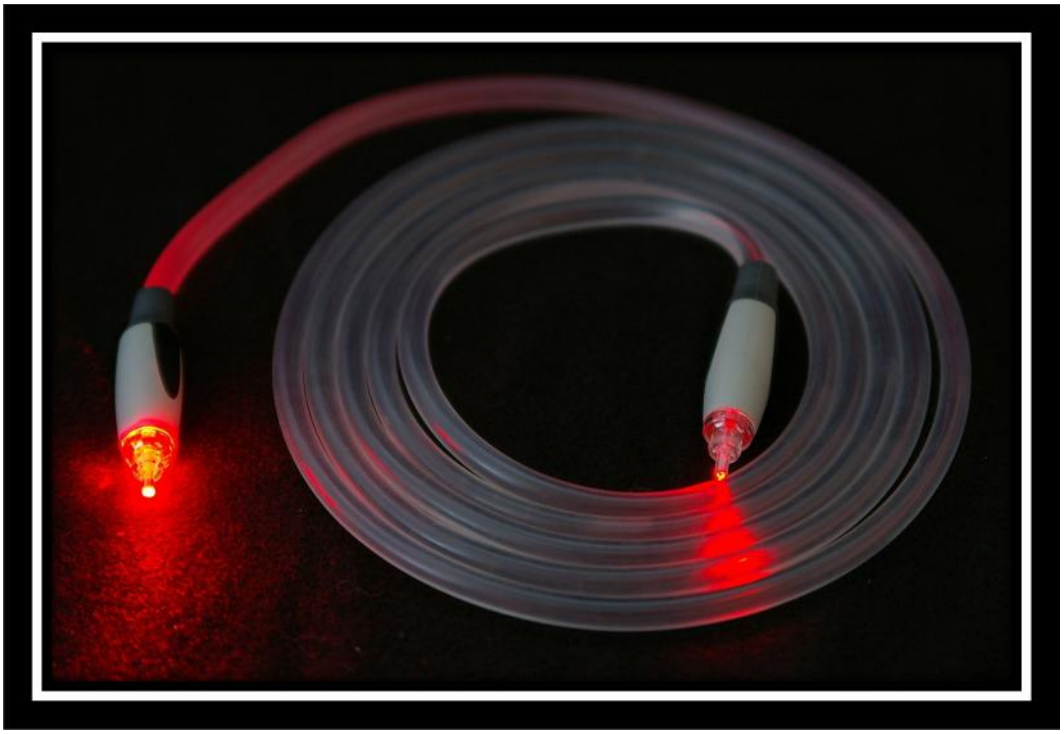


Figure (1-9) Cable optical fiber [32

1.5.1 Single Mode Fiber

A single-mode fiber (SMF) is an optical fiber intended to transmit light in adown direction - the transverse mode. Waves can require the same mode but have altered or different frequencies. In single-mode fibers, waves appeared with different frequencies and with the same mode, means that they are distributed in space in the same way, and that provides a single beam of light. Though the ray movements parallel to the length of the fiber, it is called transverse mode meanwhile its electromagnetic alternations occur perpendicular (transverse) to the length of the fiber. **)**.show in figure (1-10) [34, 35]

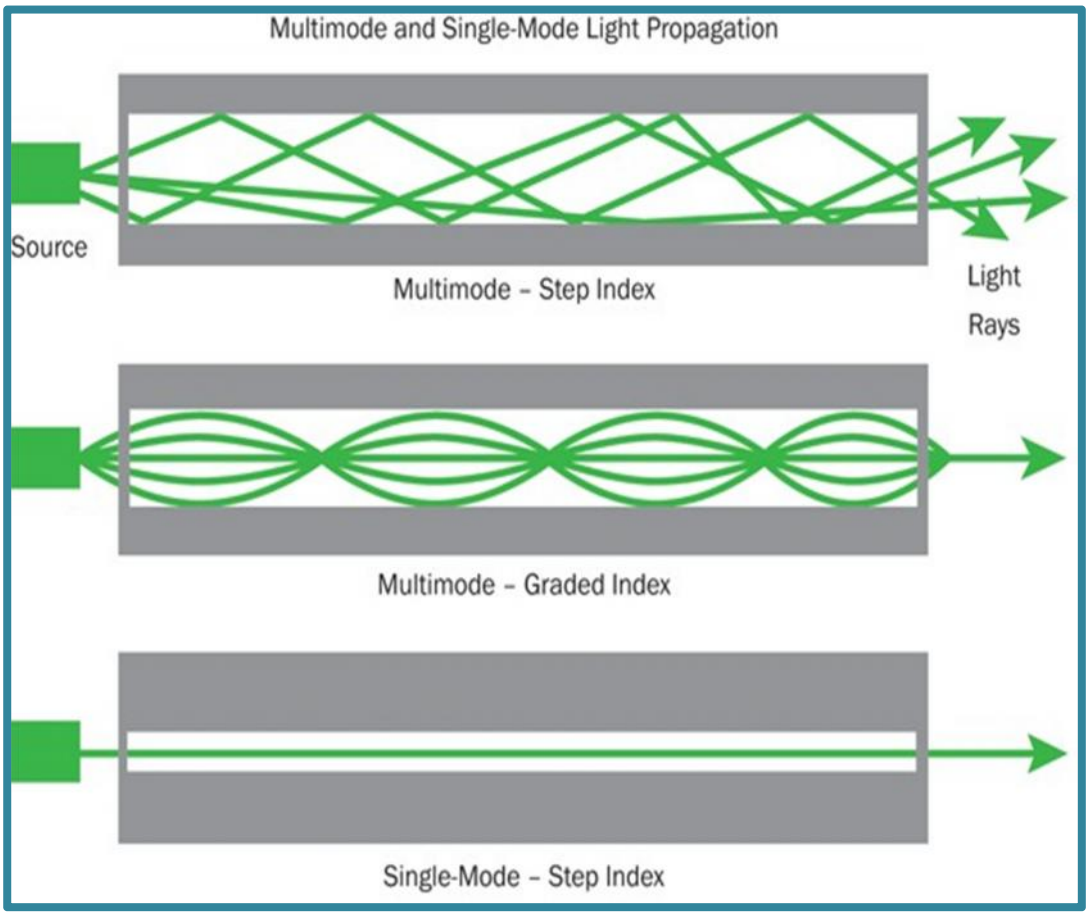


Figure (1-10): Single mode and Multimode fiber [36]

1.5.2 Multi-mode Fiber

Multimode fiber mainly associated with a core large in diameter (More than 10 micrometers). Emissions of light in multimode fiber Are directed along core by total internal reflection .Emission of rays that encounter the boundary at a short angle are diverted from the core into the cladding, and do not transportation light, figure (1-11) showing the diffrence among Single mode and Multimode fibers. [37,38]

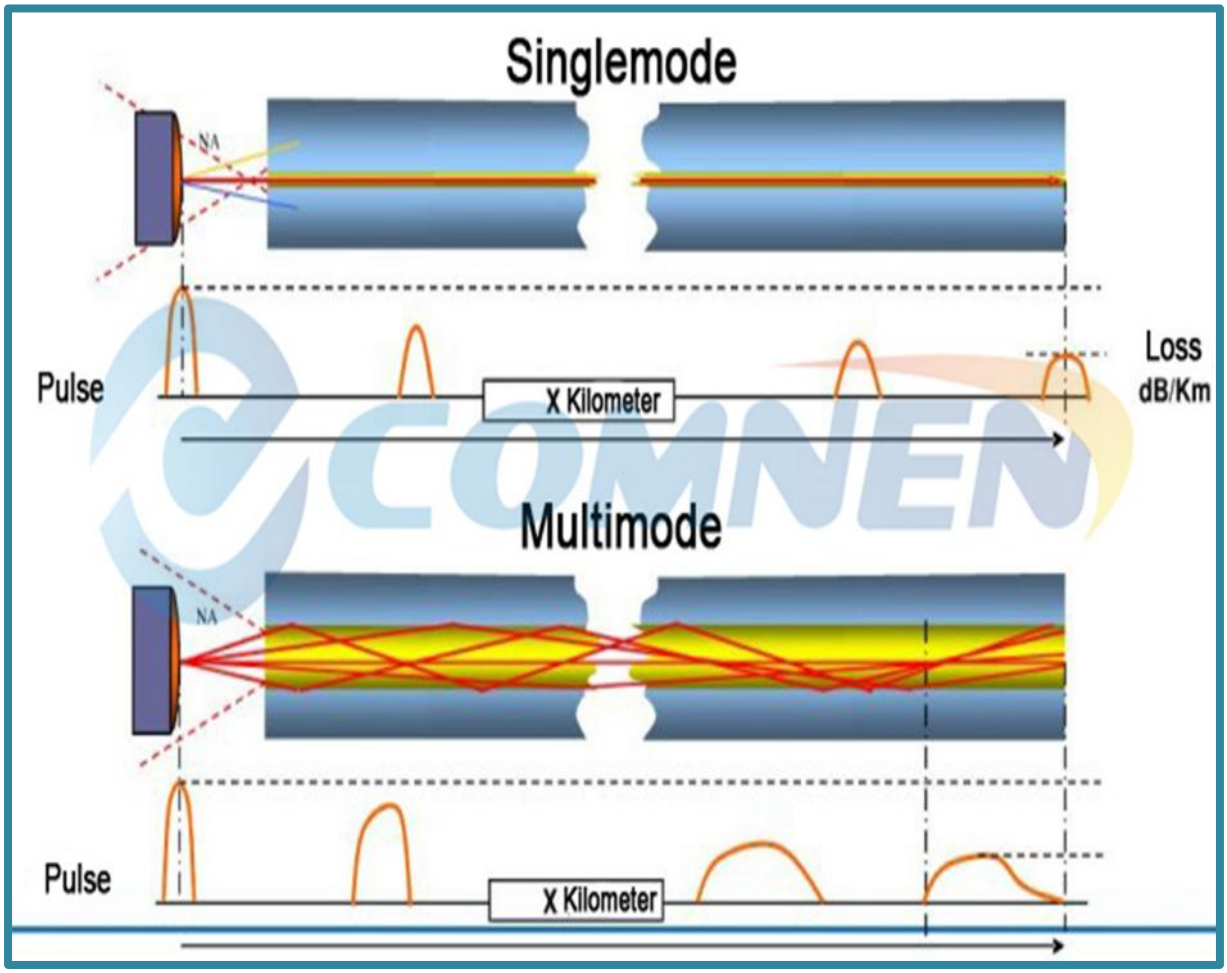


Figure (1-11): Difference concerning Single mode and Multimode Fiber Patch Cables cords. [39]

1.5.3 Photonic crystal fiber (PCF)

This type of fiber are also named as microstructure or holey fibers. Photonic crystal fibers (PCFs), have an intermittent collection of micro holes that track length ways the whole length of fiber. The light-guiding mechanisms in case that means of a modified total internal reflection [40, 41]

Exceptional guiding mechanism and modal properties of photonic crystal fibers discuss the number, size, shape, and the parting between the air-holes as well as the air-hole organization [42, 43].

A PCF using LMA 10 fiber, created the information that higher order modes can remain in the core of a PCF with a small length showing in figures (1-12A, b), (1-13). [44, 45]

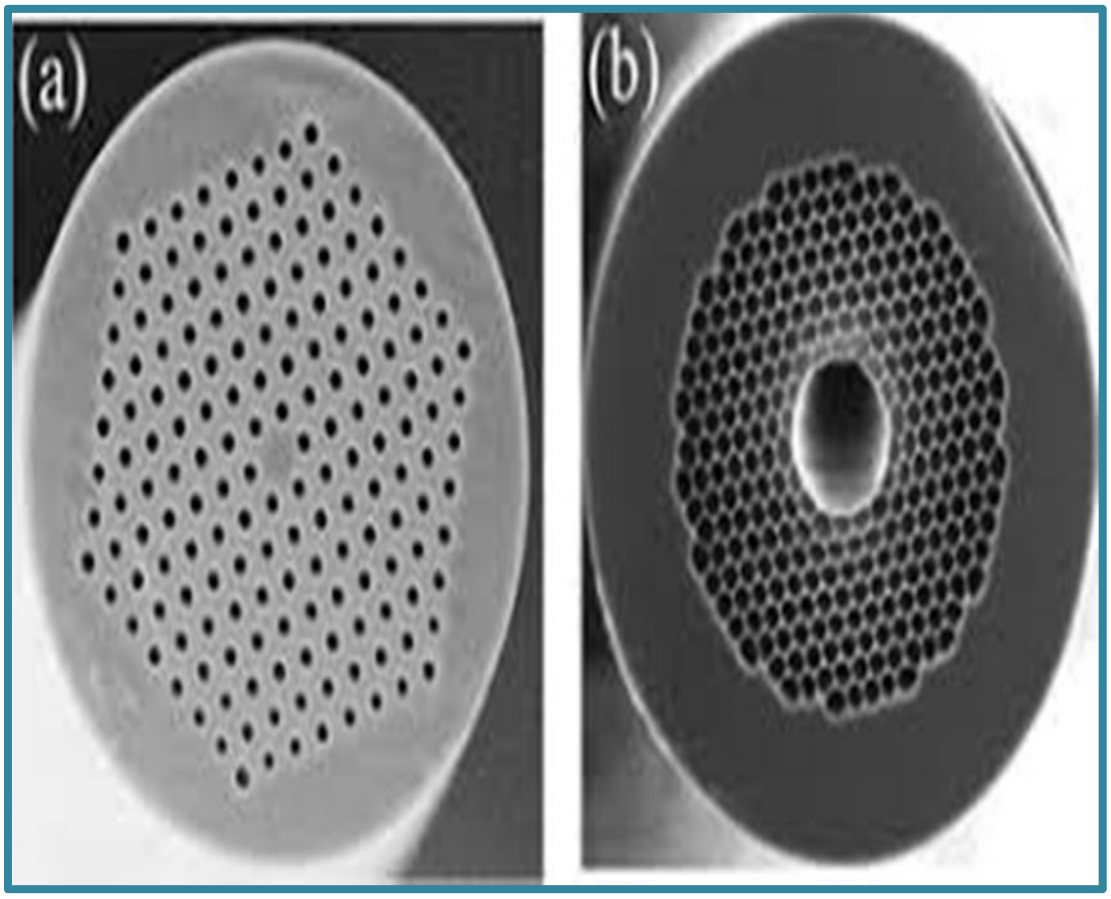


Figure (1-12) :(a) solid core (b) hollow core [46]

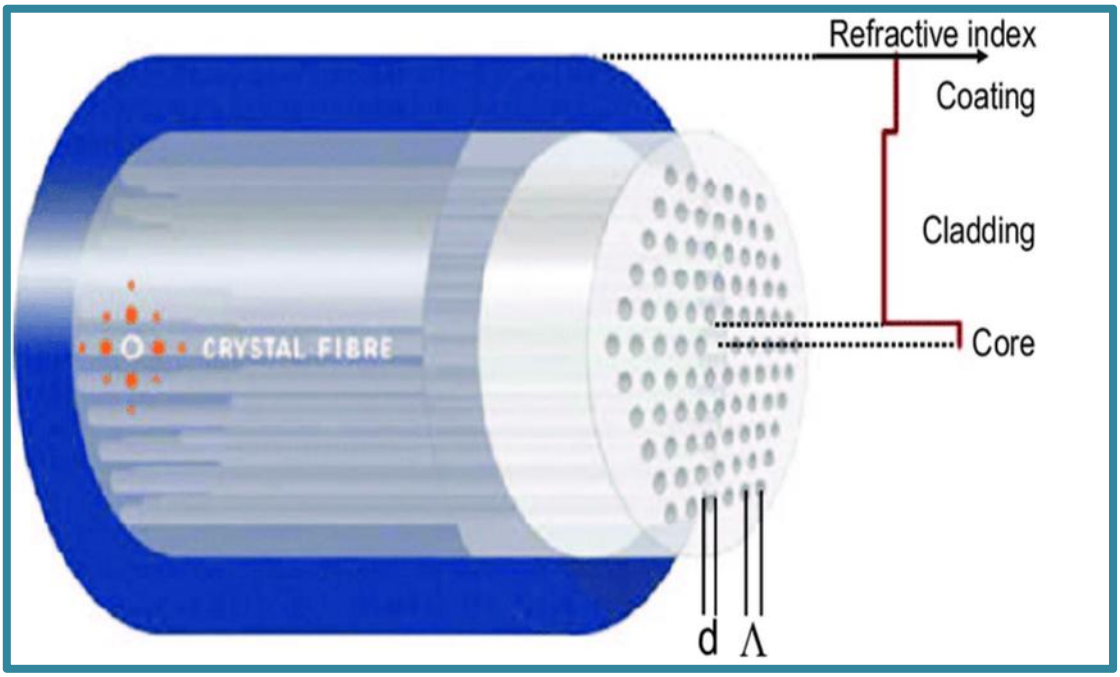


Figure (1-13): Photonic crystal fiber with a solid core. Air holes decrease the effective index of the cladding below that of the core which can then guide light. [46]

1.5.3.1 Large Mode Area Photonic Crystal Fibre (LMA)

The special feature of photonic crystal fibres is the geometrical properties of its cross-section, by controlling on these properties, PCFs with large effective area can be designed .This type of optical fibres called large mode area (LMA) or single-mode photonic crystal fibre which can be designed by making a small relative hole size and large hole-to-hole spacing in a considerable range. This

Type of fibre can be kept away the material damage with high power lasers. Thus, large mode area can be employed for propagating a high power laser. In spite of that the photonic crystal fibres and standard optical fibres can give the same mode areas at a given single wavelength, large mode area PCFs have a different advantages for broadband and high beam quality applications due to

Their capability to be a single mode with large mode area along a broad wavelength range. [47, 48.49]

1.5.3.2 In-Line PCF Mach-Zehnder Modal Interferometer with Mic-Hole Collapsing

Micro-hole collapsing is a simple kind of MZI because its fabricati only need to cleave and splicing the optical fiber. By changing the parameters of the fusion splicing, the collapsed region can be controlled. At region where the holes are collapsed, there is no fiber cladding and the photonic crystal fiber is no more in single mode. This way can be designed by splicing PCF between two SMFs as shown in figure (1-14). At the first collapsed region, the air hole is completely collapsed, the beam mode of the core is diffracted, and worked as a splitter. In the PCF core, the beam will be expanded and a part of core mode can be coupled to the PCF cladding modes at the collapsing region. After propagating the length of the PCF (L) and when they reach of second collapsed region, they will recombine. [50]

By using Gaussian beam approximation, broadening of a fundamental mode can be measured. Mode field diameter (MFD) at the splicing point (z) can be :calculated from

$MFD = 2w\sqrt{1} + (2)$

Where (n) is the refractive index of the pure silica (n silica=1.45), λ is the light wavelength and (w) is the light spot size. In general interference pattern which is depending on the length of optical path, shows the displacement between two-arms of interferometer because of core mode has a higher effective index than the cladding mode. Therefore, the physical lengths of interferometer arms are similar; the spatial frequency of the wavelength spectrum is directly related with the different between the effective indices of the cladding and core

Mode [51]. The intensities of core and the cladding modes can be measured as function of a physical length (L), wavelength and phase difference (φ): $I = ICO + ICL + 2\sqrt{ICOICL}\ (\Delta\varphi)$

Where ( ), (ICO) are the mode intensities of cladding and core mode respectively.

Where L is the interaction length (the sensing area length), is the wavelength in the vacuum, and $\Delta n = ncore - nclad$ is the change in the effective refractive index of the mode. By changing the length of the photonic crystal fibre, the fringe or period spacing in these interferometers can be easily control [52]

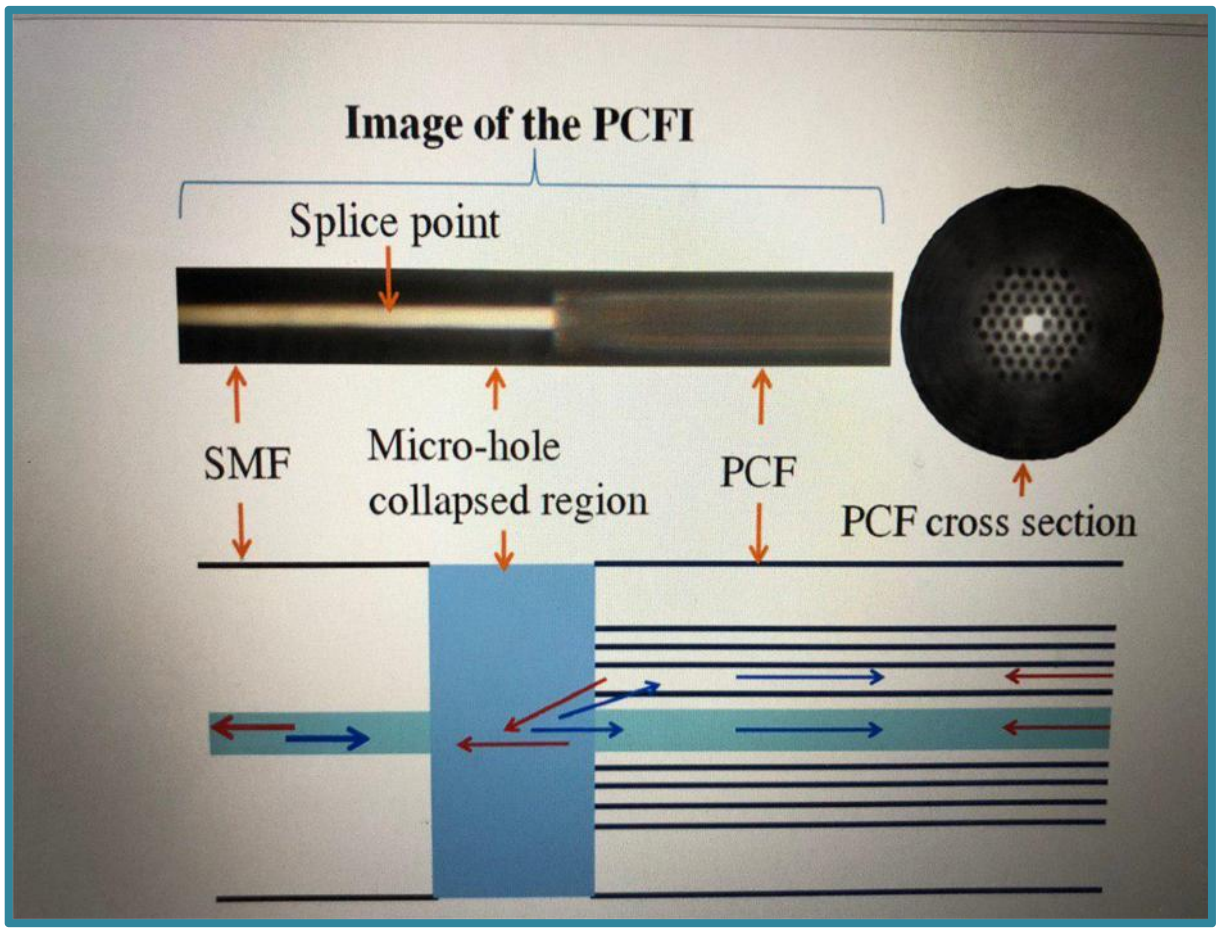


Figure (1.14) Diagram illustrate transmission of light between single Mode fiber and photonic crystal fiber, excitation and recombination Of modes in the whole collapsed region. [53]

1.5.3.3 Unclad Evanescent Wave Optical Fibre Sensor

Evanescent wave technique is used in a particular class of optical fibre sensor for monitoring and measurement of asset of chemical and physical variables [54]. An evanescent wave is created whenever light undergoes TIR at the core cladding interface. The evanescent wave penetrating a small distance into the cladding of optical fibre. Figure (1-15) shown the evanescent wave which decays exponentially from the interface of core and clad and propagating parallel to it. [55]

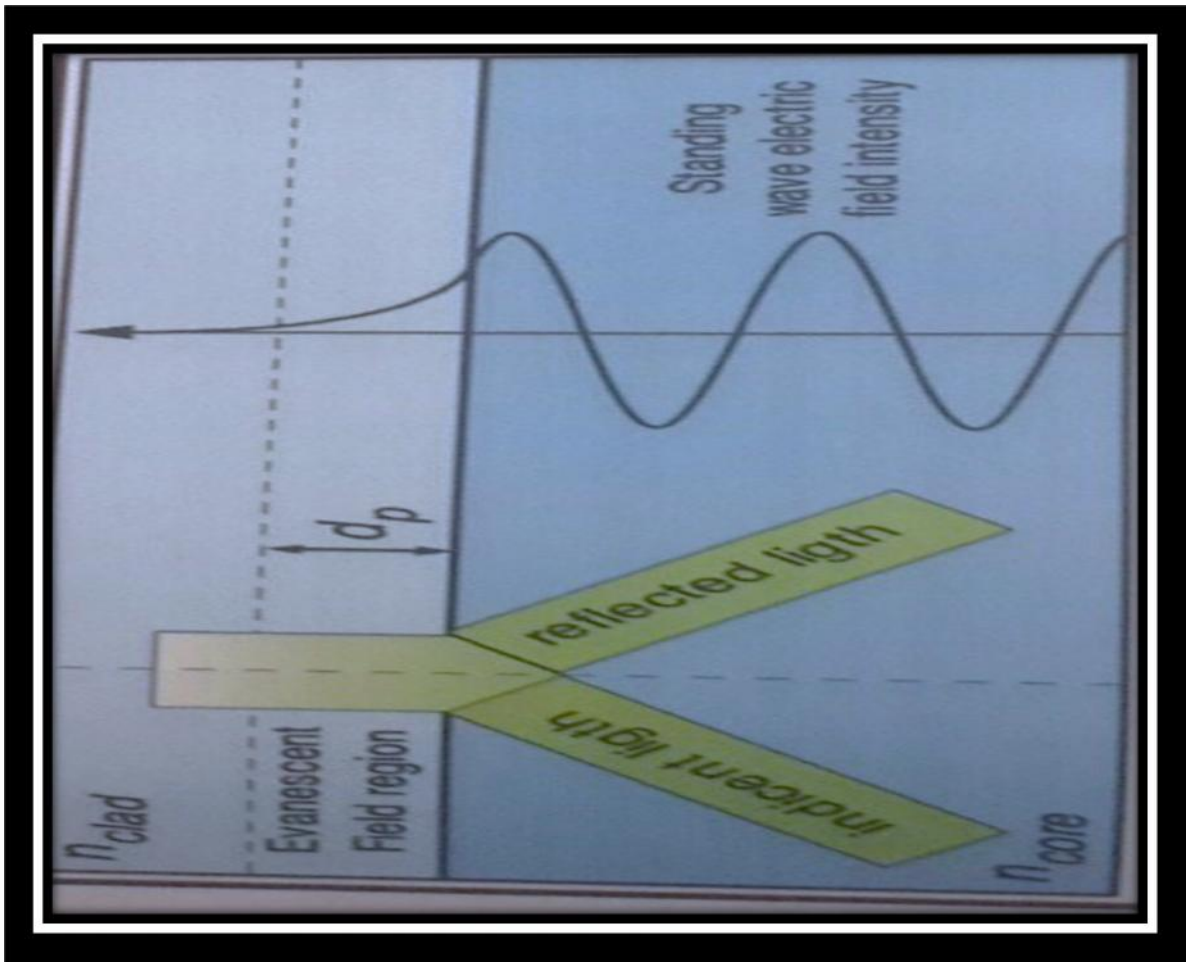


Figure (1-15) evanescent wave in the optical fibre cladding [54]

1.6 BIOSENSOR

Biosensors can be well-defined as analytical procedures which contain a combination of biological sensing elements like sensor system and a transducer they includes a biologically active element with an suitable physical transducer to yield a quantifiable signal proportional to the concentration of chemical species in any type of sample show in figure (1-16) [56,57 ,58].

The main construction advantages and applications of biosensors are stability, cost, sensitivity, and reproducibility shown in figure (1-17).

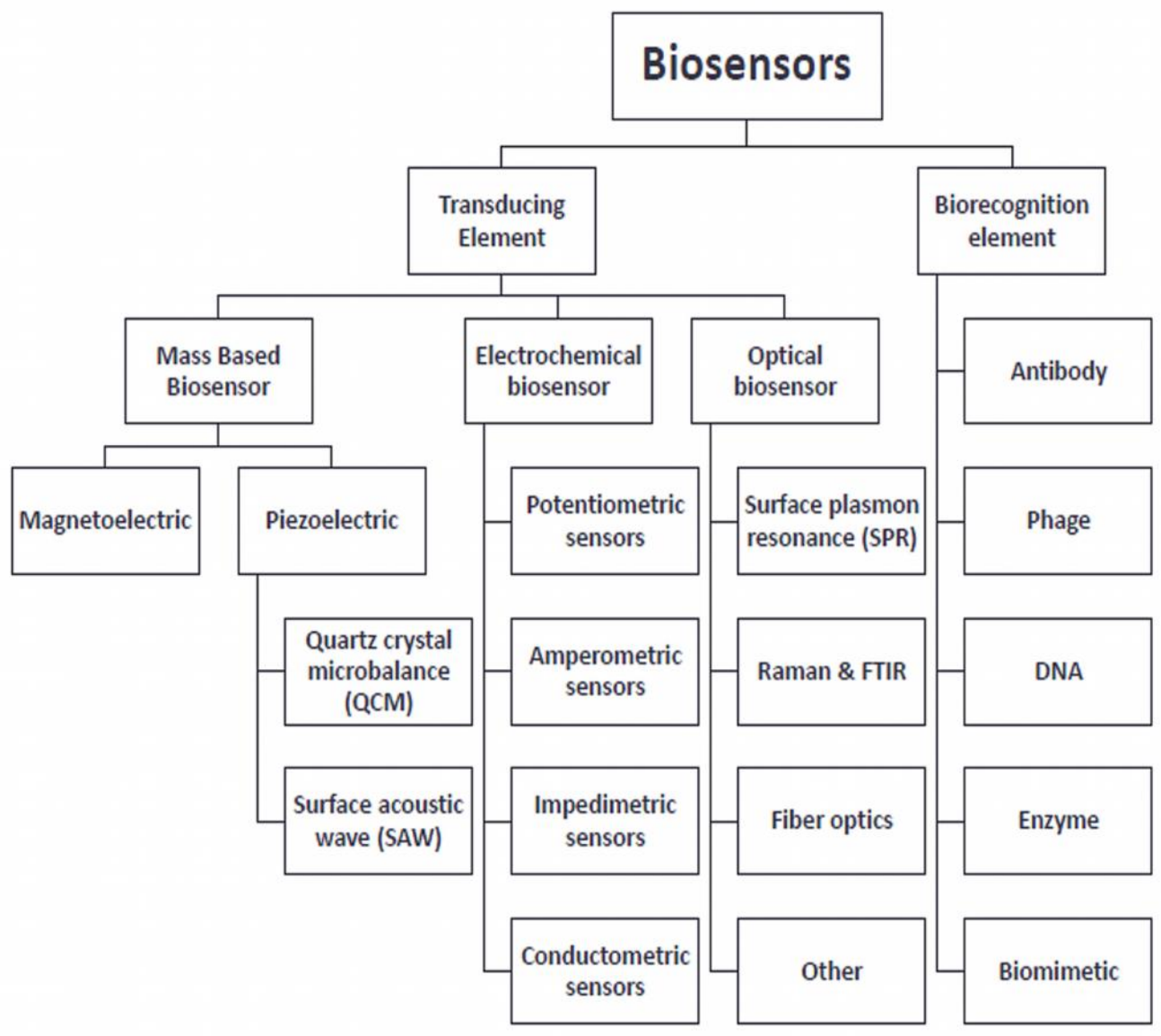
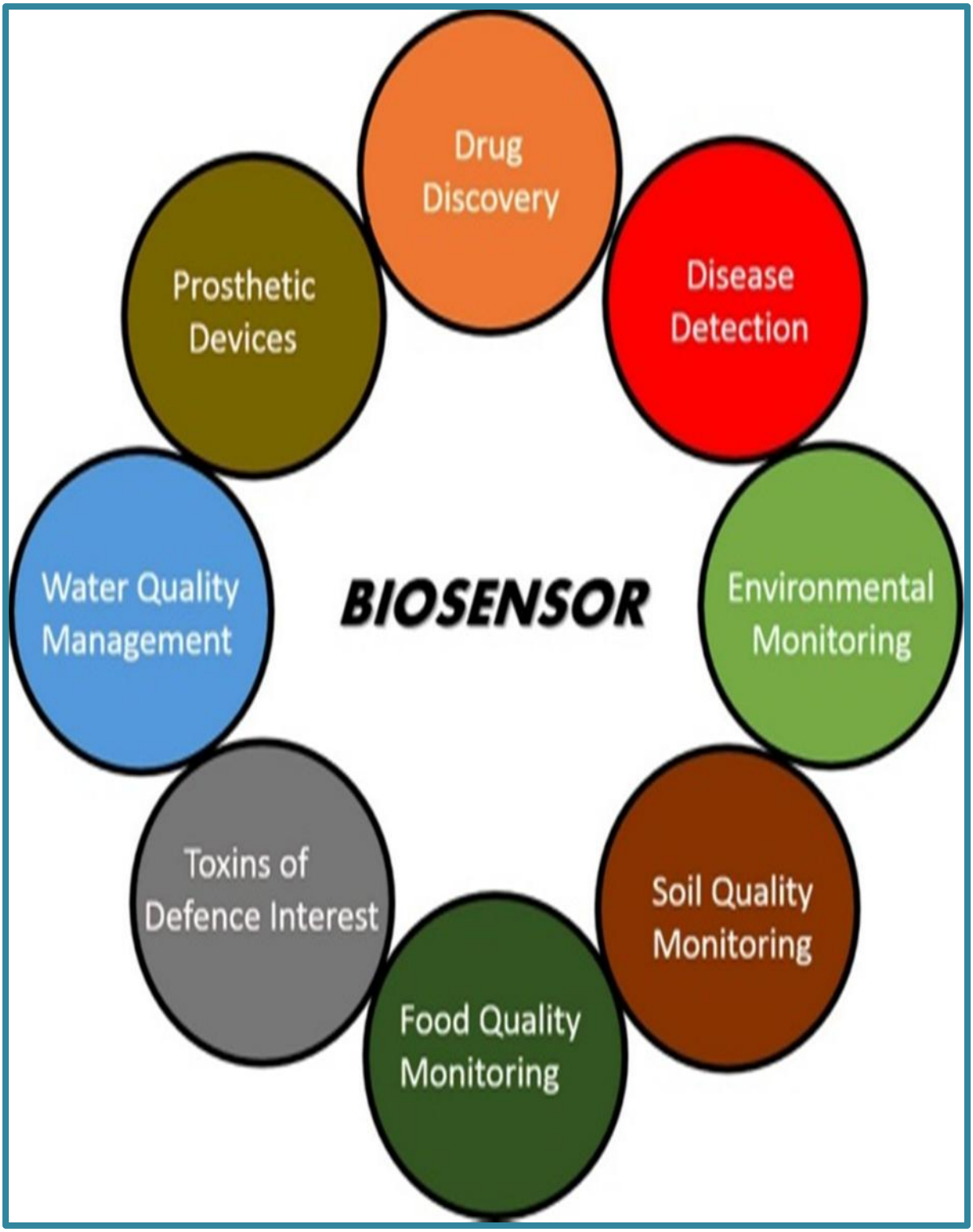


Figure (1-16) Construction of biosensor [59]



Schematic Figure (1-17) application of biosensors [60]

1.6.1 Biosensors Applications

In recent years, these sensors have become very popular, and they are appli cable in different fields .

- Common healthcare checking ,Metabolites Measurement .Screening for sickness ,Insulin treatment ,Clinical psychotherapy & diagnosis of disease
- In Military ,Agricultural, and Veterinary applications ,drug improvement, offense detection ,Processing & monitoring in Industrial ,Ecological pollution control.

From the above article, finally, we can conclude hat biosensors and bioelectronics have been used in a lot of areas of healthcare, life science research, environmental, food & military applications. Further, these sensors can be enhanced as Nan biotechnology shown in figure (1-18). [61]

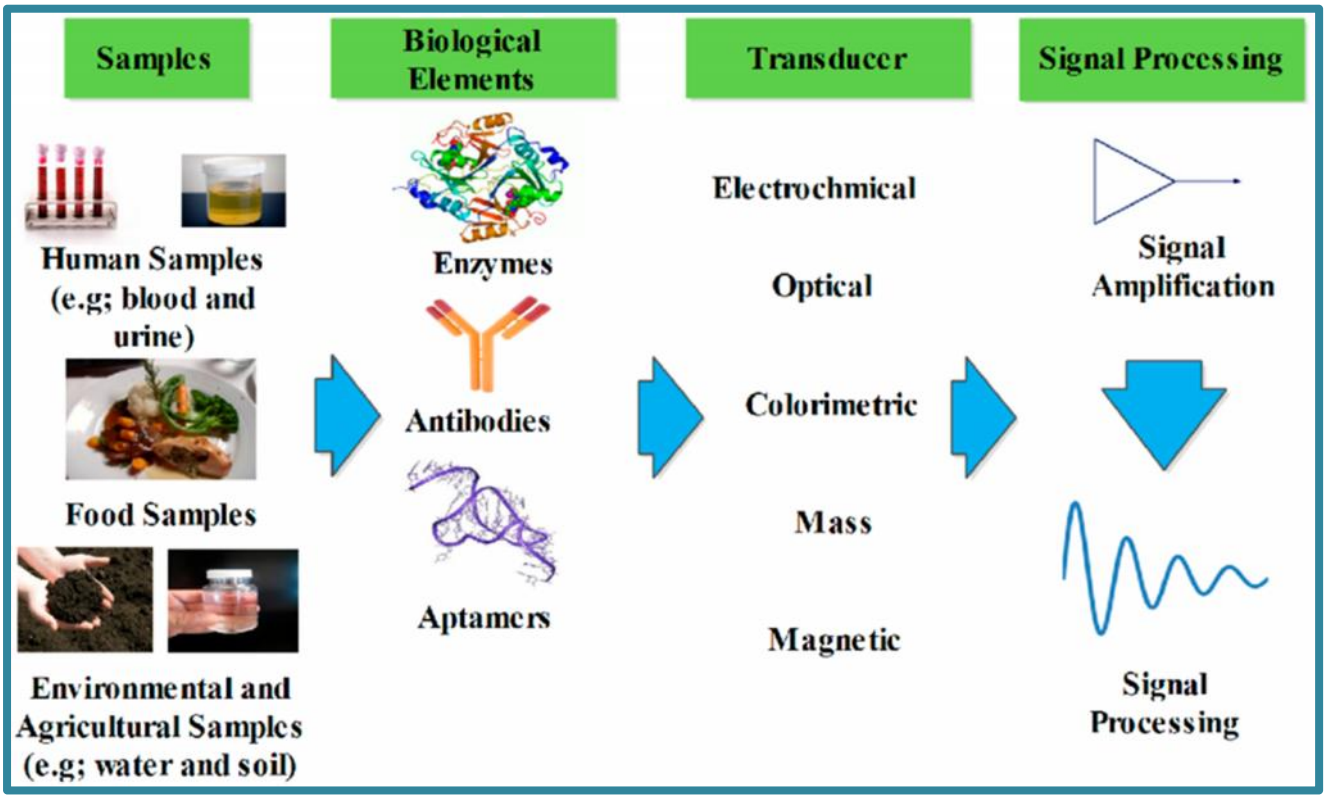


Figure (1-18) Schematic biosensors of different types containing biological recognition elements, transducers, and detectors. [61]

1.6.2 Optical Biosensor

Several optical phenomena can be used in optical biosensors such as fluorescence, luminescence, absorption, interferometry, evanescent wave and Surface Plasmon Resonance, to change biological informations into the measurable optical signal. Absorption is a simple phenomenon can be applied in bio-sensing. Absorption is a process in which all or part of photon energy is transformed to other types of energy, like heat. The absorbance can be measured by directing a beam of radiation at the sample and sensing the intensity of the radiation that originates across it. Some techniques of absorption spectroscopy have been progressive to determine the absorption as a function of wavelength, such as ultraviolet-visible, infrared and X-ray absorption spectroscopy [62]. Intensity modulation was used in early optical sensor growth owing to its low cost, simplicity, and reliability [63].

The intensity of light source approved through a sensor head from a light differs in accordance with the measured. The intensity of modulated light can be measured by a light receiving component and transformed into an electrical signal. The electrical signal variation is proportional to the change of the measured. Different sensors, like mass and refractive index sensors have been realized based on this modulation technique .[64, 65].

A typical biosensor is characterized in figure which it involves of the following components:

Analyte: A substance of concern that needs recognition. For example, glucose is an ‘analyte’ in a biosensor planned to detect glucose. Bioreceptor A: molecule that specifically identifies the analyte is well-known as a bio receptor. Enzymes, cells, aptamers, deoxyribonucleic acid (DNA) and antibodies are some examples of bio receptors. The process of signal generation (in the form of light, heat, pH,

Charge or bulk change, etc.) upon interaction of the bio receptor with the analyte is called bio recognition.

• Transducer: The transducer is a component that alters one form of energy into another. In a biosensor the role of the transducer is to change the bio-recognition event into a determinate signal. This process of energy alteration is known as signalisation. Most transducers yield either optical or electrical signals that are usually proportional to the amount of analyte bio receptor interactions.

• Electronics: A part of a biosensor that processes the transduced signal and formulates it for display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analogue into the digital form. The processed signals are then counted by the presentation unit of the biosensor.

Display: The display involves of a user interpretation system such as the liquid crystal demonstration of a computer or a direct imprinter that produces numbers or curves understandable by the user. This part often consists of a mixture of hardware and software that makes results of the biosensor in an accessible manner.The output signal on the display can be numeric, graphic, flat or a copy, depending on the supplies of the finish user shown in figure (1-19). [66]

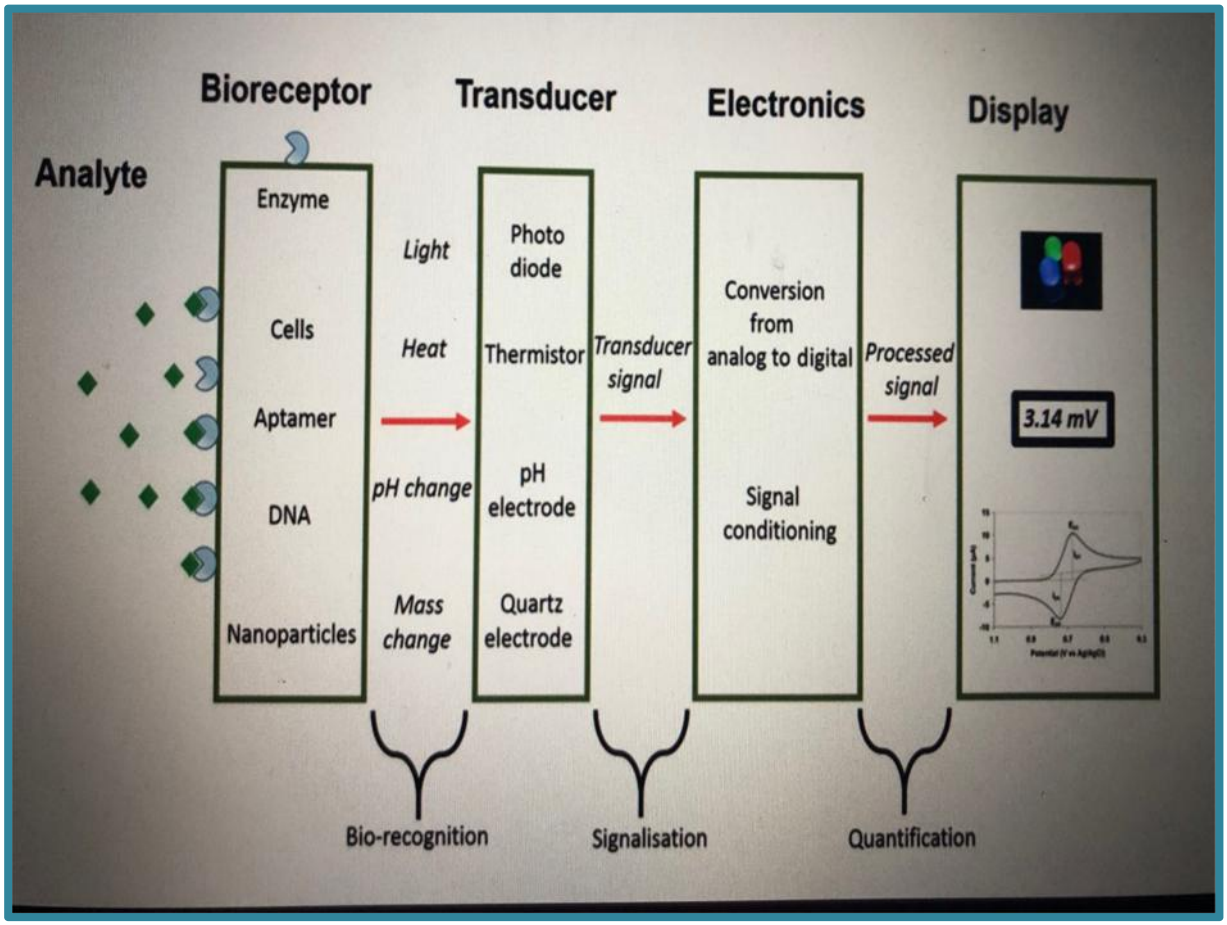


Figure (1-19) the Schematic representation of a biosensor [66].

1.6.3 Optical Biosensor Properties

The Optical biosensor is a device that procedures an optical measurement standard. They use the fiber optics as well as optoelectronic transducers. The term optrode characterizes a compression of the two terms optical & electrode. These sensors mainly include antibodies and enzymes similar the transducing elements.

The optical biosensors are categorized into two type's namely direct optical detection biosensor and labelled optical detection biosensor. [67]

1-Selectivity Selectivity is perhaps the most important feature of a biosensor. Selectivity is thecapability of a bioreceptor to notice a specific analyte in a sample containing other admixtures and contaminants. The finest example of selectivity is represented by the interaction of an antigen with the antibody. Classically, antibodies act as bioreceptors and are immobilised on the surface of the transducer. A solution (usually a buffer containing salts) surrounding the antigen is then exposed to the transducer where antibodies cooperate only with the antigens. To build a biosensor, selectivity is the main consideration when selecting bioreceptors. [68]

2-Reproducibility

Reproducibility is the capability of the biosensor to produce matching responses for a copied experimental set-up. The reproducibility is characterised by the accuracy and accuracy of the transducer and electronics in a biosensor. Precision is the capability of the sensor to offer alike results every time. a sample is measured and accuracy designates the sensor's capacity to provide a mean value near to the accurate value when a sample is measured more than once. Reproducible signals afford high reliability and strength to the inference made on the answer of a biosensor.[69, 70]

3- Stability

Stability is the degree of susceptibility to ambient disorders in and about the biose nsing system. These disorders can aim a drift in the output signals of a biosensor under measurement. This can found an error in the measured concentration and can affect the precision and accurateness of the biosensor. Stability is the most critical feature in applications where a biosensor requires long incubation steps or continuous monitoring. The reaction of transducers and electronics can be temperature-sensitive, which may affect the stability of a biosensor. Therefore, appropriate tuning of electronics is mandatory to confirm a

Stable response of the sensor. Bioreceptors with high affinities inspire either strog electrostatic connection or covalent linkage of the analyte that strengthens the stability of a biosensor. Another factor that disturbs the stability of measurement is the deprivation of the bioreceptor over a period of time. [71, 72]

4-Sensitivity

The minimum amount of analyte that can be noticed by a biosensor describes its boundary of detection (LOD) or sensitivity. In a number of medical and environmental monitoring applications, a biosensor is essential to sense analyte concentration of as little as ng/ml or even fg/ml to check the presence of traces of analytes in a sample. For instance, aprostate-specific antigen (PSA) concentration of 4ng/ml in blood is associated with prostate cancer for which doctors propose biopsy examinations. Hence, sensitivity is considered to be a significant property of a biosensor**.** [73,74]

1.6.4 Optical fiber based Biosensors

Optical-based biosensors are original, minute, flexible platforms that are being used with increasing frequency as biosensor transducers. Optical fibers arecapable to make fast and sensitive responses, and can be working as an intrinsic or extrinsic biosensor [75]

Optical fibers are a suitable material for optical sensor project because they can be economical and offer informal and efficient signal distribution They are currently among the most common of all fiber-based optical sensors for inspecting load, straining, temperature, vibration, and RI Optical fibers convey light on the base of the principle of total internal reflection (TIR). Fiber optic biosensors are based on the transmission of light along silica glass fiber, or

Plasti c optical fiber to the site of analysis. Optical fiber biosensors can be used in arrangement with different kinds of spectroscopic technique, e.g. absorption, fluorescence, phosphorescence, surface plasmonresonance (SPR) shown in figure (1-20). [76].

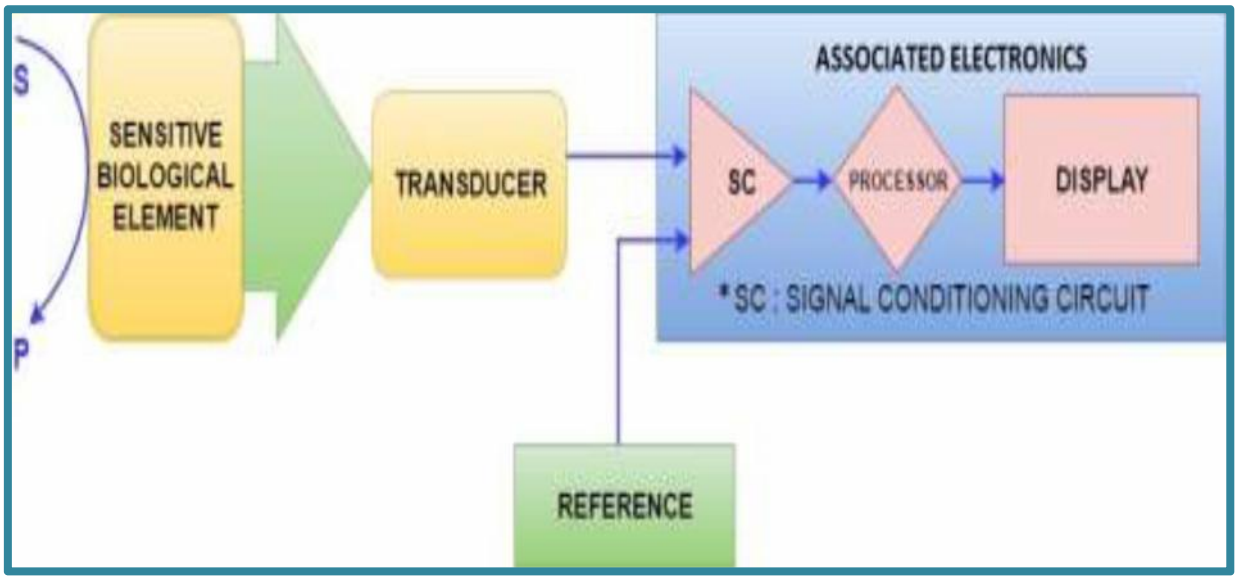


Figure (1-20) the Schematic diagram showing main components of a biosensor [77]

Cladding detached evanescent wave (EW) maybe the simplest way to attain direct communication of ligh twith the medium adjacent the optical fibre is through the elimination of the cladding to permit access to the EW, change of the optical properties of the surrounding medium, for example, RI or spectral

Absorption, will lead to variation of the light spreading in the optical fibre. this fibre modification enables E spectroscopy which is a highly subtle and powerful technique that is used to measure quantitatively and qualitatively the chemistry of the atmosphere surrounding the optical fibre [78,79].

The absorption spectrum of the medium surrounding the fibre effects the weakening of the EW, and thus of the mode, according to the Lambert –Beer law:

where α is the molar extinction coefficient, c is the concentration of the absorbing substance, L is the path-length that light journeys through the sample and Io and I are the light intensities at the input and output of the optical fibre, i.e. before and after interaction with the absorbing substance, respectively [80,81].

In its simplest form, connection the output from a broadband optical source into the proximal end of the fibre and detecting the spread light by connection the output from the distal end to a spectrometer will permit the spectroscopy of the surrounding medium to be measured. There is also an application advantage as the source and detector are located on the same side of the medium under research.It is also worth revealing that the low weakening of chalcogenide glasses in the infrared (IR) region (1–10μm), where specific absorption landscapes of organic molecules are situated. Creates the use of these fibres attractive for EW spectroscopy shown in figure (1-21) [82].

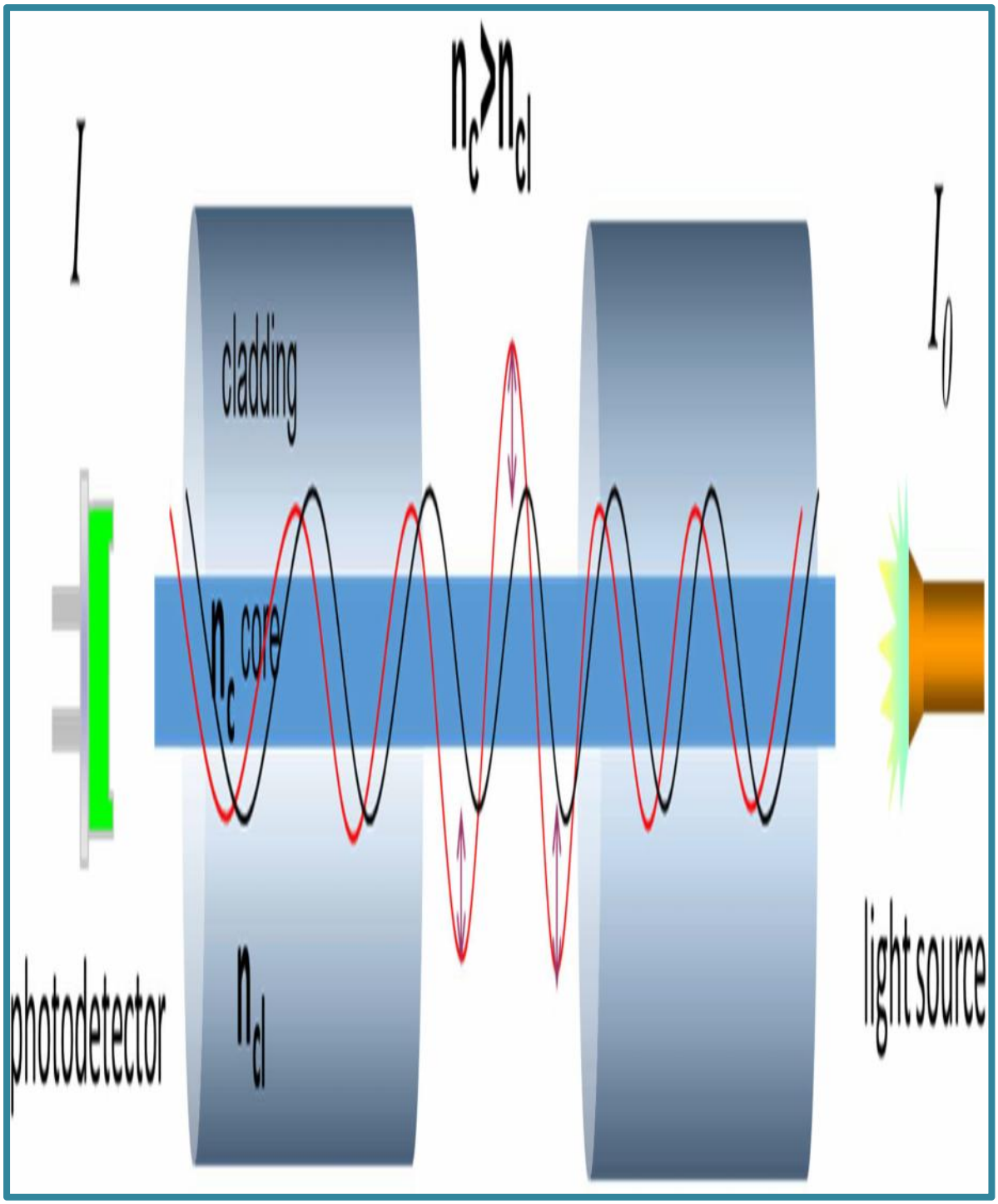


Figure (1-21) illustration of an evanescent wave with removed cladding. [82]

1.7 Literature survey

The field of optical biosensor reflect as multidisciplinary region of research that bonds the basic sciences principles (biology, physics and chemistry,) with essentials of medical application, nano-technology and its application in electronics. The history of biosensors demonstrated that the first 'true' biosensor was established by Leland C. Clark in 1956 [83] to detect oxygen and establish the first bubble oxygenator for tradition in cardiac surgery in1962, he is identified as the 'biosensors father 'and the origination of the oxygen electrode bearings his name (Bhalla et al 2016) [84]. The Clark oxygen electrode put the basis for the first glucose biosensor (the first biosensor of any type) and proved an amperometric enzyme electrode for the detection of glucose, the rate of reaction current is limited by the diffusion of both glucose and oxygen, this diffusion can be well characterized for a membrane for both the oxygen and glucose, leaving as the only variable the oxygen and glucose concentrations on the analyte-side of the glucose membrane, which is the quantity being to be measured (Severinghaus and astrup 1986) .

Banerjee et al 2007, [85] used a plastic cladded sensor fiber to calculate refractive index of liquid. They designed the ratio of intensity as a role of refractive index of copper sulphate and fructose aqueous solutions, in order to estimate the influence of optical absorption on refractive index, the result showed that optical absorption will increase.

Villatoro and Badenes 2008, [86] designed a model interferometer based on reflection. They used photonic crystal fiber (LMA) in a length of (24mm) and joined it with single mod fiber standard in type. Connection and recombining of cladding mode and core when the air holes were completely

Collapsed using a various refractive index, high stability was observed in the device over time.

Jha et al 2008, [87] observed and established a biosensor using single-mode taperedfiber that has been immobilized with bio recognition molecules to i ntelligence targeted proteins.

An interferometry-patterned spectrum interaction of evanescent waves with the external medium surrounding the tapered region are produced, which shifts corre spondingly to any variations of refractive index (RI) in the external medium. The projected setup achieved to find an RI sensitivity and concentration sensitivity of 2526.8 nm/RIU and 20.368nm respectively is highly sensitive when compared with previous trainings. The dynamic presentation, upright specificity, and great sensitivity of the projected method highlight a massively beneficial choice for immunological diagnostics.

Park et al 2010, [88] constructed a sensor generated on reflection. The sensor made of a solid silica core photonic crystal fiber (LMA-10) collapsed at the middle and spliced with a silica fiber, later dipped in oil with refractive index in range (1.410−1.430). Its simple manufacture process, they could suppose mass production with low cost.

Mathew et al., 2010 [89] ,Cárdenas-Sevilla et al., 2011, 16 they had many training in construction of photonic crystal optic fiber (PCF) interferometer by using two arm interferometer and modal interferometer. Splitting and recombining of two - arm interferometer involves two monochromatic optical beams that spread in different fibres which requires some meters of optical fibre and one or two couplers. modal interferometer activities the relative phase displacement between two modes of the fibre. Photonic crystal fibre interferometers built on

micro-hole collapse have achieved great importance in recent times owing to the simple fabrication process convoluted and excellent sensing performance.

Wang and Tang 2012, [90] photonic crystal fiber (PCF, LMA-10) are used to design a refractory index sensor. A minor section of PCF has been joined amongst two standard single mode fibers (SMFs), entirely collapsed of the two regions of union, splice permit cladding modes and the core to joining and recombining to produce a Mach-Zehnder interferometer (MZI), immersion of the sensor at diverse concentrations of sucrose solution.

Cárdenas et al. 2013, [91] exhibited a sensor built up of a small piece photonic crystal fiber (PCF LMA-10) in the range of (10−12) mm fused and spliced to two single mode fibers (SMFs the coupling and recombining of core and cladding modes in the PCF are made. Thus, most of the chemical and biological sensors can be applied with the sensor planned.

Kami land Abu Baker 2015, [92] designated a new type of biosensor expenses tapered type of single mode fiber with molecules bio recognition type to intelligence directed molecule of proteins. Interface of evanescent waves with the outside average adjoining the area which is tapered products an interferometry-patterned spectrum, which changes similarly to variations of refractive index (RI) in the exterior medium. The planned arrangement achieved toward get and RI sensitivity and concentration sensitivity of 2526.8 nm/RIU and 20.368 nm/μM, respectively, is highly sensitive, the sensor represent ahight specificity, and great sensitivity of the planned technique highpoint a massively excellent for immunological diagnostics.

Rawaa and Hanan 2015, [93] they constructed a sensor chemical in type invented it by cutting and splicing photonic crystal fiber (PCF LM-10) with standard single-mode fibers (SMF-28) at different length. A microscopic collapsed region in the PCF is are examined under the microscope and consider the key element for stimulating and recombining two core modes, they decided

That the interferometers demonstrate regular interference patterns which change remarkable when the spaces of the fiber are penetrated with molecules of Instable compounds and Acceptance of the system with a fast sensing information.

Li et al 2016, [94]demonstrated an optical microfiber coupler (OMC) sens or functioning near the turning point of active group index to achieve high refractive
index (RI) sensitivity. Experimentally established an ultrahigh sensitivity of 39541.7 nm/RIU at a low ambient RI of 1.3334 based on an OMC with the diameter of 1.4 lm. Higher sensitivity can be attained by transport the measurements at RI nearer to the turning point. The resulting ultrasensitive RI sensing platform proposals a substantial impact on a variety of applications from high performance trace analyte recognition to small molecule recognising.
Jamal 2017, [95] designated and constructed a laser biosensor photonic crystal fibers (PCF) using different lengths of PCF (LMA-10) cleaved and spliced with conventional optic fiber single mode (SMF-28) for blood and urine test (haemoglobin concentration ,different types of anaemia ,pregnancy test and general urine examination). The extreme absorbance started in the wavelengths range (470-590) nm of blood, urine sample is in the range of (590-670) nm. The variation in the intensity (or the absorbance of urine sample) is increased by increasing the number of the biological components. The sensitivity is enlarged by increasing the length of the PCF inside the used fiber, refractive index of the blood is improved by the increasing the level of the haemoglobin concentration, the pregnancy state foundations are increased with increasing refractive index of the female urine sample and different types of anaemia revealed decreasing in the refractive index of blood sample.

Arora andSaini 2017 [96] 14reported Catalytic enzyme based sensor recognition elements are very smart and pretty for biosensor applications due to a diversity of measurable reaction products ascending from the catalytic process, which contain protons, electrons, light, and heat. The enzyme urease has been widely Used as a sensor bio recognition element owing to an essential for urea determination/monitoring for both medical and environmental applications. The very seeming characteristic, regulatory environment of allosteric enzymes gives great potential for use as biosensor recognition elements.

Although a variety of glucose sensors are presented, the glucose biosensor has different little in principle over the year's glucose biosensors utilize glucose oxidase as their recognition element that catalyses the oxidation of glucose to gluconolactone. Chakma et.al 2018, [97] designated modern sensor consist of two-layer photonic crystal fiber (PCF) Surface based on the Plasmon Resonance (SPR) to gain high sensitivity for the detection ofunknown analytes SPR sensor, achieving its duty.

These parameters essentially be selected by considering a way that will offer an easy interaction between the evanescent field and the metal surface. The capable excitation of metal surface is used as a key factor of plasmonic phenomenon. The resonance can generate and the surface may excite by the incident field at a fixed wavelength, the importance of this work is to upsurge the sensitivity through making a potential coupling within the core guided-mode and SPP mode. A plasmonic chemically inactive material gold (Au) with thickness 35 nm is used to the outside of the PCF structure which exhibits negative real permittivity. These parameters must be selected by sighted a way that will afford an easy interaction between the evanescent field and the metal surface. The capable excitation of metal surface is applied as a key factor of plasmonic phenomenon. The resonance can produce and the surface may

Excited by the incident field at a fixed wavelength amid to increase the sensitivity through making a potential coupling within the core guided-mode and SPP mode.

Aim of study

The aim of the present study is the detection of the level of Alanine transaminase ALT and Aspartate aminotransferase AST liver enzymes and its concentration by using different set ups laser biosensors as a new method.

Chapter Two

Materials & Methods

2 .1 Introduction

The present chapter are focused on, instruments, materials and equipment's that have been used in the study. The first aspect is biological aspect focuses with the measurement of refractive indices, concentration of ALT and AST liver enzymes manually in the hospital using Randox kit. The second aspect focuses on the construction of 3 types of biosensors (single mode, multimode and photonic crystal fiber) by cleaving and splicing. The third and last aspect focuses on the detecting the spectra of ALT and AST enzymes by Laser biosensor.

2.2 Sample collection

Twenty four blood samples were collected from males patients (9) and females (15) in age ranging from (15-72) years admitted to Ibn -Alnafees hospital complaining mainly from heart disease and also from liver diseases.

From each patient10 ml of blood were taken centrifuged and divided into 2 parts, (5ml) used in traditional method for detection the concentration of ALT and AST enzymes, examined manually in the hospital using RANDOX kit and spectrophotometer at a wavelength (546) nm.

The other (5ml) used to detect the concentration of enzymes by using 3 setup of constructed laser biosensor device. According to the optical properties of ALT and AST enzymes standard wavelength 530-550 nm) as listed in the kit and the refractive index of ALT and AST standard using abbey refrectometer,1.3460 au , 1.3470 au respectively choosing the suitable laser green light diode (semiconductor) at wavelength 532 nm, Power <1000 mW.

2.3 Materials and instruments

Table (2-1) Materials and Instruments

Number	Materials and Instruments	Manufacture	Origin
1-	Micropipette (100- 1000) liter	Nova	China
2-	Tips of pipet 1ml	Nova	China
3-	Plane tube 5ml	Nova	China
4-	Gel tube 3ml	GC	America
5-	Eppendorf tube 1.5 ml	Nova	China
6-	Disposable syringe 5ml and 10 ml	Nova	China
7-	Disposable masks and gloves	Nova	China
8-	Sterilized cottons	Iraq	Kemadia
9-	Ethanol alcohol 96%	GSF chemical	Germany
10-	Pieces of towel	Comfit	Malaysia
11-	Rack of plane tube (large)	GC	America
12-	Rack of Eppendorf tube (small)	GC	America
13-	Ice box		(Germany
14-	Types of fibers, single mode fiber (SM-28), multimode fiber (step index), and photonic crystal fiber	NKT Photonic A/S	
15-	Plastic piece		China
16-	Silicone adhesive		Germany
17-	Hydrofluoric acid HF 40%	HI media	Germany
18-	Acetone 40 %		U.K.D
19-	Adapter (FC Design)		China
20-	Cutter		Japan

21-	Autoclave	Moocow	Italy
22-	Centrifuge device	Kokusan	Italy
23-	Spectrophotometer range 546 nm	Cecil 2031	England
24-	Freezer mince 20	Panasonic	Germany
25-	Abbey refractor meter	BOECO	Italy
26-	Fiber striper CSF-3		China
27-	The cleaver machine (Fujikura CT30)		China
28-	Ocean HR2000 (Ocean Optics)		China
29-	Power supply		China
30-	Computer (HP)	Pavilion	China

2.4 Types of fibres used in this work

Three types of optical fibres were used to detect the concentration of ALT and AST enzymes in serum samples:

1-Single mode fibre (SMF-28)

2- Multimode fibre (MMF)

3- PCF Machzender- interferometer Photonic crystal fiber (PCF- LMA-10) figure (2-1). The ALT enzyme sensing in wavelength 531.62 nm and the AST enzyme sensing in wavelength 531.16 nm in 3 setup.

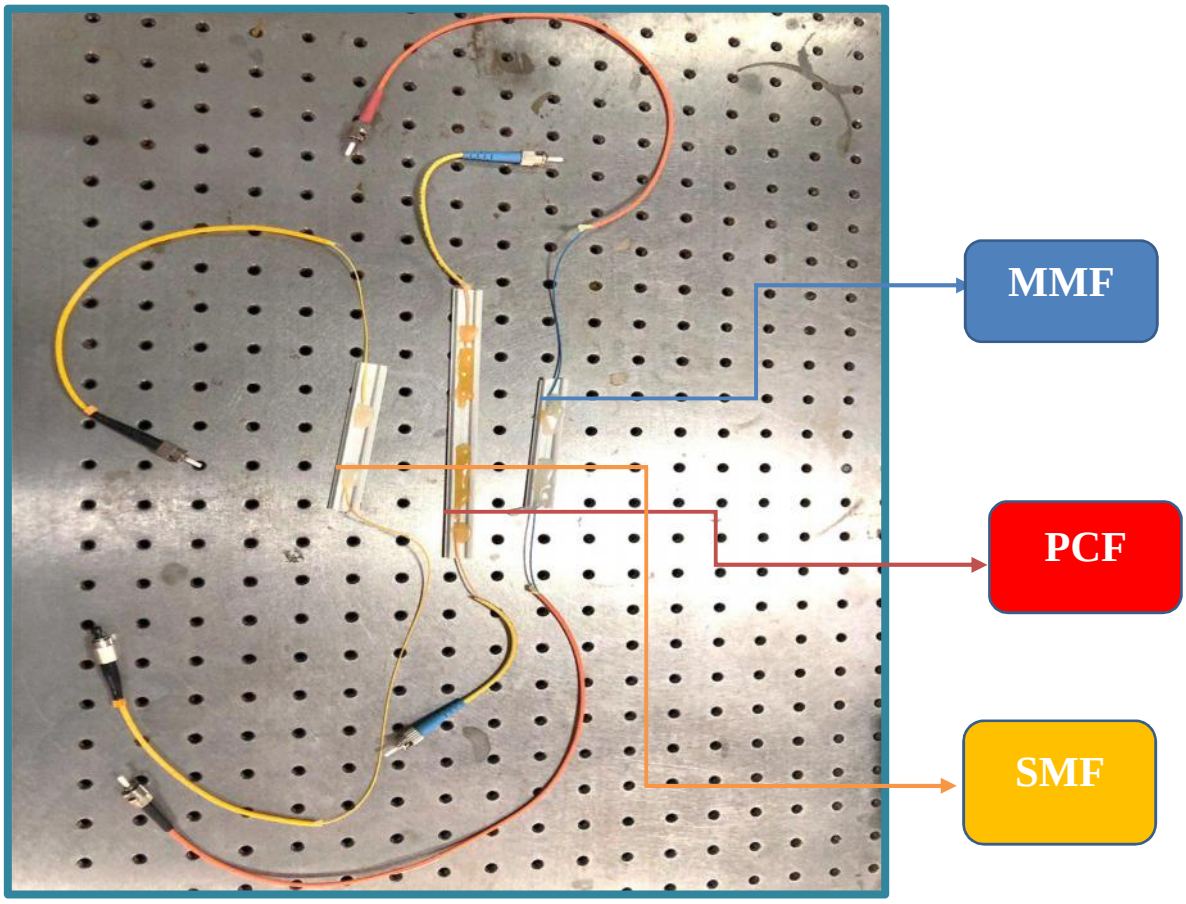


Figure (2-1) showing three types of biosensors single mode fiber, Multimode fiber and photonic crystal fiber.

2.5 Refractive Index Measurement for Biological Samples

Abbe refractometer which is shown in figure (2-2) were used to quantify or measurement of the refractive index of the biological samples as following:

1-The light is turn on.

2- The incident prism is opened and prism face is cleaned by using alcohol and dried with a delicate task wipers.

3- Volumes of 0.5 ml of standard and serum were taken and examined, then positioned on refined surface of lower refracting prism, closed upper incident prism so distribution of the liquid occurred on the surface of refracting prism.
4- The lower large knob is scanned till a divided image of dark and light can be appreciated.
5- A sharp boundary between light and dark is seen after adjustment of upper smaller dispersion correction knob, scanning the lower large knob is done until a divided image of light and dark.
6. The refractive index is readed from green scale below the boundary of refrectometer.

Figure (2-2) The Abbe Refractometer.

2.6 Preparation and sterilization

All the instruments used in this work were prepared and cleaned. Pipette tips and Eppendorf were firstly cleaned using a mixture of chloride solution 10% and distillate water 90% for 30 minutes, then washed with

Distillate water and sterilized using autoclave in temperature 273 ºc for 35 minutes figure (2-3).

Figure (2-3) all instruments used in the study.

2.7 Biological analysis methods of liver enzymes (ALT and AST)

The two enzymes (ALT and AST) were measured by two different kits to obtain the concentration of enzymes in blood sample manually. The same procedure were applied for the two enzymes but the differences are in the chemical composition of the each kit.

2.7.1 RANDOX ALT kit content (Figure 2-4) (appendix 1):

1-Buffer: Reagent 1(R1):

2- 2, 4 dinitrophenyle –hydrazine: Reagent 2 (R2):

3-Sodium hydroxide- Reagent 3 (R3):

4: Pyruvate as standard (undiluted) - CAL:

NOTE: Standard (or CAL) is the normal value of e enzyme ALT in blood

Figure (2-4) Showing the RANDOX ALT kit.

2.7.2 RANDOX A ST kit content (Figure 2-5) (appendix 2):

1- Reagent 1 R1: buffer

2- 2, 4 dinitrophenyle –hydrazine. R2 Reagent 2

3-Sodium hydroxide. - R3 Reagent 3

4- Pyruvate as standard dilute 1.5 ml of CAL Standard (CAL) with 4.5 ml of buffer (R1) Immediately before measurement.

NOTE: Standard (or CAL) is the normal value of enzyme AST in blood.

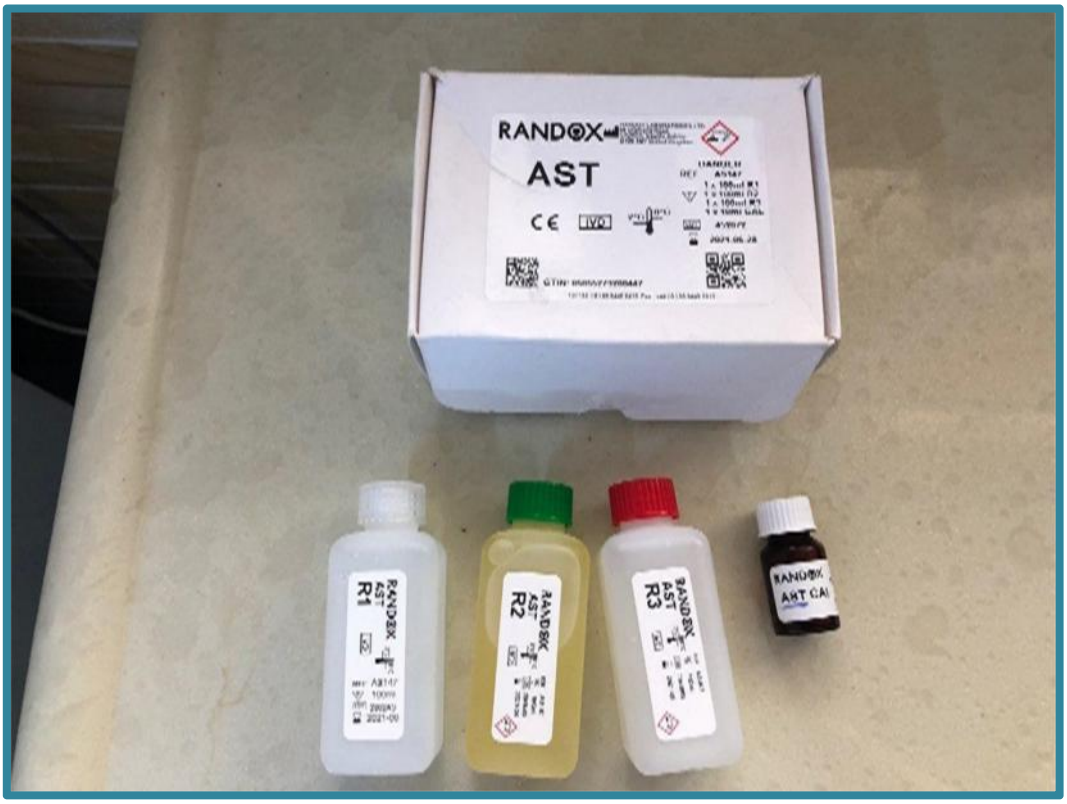


Figure (2-5) RANDOX AST kit content.

2.7.3 Procedure

Sample collection: Volume of 10 ml of blood sample was taken from each patient, each sample was labelled, centrifuged then divided into two parts .The first 5 ml used to detect AST and ALT enzymes manually in the hospital, the other 5 ml was treated and deep freezed, later on are used to detect the two enzymes by using three types of constructed biosensors.

2.7.3.1 Measurement using RANDOX kit

The first step was adding of 1ml of serum by pipette to 0.5 ml of with 0.5 ml R1, then mixing and incubation for exactly 30 min at 37 c in water bath figure (2-6 and 2-7).

Figure (2-6) serum samples treated with reagent 1(R1)

Figure (2-7) Incubation of the sample in water bath.

The second step: adding reagent 2 in amount about 0.5 ml, permit to Stand for 20 min at 25 c exactly at room temperature shown in figure (2-8).

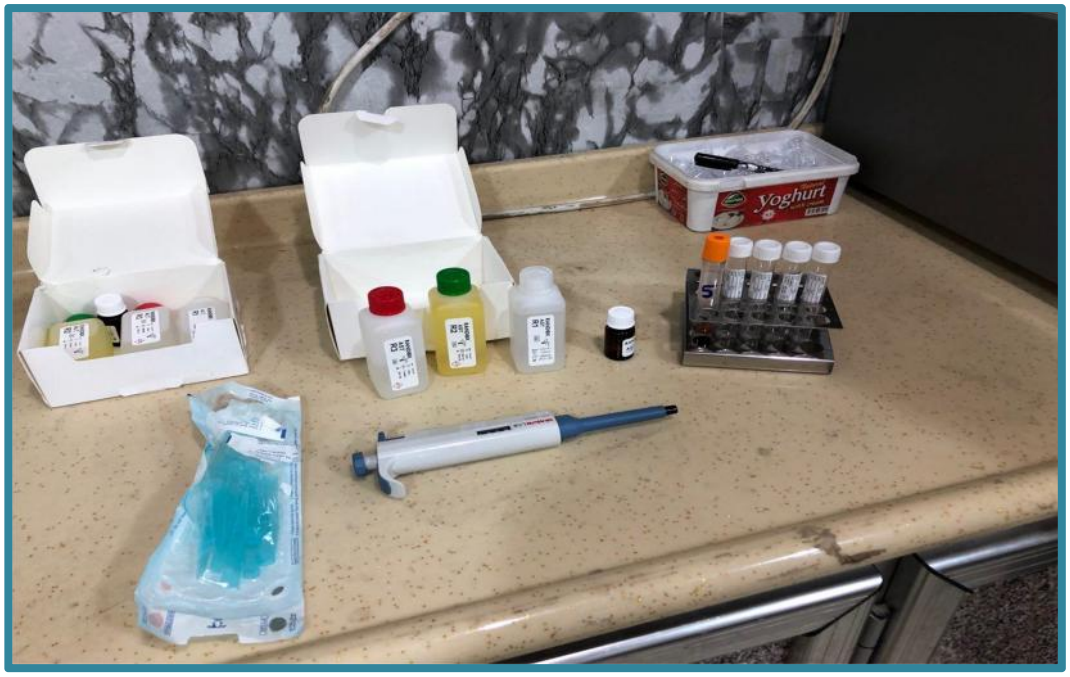


Figure (2-8) Sample treated with reagent 2 (R 2).

The third step was adding of 5ml R3 to deliver the absorbance of sample after Five minute against the reagent blank, the results were obtained by using spectrophotometer 546 nm (figure 2-9).

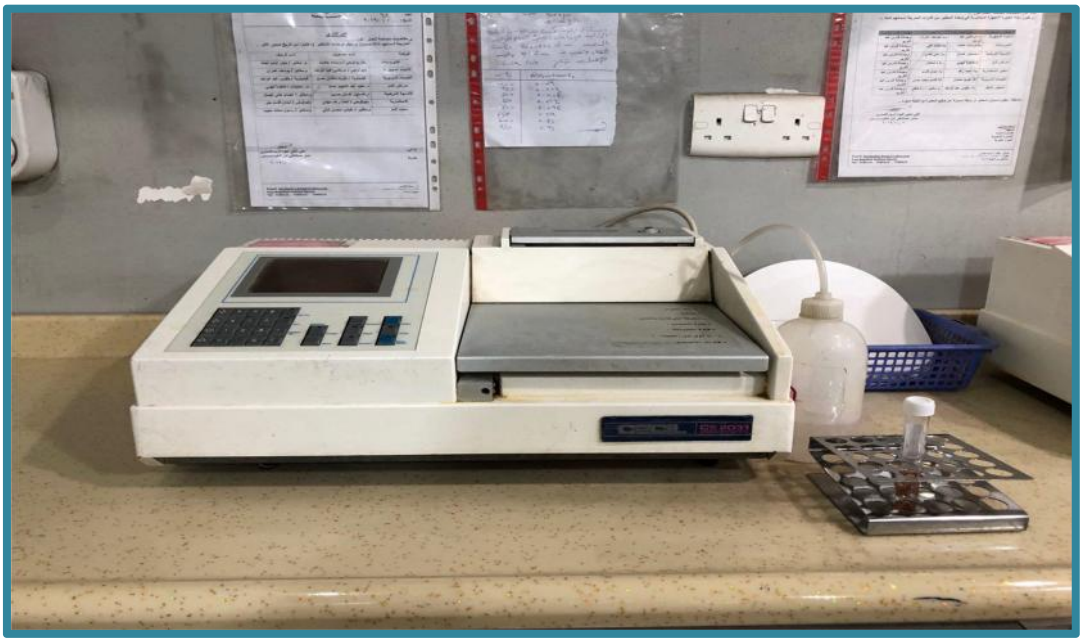


Figure (2-9) Measurement of absorption using spectrophotometer.

2.7.3.2 Preparation for measurements using three setups of constructed biosensors

Another 5 ml of blood samples from each patient was placed in gel tube, centrifuged for 5 minutes and then divided into 5 parts in Eppendorf tube, deep freeze in -20 ºC after short period of time measured by 3 types of constructed Biosensors **.**

2.8 Construction of three types of Biosensors (SMF, MMF and PCF)

2.8.1 Construction of single mode fiber (SMF) and multimode mode (MMF) set up

In order to construct a single mode fiber, a standard single mode type of optical fibre (SMF-28) (appendix 3) is taken show in figure (2-10), this type recycled in private network and telecommunication application in the spread of video and voice service, the capacity of fiber aimed at carrying the information is the highest in

the wavelength region of 1310 nm (the transmission window), the dispersion in this wavelength region is the lowest. , typically fiber comprises of three layers; the core of fiber made of silica with a core diameter (typically 2–10μm) and drugged by a material like germanium to rise the refractive index (RI), using silica pure in type as cladding of diameter 125μm and a buffer 250mm, this coating had no effect in light guiding, however the fiber are kept from mechanical damage and compromises mechanical strength, the same procedure for construction of single mode and multimode fibers ,the core of multimode fiber 55 micrometer .

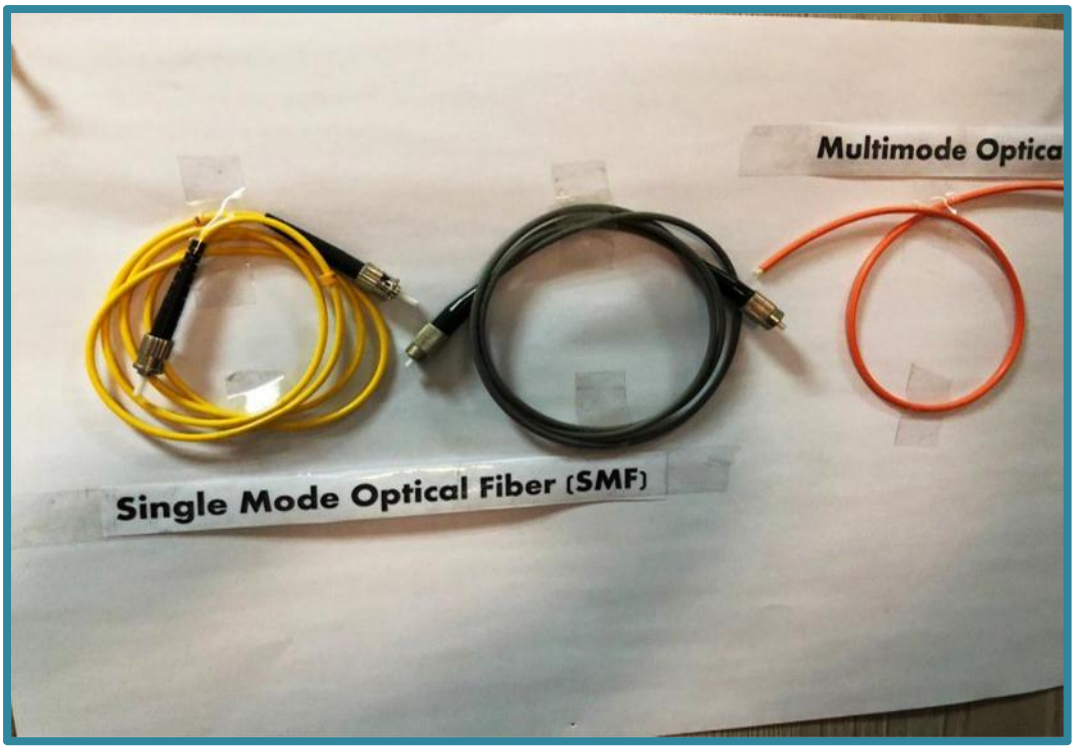


Figure (2-10) Single mode and multimode fibers.

1- Fiber sensor 30cm in length was considered as the conventional optical fiber, segment about 2 cm in length was made in the middle of the fiber using cutter to make a grooves in each side .

2-The buffer was removed by dipping the segment in 40% acetone concentration for 30 minutes.

3- Washing with distilled water for cleaning.

4- Dipping the segment in pure hydrofluoric acid (HF) 40% to remove the cladding of fiber for 10 minutes then washing with distilled water (figure2-11).

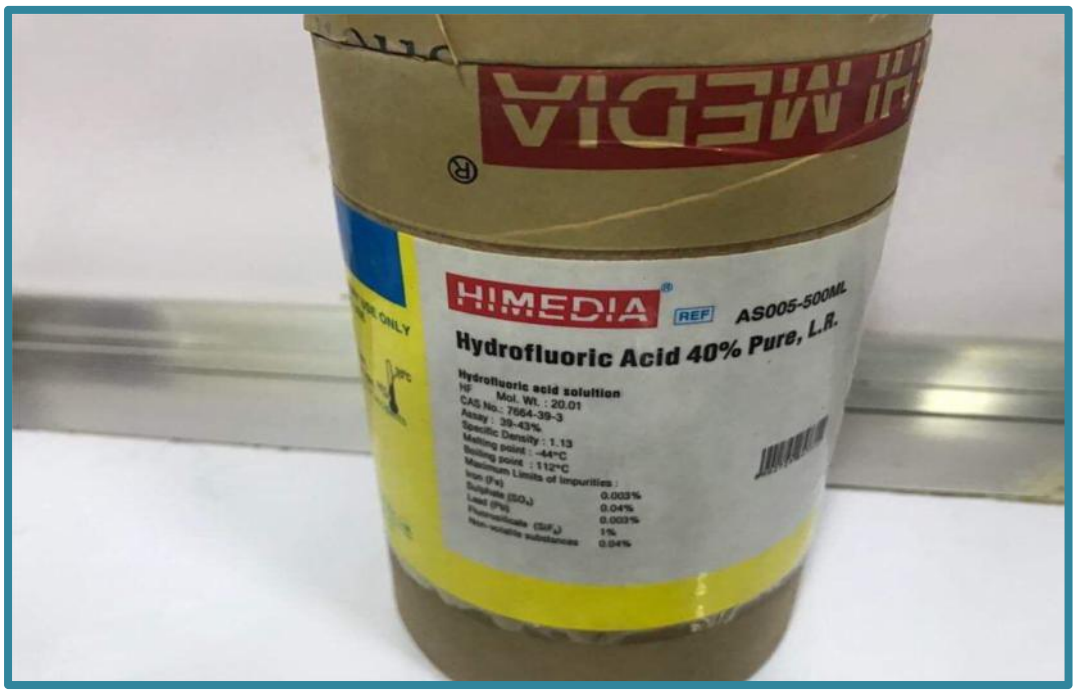


Figure (2-11) pure hydrofluoric acid (HF) 40%.

5-The whole fiber (30) cm was put in aplastic device using silicone adhesive.

6-The two ends of fiber were connected with adapter device.

Terminal equipment were connected to optical fiber

by optical fiber connector, a standard type connectors such as FC, SC, ST, LC, MTRJ, or SMA.

Optical fibers linked to each other by connectors. Splicing were used for Joining two fibers organized to form a unending optical waveguide.

7-First end connected with laser source (diode laser) Show in figure (2-12) and power supply.

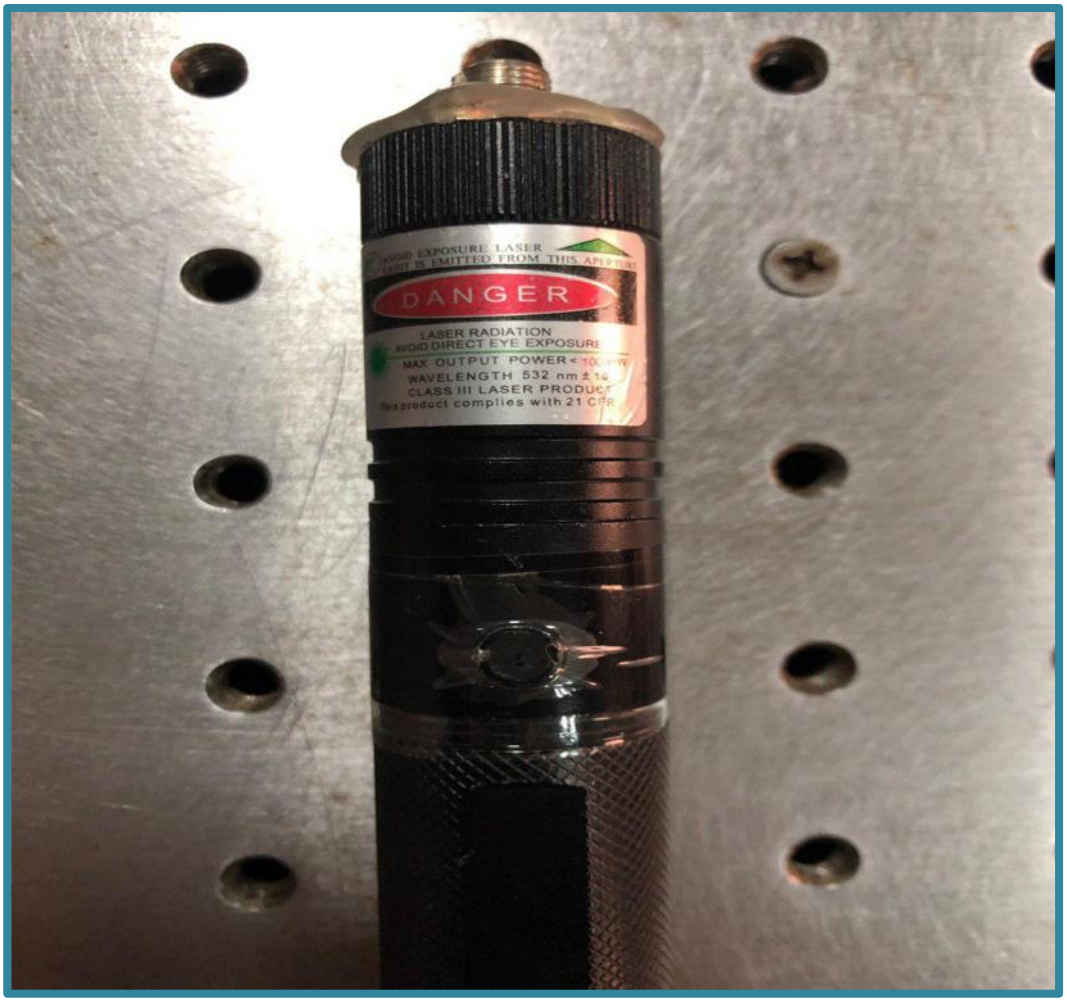


Figure (2-12) Diode laser 532 nm.

The light source had been used in this experiment was green laser with λ=532nm and output power =12.2 mw .This source of laser has a power supply which is stable at all the time of use it. Selecting this light source was because the absorption spectra of the blood sample covered this wavelength**.**

The second end connected with spectrometer (ocean HR2000) and computer to obtain signal of intensity.

An optical spectrum analyzer (ocean optics HR2000) with (0.065nm) resolution was used to display transmission interference spectrum of the sensor.

The spectrometer type (ocean optics HR2000), have the following characteristics:

1- Operating in the wavelength range from 200nm to 1100nm.

2- Resolution are 0.065nm of the high wavelength.

3- At full signal, the signal-to-noise – ratio are 250:1

4- Applicable to SMF, MMF and PCF.

The final setup of single mode of biosensor shown in figures (2-13).

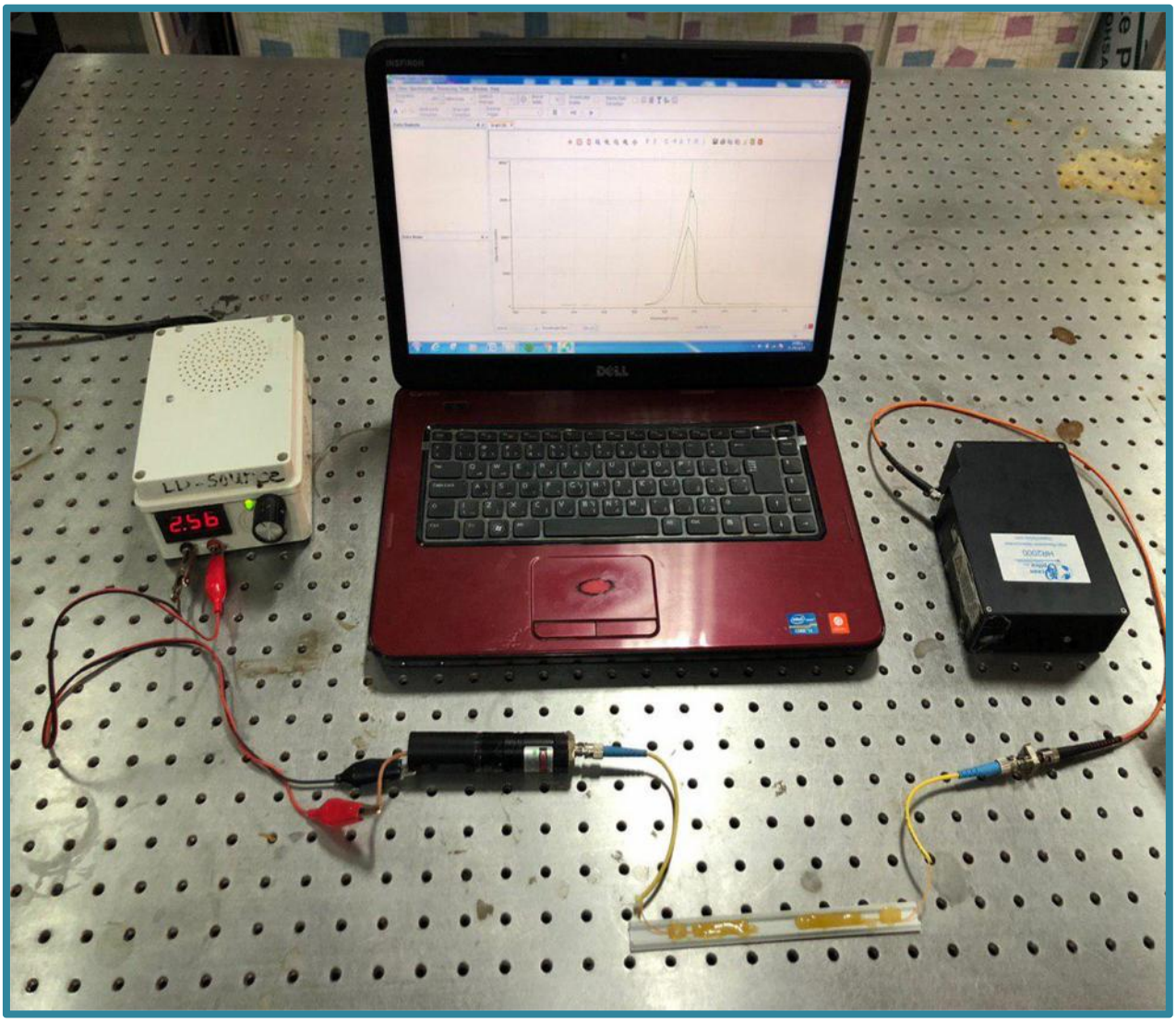


Figure (2-13) Setup of single mode biosensor.

2.8.2 Construction of PCF Machzender- interferometer

Photonic crystal fiber (PCF- LMA-10) set up

For the construction of the Photonic crystal biosensor, single mode fiber (SMF-28) as a standard had been used and the (PCF-LMA-10) (appendix 4) photonic crystal fiber ,solid core wereused which contain four circles of air holes hexagonal in shape ,Outer cladding diameter has (125 ± 2 μm), diameter of core were (10.1 ± 0.5) μm, air hole diameter 3.1μm and spacing between two holes were 6.6μm. Show in figure (2-14).

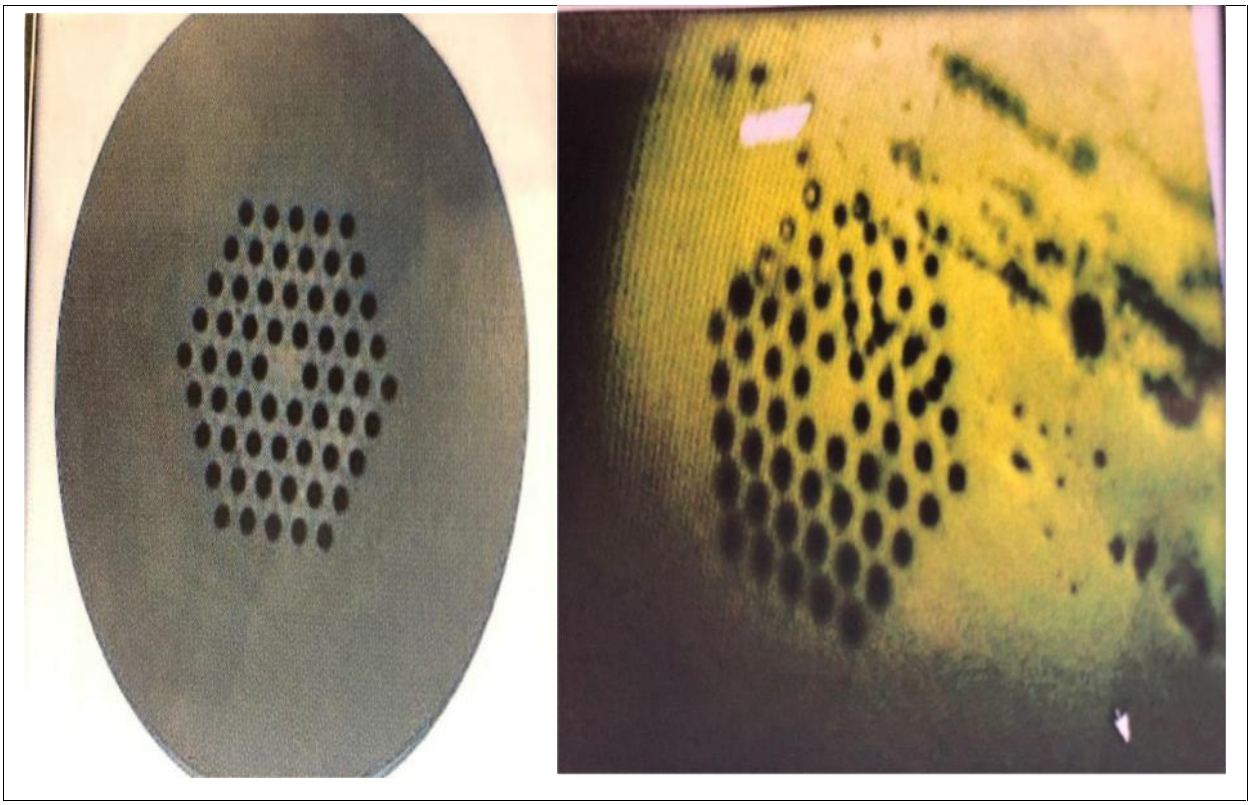


Figure (2-14) Photonic crystal fibre (PCF) (LMA-10) cross section.

The cladding and core were made from a single material (fused silica), the photonic crystal fiber has a mode area larger than the standard single mode optical fiber.

1- The preparing process for the optical fiber cleaving and splicing was to remove the buffer covering along the length of the optical fiber, by using the fiber stripper CFS-3 with a 125µm cladding diameter. All commercial optical fiber stripper had three holes. stripping (1.6-3) mm by the first hole, buffer stripping coating equal to (600-900) µm by second hole, and finally stripping the acrylate coating of (250 µm) thickness by the third whole.

2- The optical fiber cleaving allows the clamping of the optical fiber into the specified place also makes a longitudinal finish appearance of the optical fiber and perfectly smooth by the cleaver machine **(Fujikura CT30)**. the edge of the optical fiber were put above the cutting blade of the cleaving machine, a cleaved optical fiber with 90º angle and flat surface can be obtained.

3- An alcohol or other solvent to clean SMF and PCF can be used. The tip of PCF should not be exposed to solvents or liquids for cleaning fiber after cleaving to avoid infiltration. This infiltration may cause failure in the sensing and connecting process and possible lead to damage.

4- To connect two conventional optical fibers or PCF and SMF splicing the process of Fusion Splicing were used after cleaving the both sides.

In this work, Splicing machine **Fujikura (FSM-60S)** was used to merging splice (SMF-28) and PCF (LMA-10) figure (2-15 and 2-16)

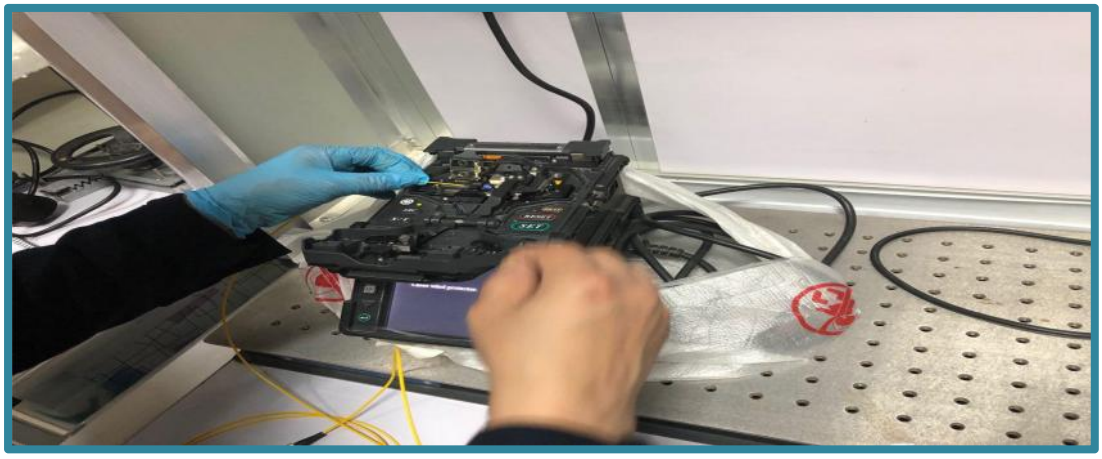


Figure (2-15) Fusion single mode with photonic crystal fiber

Figure (2-16) Smooth cutting of optical fiber as shown in the digital machain.

5-The splicing region and the regions surrounded by must be strong enough as the original fiber. Thus, when the light passing through fiber, it does not scatter. Electric arc are the source for this process can be getting from are the softening point for the conventional single mode fiber is different for PCF due to the microstructure of PCF .The surface tension in softening point will overcome know construction of PCF biosensor are finished .

2.8.3 Common steps for fusion splicing

To strip the optical fiber and eliminate covering plastic coated about it. By exhausting cleaving machine, the optical fibers must cleave at 900µm and then cleaned with lens tissue.

Align the cleaved ends of the optical fiber in the v-groove of the fusion splicer and leaving a smallest gap between the ends of the two fibers.

Changing the fusion splicer parameters (arc power and arc time). Turning on electric arc and heating optical fibers edges to the soften point and thrust the optical fiber edges collected or together.

2.9 Detection of ALT and AST enzymes by Laser biosensor

The experimental of three setup designs is shown in figure (2-17). The beam of the light emitted from the laser source toward the spectrum analyzer transmitting within the (SMF, MMF and PCF), in which the blood sample exists above. The detected signal could be obtained from the spectrometer. The light source had been used in this experiment is green laser) with λ=532 nm and output power maximum <1000mW, lower large adjustment knob this source of laser has a power supply which is stable at all the time of use it. Selecting this light source was because the absorption spectra of the blood sample covered this wavelength.

ALT and AST enzyme were detected by using three setups laser biosensors.

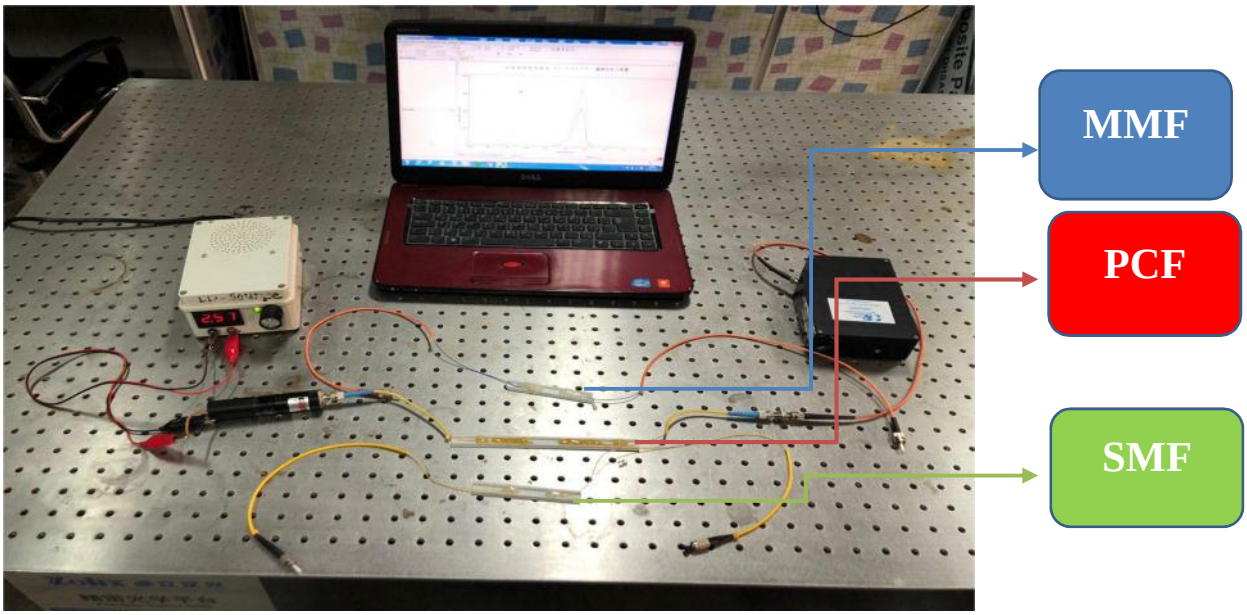


Figure (2-17) three types of biosensor (SMF, MMF, PCF)

2.9.1 Procedure

1- The power supply was connected, the output power was lower than 1000 mw.

2- The laser source green light diode laser 532 nm was joined according to the optical properties of the standard of ALT and AST enzyme.

3- All constructed biosensors (SM, MM and PC) were connected with the laser source, on the prepared segment of fiber (2 cm) of all setup, 1 ml of standard (of all samples) were placed (figure 2-18, 2-19).

4- AST enzyme was sensing in 531.16 nm in all 3 setups while ALT enzyme sensing in 531, 62 nm in SMF, in MMF and PCF sensing in 531.16 nm.

5- An optical spectrum analyzer (ocean HR-2000) was connected with biosensors to show the signal of intensity.

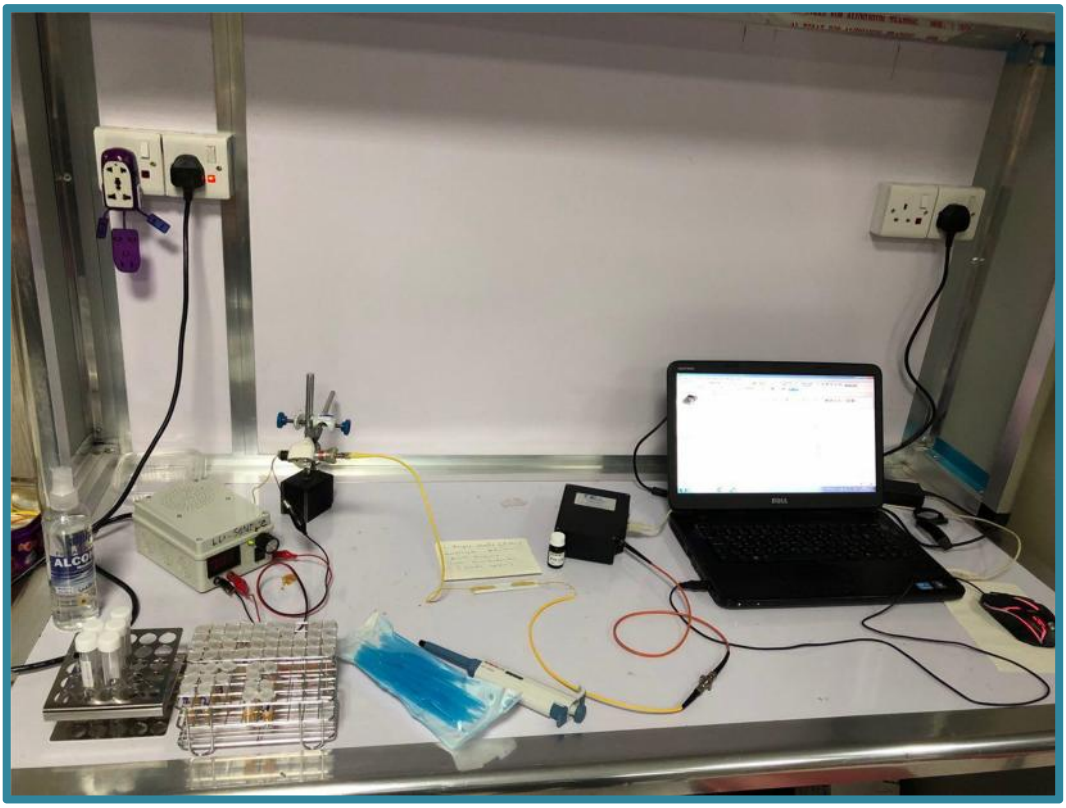


Figure (2-18) Detection of AST enzyme in serum samples.

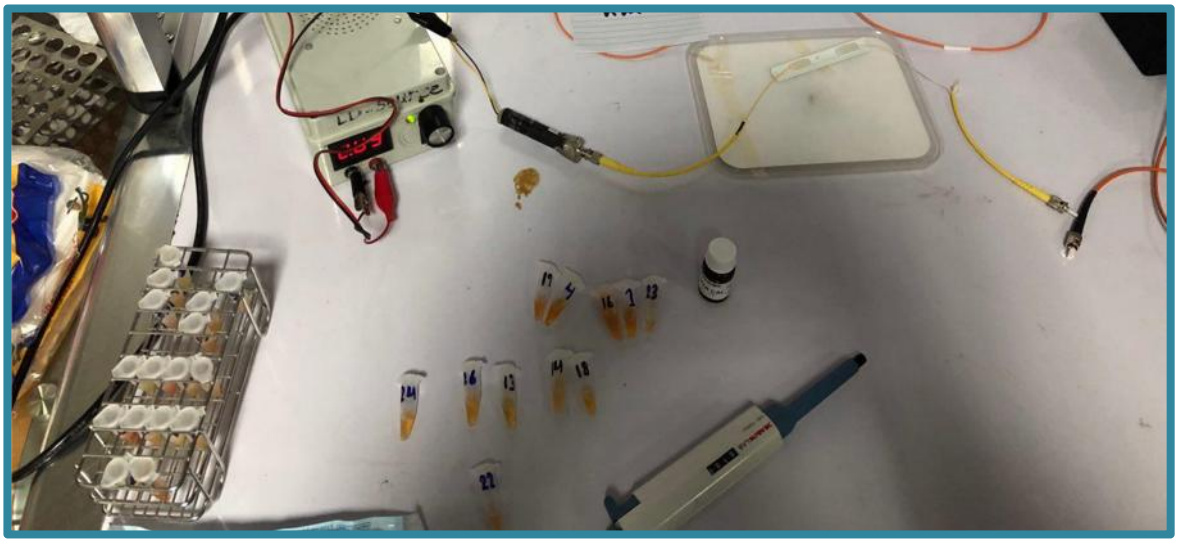


Figure (2-19) Detection of ALT enzyme

2.10 Statistical Analysis

Statistical data analysis methodologies were used in order to analyse and evaluate the results of the study under presentation of the statistical set (SPSS) ver. (22.0):

1- Descriptive data analysis:

A. Mean value, Standard Deviation, Standard Error, and (95%) Confidence interval for population Mean values and the two extremes values (min. and max.) for assuming that data under lying followed (Normal Distribution Function).

B. Graphical presentation by using:

- Stem-Leaf Charts.
- Cluster Bar Charts.
- ROC curve Charts.
- Long term trend plots.

2-Inferential data analysis:

These were used to accept or reject the statistical hypotheses, which included the following:

A- The One-Sample Kolmogorov-Smirnov (K-S) test.

B- The One-Way ANOVA test.

C- Levene test.

D- GLM Univariate procedure provides regression analysis and analysis of variance for one dependent variable by two factors.

E- Screening tests: Tests for mining data and estimating several indicators, such that (Sensitivity Rate, Specificity Rate).

F- Receiver Operation Characteristic curve [ROC] curve and estimating Area, as well as estimating 95% confidence interval, with standard error, asymptotic significant level Receiver Operation Characteristic [ROC] curve.

Cutoff Point: Using estimation of the low distance between angle front to curve and the curve:

G-Linear and Non-Linear Regression Models such as (Logarithmic, Inverse, Quadratic, Compound, Power, S-Shape, Growth, Exponential, and Logistic).

For the abbreviations of the comparison significant (C.S.), we used the followings:

- NS : Non significant at $P>0.05$
- S : Significant at $P<0.05$
- HS: Highly significant at $P<0.01$ [98].

Chapter Three
Results & Discussion and future work

Results and discussion

3.1 Introduction

The present work include the investigation of concentration of ALT and AST enzymes from patient attending Ibn- Alnafees hospital complaining from heart and liver diseases in age ranged from (15-72) years ,females were (62.5%) and males were (37.5%) . All the works are done under the condition of scientific laboratory, temperature of working room was 27ºc and the humidity was very low, experimental setup construction of biosensors are based on intensity modulation.

Detection the concentration of ALT and AST enzymes examined manually in the hospital using RANDOX kit and spectrophotometer in wavelength (546) nm. The absorption spectra for samples (ALT and AST) enzymes from 530-550 nm were measured to select the suitable wavelength of laser using green laser (diode laser) of 532 nm as a laser source for laser biosensor of blood test.

The refractive index measured by Abby refractometer for standard (normal) and all samples.

3.2 Biological results

Samples were collected from patients,10 ml from each one divided into two parts ,5 ml were examined manually in the hospital by Randox kit to detect the concentration level of ALT and AST enzymes ,the last 5ml were prepared to be examined with constructed biosensors (SMF,MMF and PCF) fibers.

3.2.1 Concentration measurement of ALT enzyme

Table 3-1 demonstrated the sequence of measured concentration of ALT enzyme (from higher to lower) manually by Randox kits were taken from 24 patients.

Table (3-1) illustrate the sequence of concentration of ALT enzyme measured by Randox kit.

Numbers	Sequence of ALT samples from high to low concentration	Concentration of ALT enzymes U/L
1	23	101.9
2	1	86
3	16	72
4	4	43
5	19	35
6	13	34.1
7	26	32.1
8	14	21.6
9	18	27.5
10	24	24
11	22	23.8
12	7	20
13	6	20
14	5	19
15	20	17.4
16	3	17
17	2	16
18	11	15.8
19	8	15.2
20	9	14.9
21	21	14.4
22	10	14.2
23	12	12
24	15	9.9

As seen in table (3-1) the highest concentration of ALT enzyme are 101.9 U/L while the lowest concentration of enzyme are 9.9 U/L.

3.2.2 Concentration measurement of AST Enzyme

Table (3-2) explained the sequence of measured concentration of AST enzyme (from higher to lower) using Randox kit were taken from 24 patients.

Table (3-2) illustrate the sequence of concentration of AST enzyme measured by Randox kit.

Numbers	Sequences of AST samples from high to low concentration	Concentration of AST enzymes from high to low concentration U/L
1	23	224.5
2	10	207
3	16	57
4	14	42
5	19	35
6	26	33.2
7	13	33.1
8	18	28.4
9	12	23
10	22	22.3
11	24	21
12	1	18
13	3	18
14	4	18
15	8	17.8
16	9	17
17	21	16.1
18	17	15.4
19	15	11.4
20	11	11.1
21	20	9
22	2	7
23	6	4.3
24	6	4.3

The highest concentration of AST enzyme as seen in table (3-2) are 224.5 U/L and the lowest concentration of AST enzyme are 4.3 U/L.

3.3 Laser biosensor for detection of ALT and AST enzymes

The intensity of ALT and AST enzymes are measured by using 3 types of constructed biosensors (SM, MM and PC) fibers. Diode laser of 532 nm are used as a laser source for biosensor.

3.3.1. Detection the intensity of ALT enzyme.

The intensity of ALT enzyme of all samples and standard are measured using single mode, multimode and photonic crystal biosensors as seen in table (3-3).

Table (3-3) shows the measurement of intensity of ALT enzyme using biosensors.

Sequence of ALT Samples from higher to lower concentration	Intensity au Single mode biosensor	Intensity au Multimode biosensor	Intensity au crystal biosensor
Reference	3375	3200	3325
Standard	1647	2568	2144
23	1497	1119	1301
1	1500	1172	1936
16	1585	2377	2112
4	1647	2568	2144
19	1811	2781	2163
13	1922	2809	2166
26	1958	2829	2190
14	2006	2838	2199
18	2083	2849	2208
24	2172	2863	2211
22	2246	2888	2216
7	2265	2900	2220
6	2265	2900	2220
5	2289	2905	2223
20	2293	2912	2230
3	2345	2925	2236
2	2389	2927	2246
11	2530	2941	2258
8	2579	2951	2275
9	2798	2961	2289
21	2915	2969	2292
10	2977	3003	2305
12	3224	3100	2307
15	3250	3150	2327

The initial intensity (reference) for single mode biosensor are 3375 au, for multimode are 3200 au and for photonic crystal biosensor are 3325 au. The highest intensity of ALT enzyme in single mode are 3250 au and the lowest intensity are 1497 au. In multimode biosensor the highest intensity are 3150 au and the lowest intensity are 1119 au while in photonic crystal biosensor the highest intensity are 3325 au and the lowest intensity are 1301 au.

The laser biosensors in (2cm) length and 532nm wavelength are used to measure the intensity spectra of ALT enzyme. The intensity spectra of ALT standard and the highest concentration of ALT sample in 3 types of biosensor (SM, MM and PC) fibers are shown in figures (3-1-A, 3-2-A and 3-3-A) and the intensity spectra of all standard and all samples of ALT enzyme in 3 types of biosensor (SM, MM and PC) fibers are shown in figures (3-1- B, 3-2-B and 3-3 B) respectively.

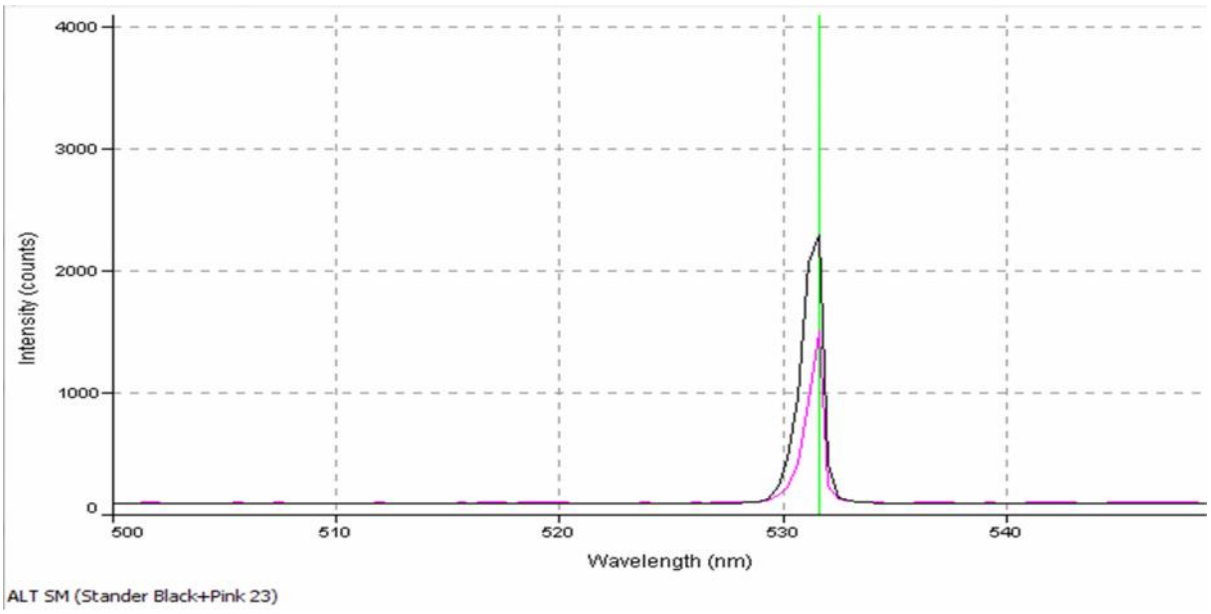


Figure (3-1-A): The intensity (spectra) of ALT standard and The highest concentration of ALT sample in (SM) biosensor.

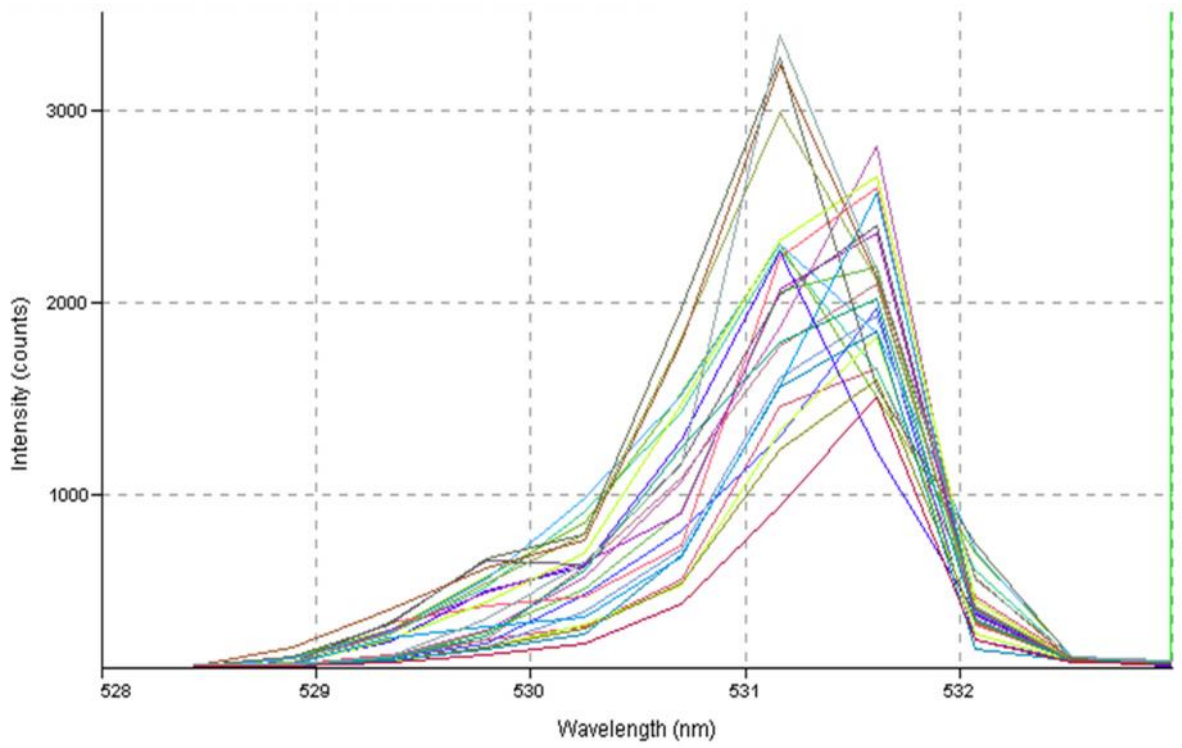


Figure (3-1-B) The intensity (spectra) of all standard and All samples of ALT enzyme in (SM) biosensor.

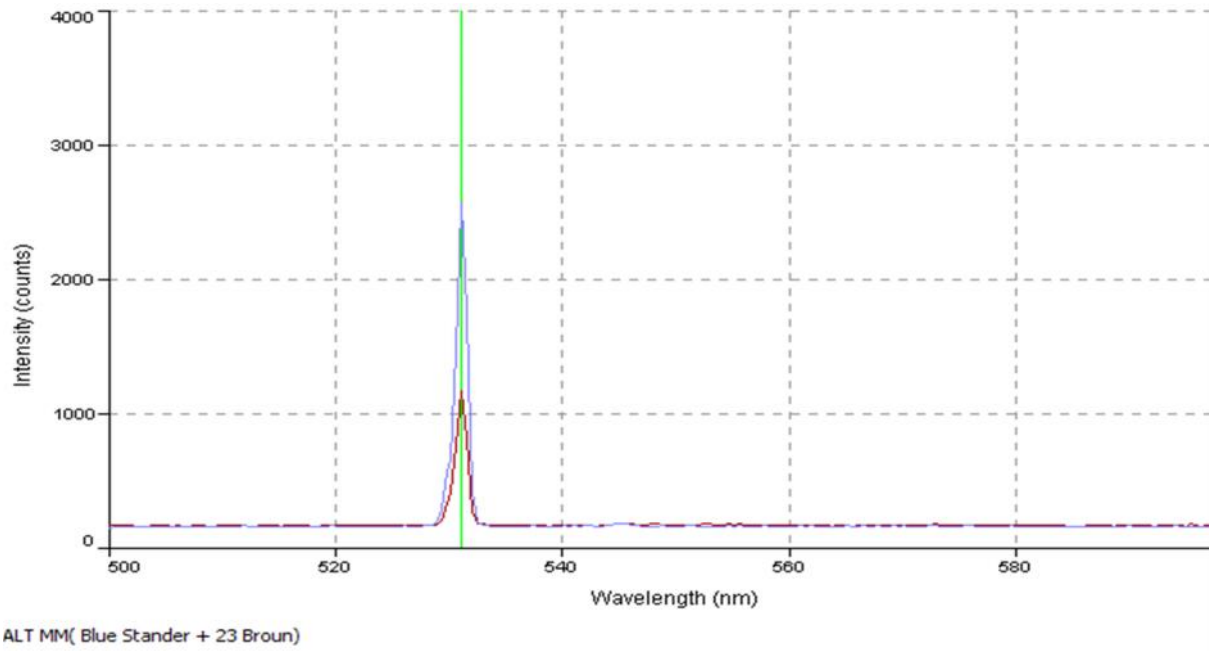


Figure (3-2-A): The intensity (spectra) of ALT standard and The highest concentration of ALT sample in (MM) biosensor.

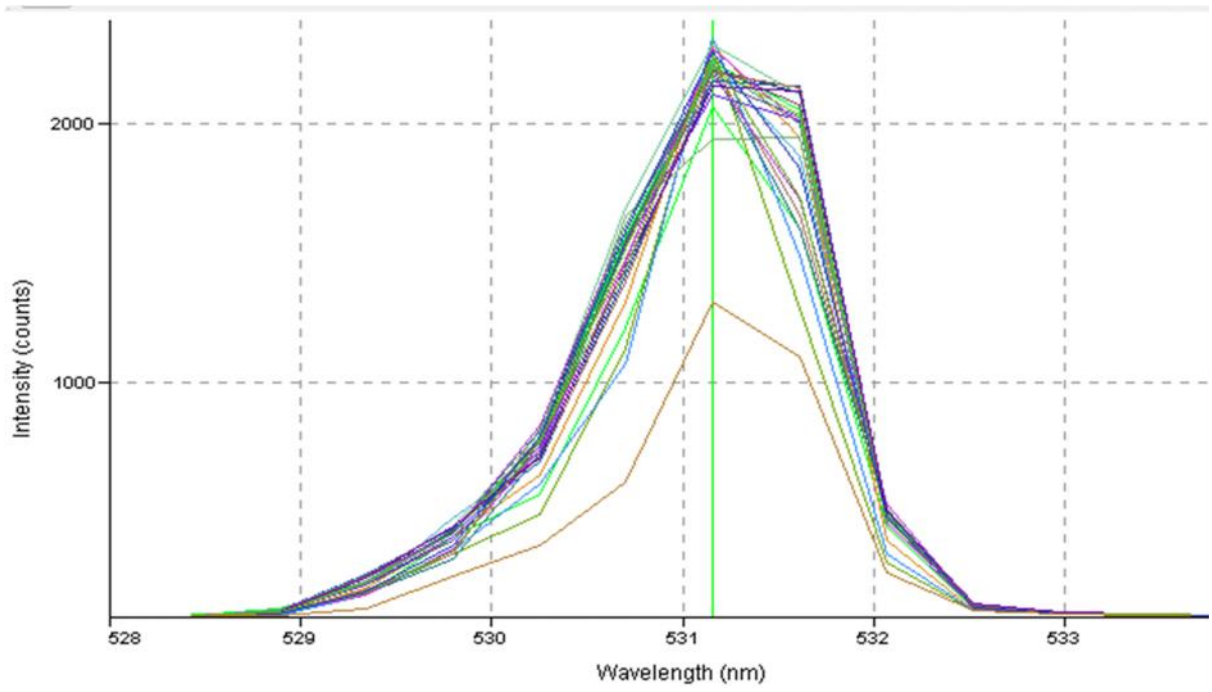


Figure (3-2 B): The intensity (spectra) of all standard and Samples of ALT enzyme in (MM) biosensor.

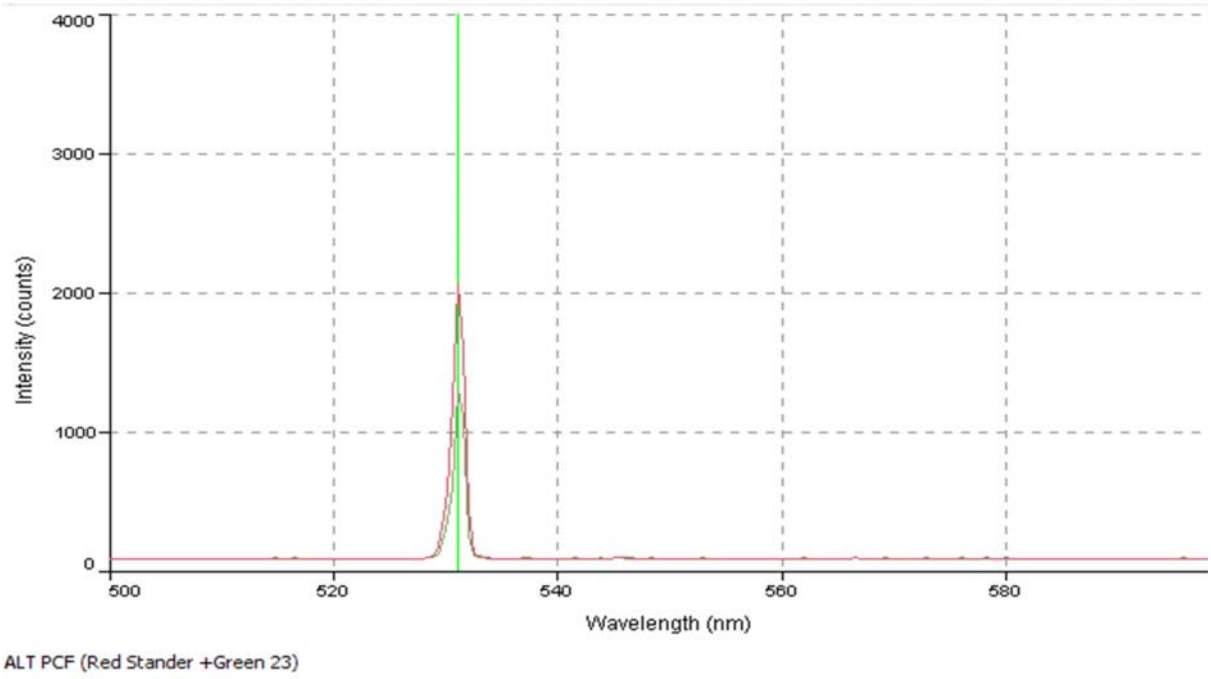


Figure (3-3-A): The intensity (spectra) of ALT standard and the highest concentration of ALT sample in (PCF) biosensor.

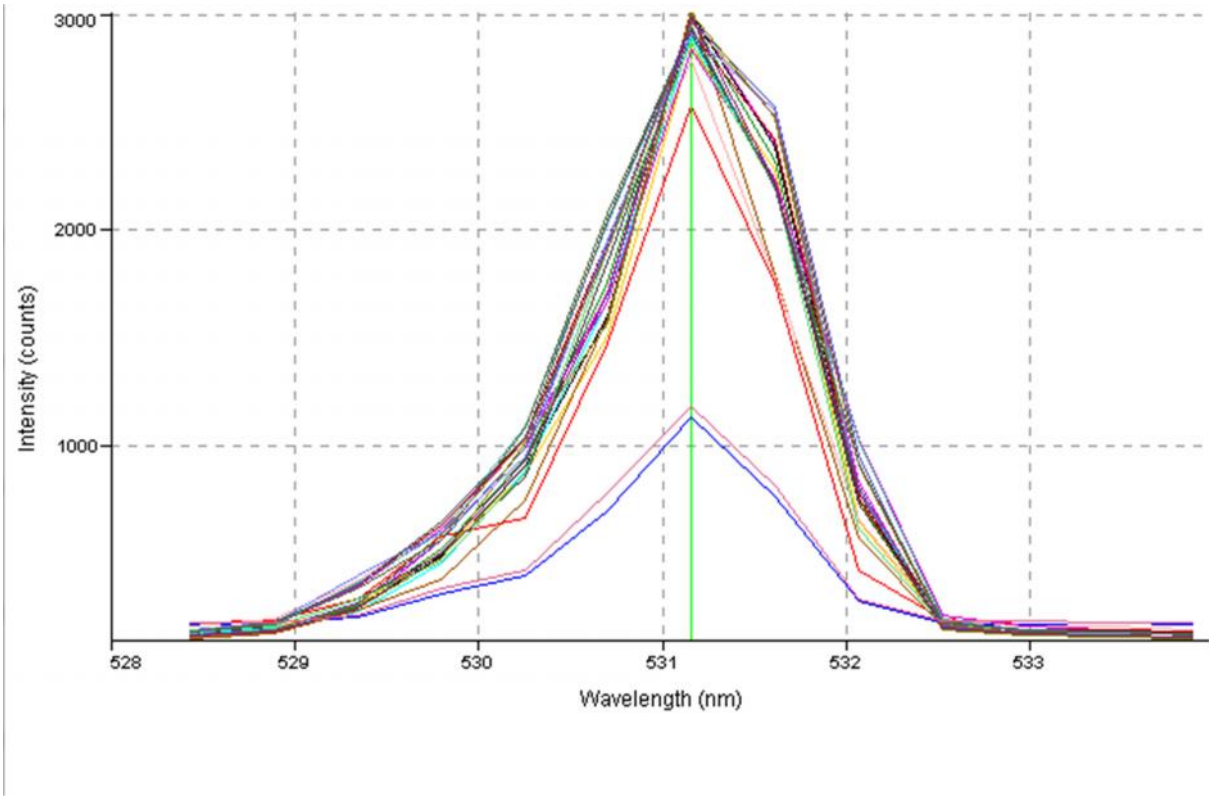


Figure (3-3-B): The intensity (spectra) of ALT standard and the highest concentration of ALT sample in (PCF) biosensor.

3.3.2 Detection the intensity of AST enzyme.

Three types of biosensors (single mode, multimode and photonic crystals) in measurement the intensity of AST enzyme as see in table (3-4).

Table (3-4) Shown the intensity of AST enzyme measured by biosensors.

Sequences of AST samples from high to low concentration	Intensity au Single mode	Intensity au Multimode	Intensity au Photonic crystal
Reference	3375	3200	3325
Standard	1468	2221	1781
23	1241	1475	1468
10	1326	1878	1531
16	1374	2013	1627
14	1468	2221	1732
19	1483	2373	1790
26	1496	2423	1893
13	1532	2461	1916
18	1722	2471	2070
12	1771	2491	2172
22	1915	2537	2300
24	2073	2550	2340
1	2198	2569	2430
3	2198	2569	2430
4	2198	2569	2430
8	2289	2588	2471
9	2425	2606	2488
21	2498	2612	2601
17	2635	2642	2678
15	2767	2680	2859
11	2876	2740	2883
20	2999	2833	2931
2	3065	2876	3132
6	3115	2887	3214

The initial intensity (reference) for single mode biosensor are 3375 au, for multimode are 3200 au and for photonic crystal biosensor are 3325 au. The highest intensity of AST enzyme in single mode are 3115 au and the lowest intensity are 1241 au, in multimode biosensor the highest intensity are 2887 au and the lowest intensity are1475 au while in photonic crystal biosensor the highest intensity are 3214 au and the lowest intensity are1468 au.

The same procedure which are used for the measurement the intensity spectra of ALT enzyme are applied to measure the intensity spectra of AST enzyme.

The intensity spectra of AST standard and the highest concentration of AST sample in 3 types of biosensors (SM, MM and PCF) fibers biosensors as shown in figures (3-4-A ,3-5-A and 3-6-A), the intensity spectra of all standard and all samples of AST enzyme in 3 types of biosensors (SM, MM and PCF) fibers biosensors are shown in figures (3-4-B) (3-5- B) (3-6- B) respectively.

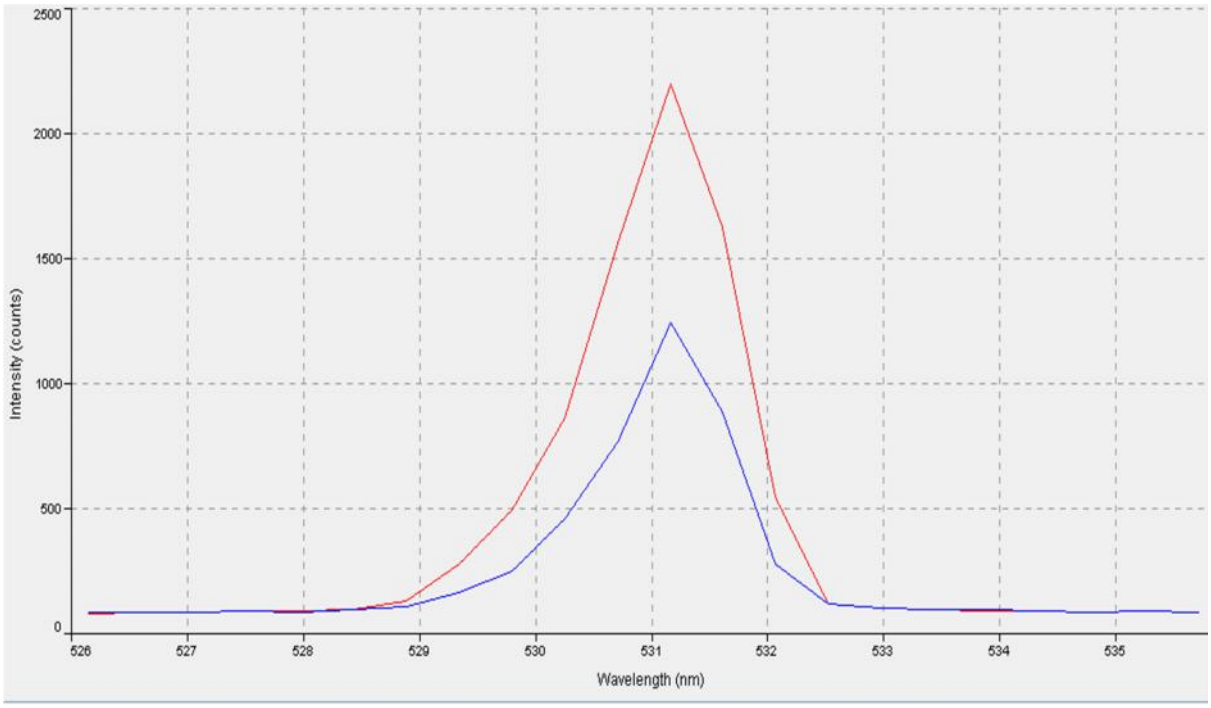


Figure (3-4 A): The intensity (spectra) of AST standard and the highest concentration of AST sample in (SM) biosensor.

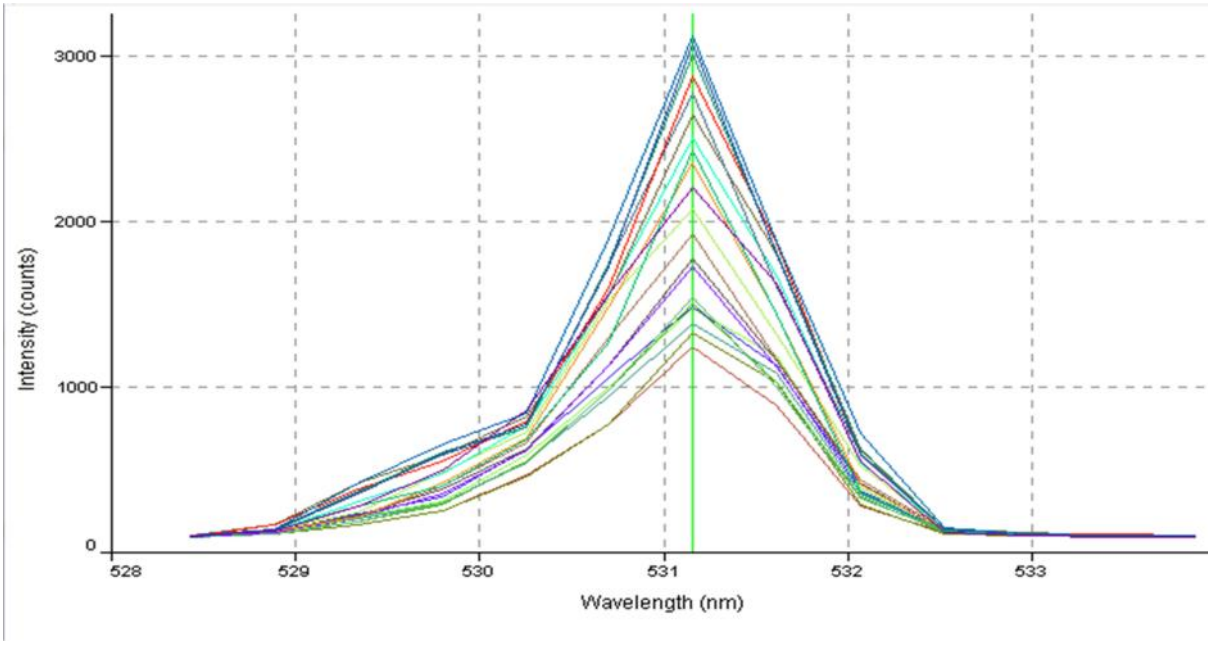


Figure (3-4-B): Show the intensity (spectra) of AST standard and the highest concentration of AST sample in (SM) biosensor.

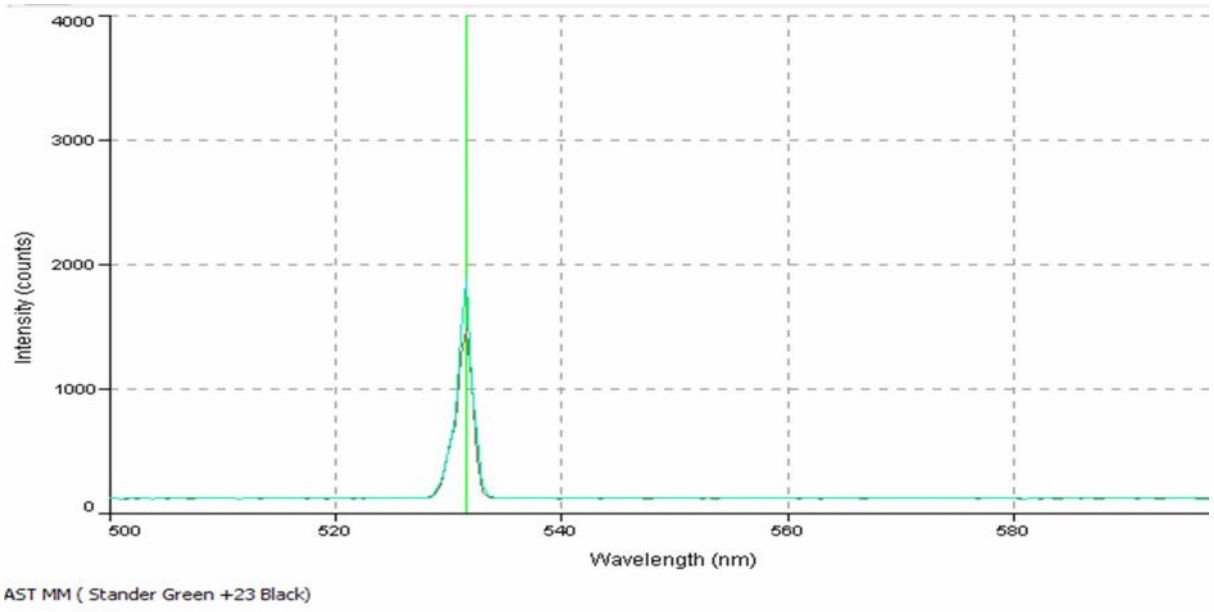


Figure (3-5-A): The intensity (spectra) of AST standard and the highest concentration of AST sample in (MM) biosensor.

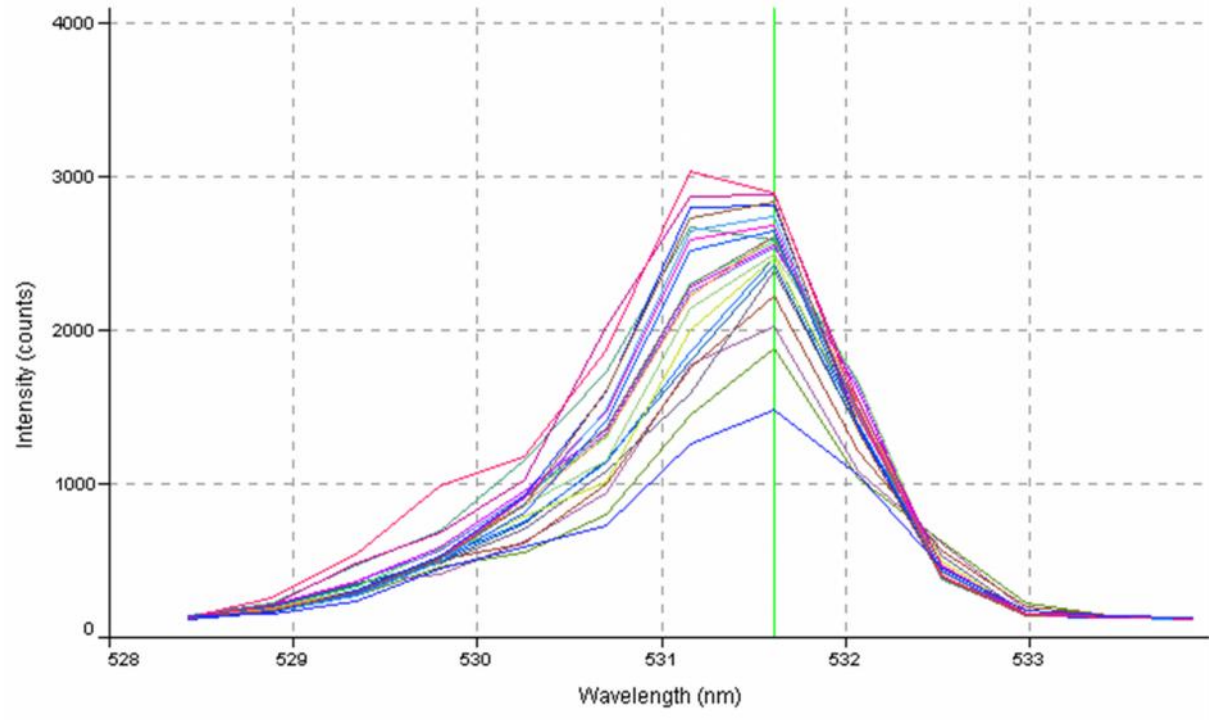


Figure (3-5-B): The intensity (spectra) of AST standard an the highest concentration of AST sample in (MM) biosensor.

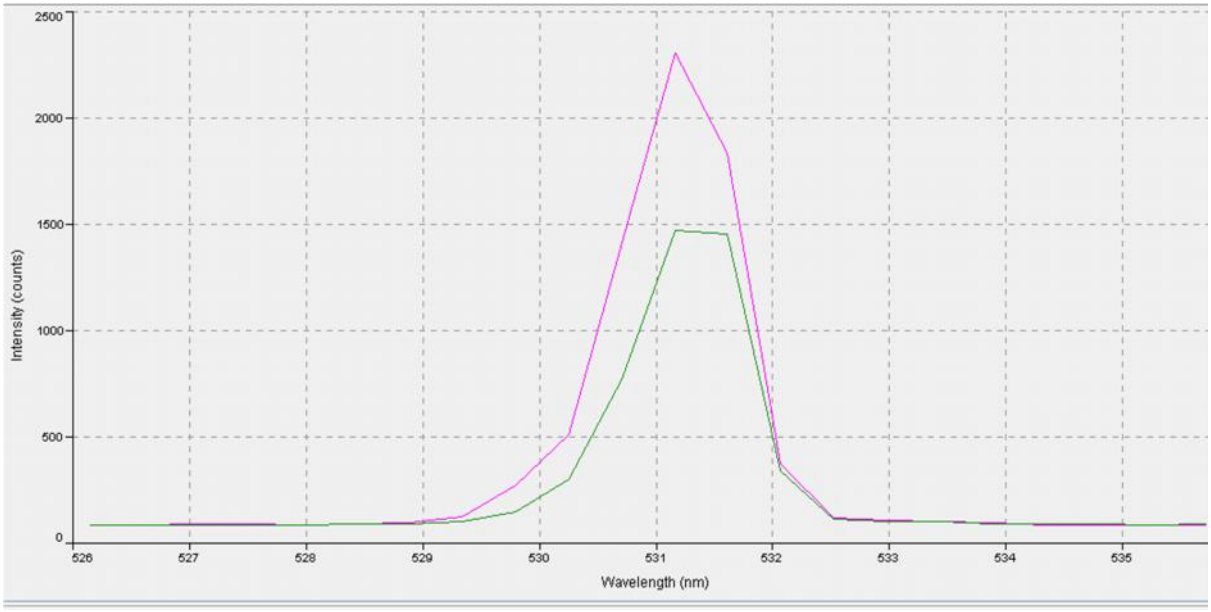


Figure (3-6-A): The intensity (spectra) of AST standard and the highest concentration of AST sample in (PCF) biosensor.

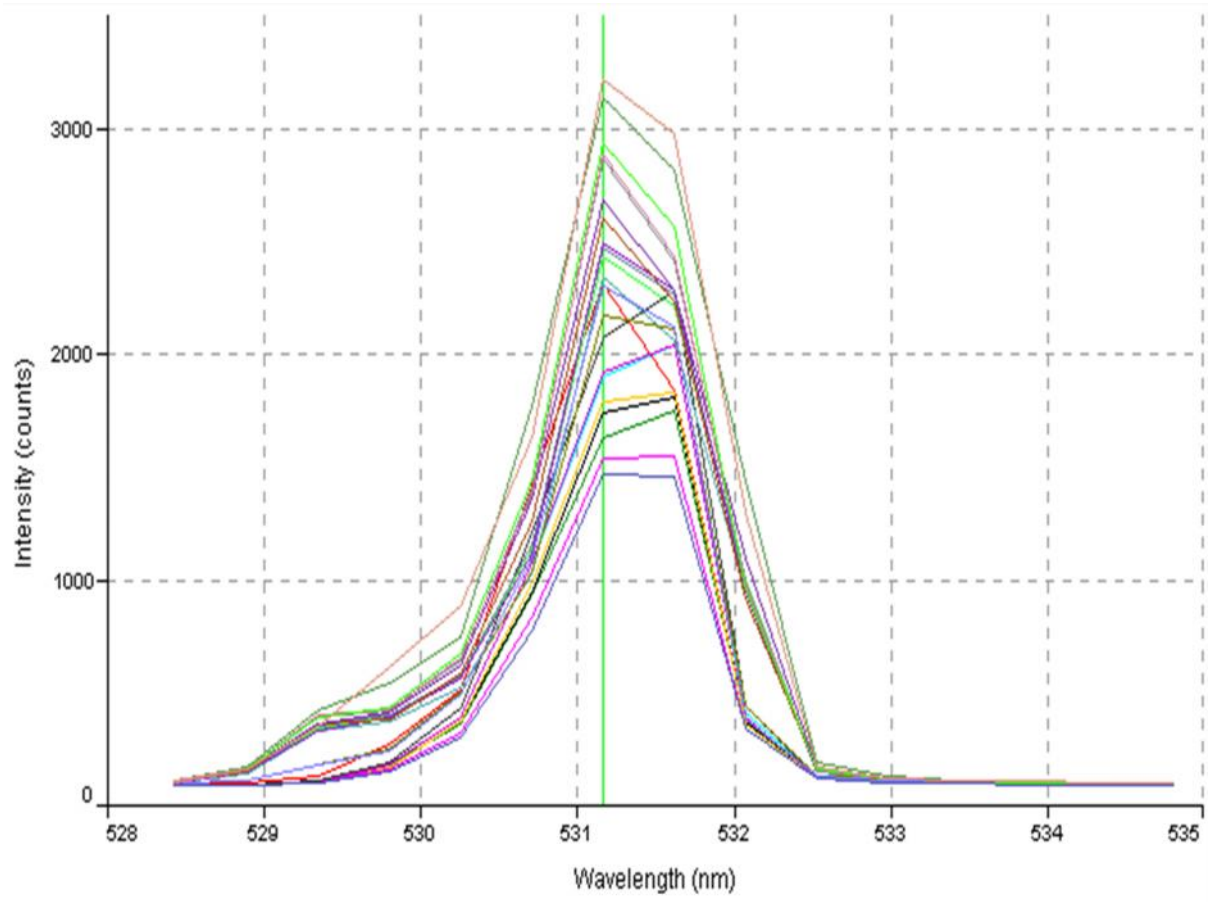


Figure (3-6-B): The intensity (spectra) of AST standard and the highest concentration of AST sample in (PCF) biosensor.

3.4 Refractive index measurement

The refractive indices for all standard and all samples of ALT and AST enzymes in the study are measured by using Abby refractometer .**Table (3-5)** demonstrate the refractive index of all samples and for the two types of enzyme.

Table (3-5) the refractive index of all standard and all samples in ALT and AST enzyme.

No. samples	Refractive index
Standard of ALT	1.3472
Standard of AST	1.3470
1	1.3587
2	1.3547
3	1.3562
4	1.3580
5	1.3540
6	1.3545
7	1.3576
8	1.3559
9	1.3591
10	1.3572
11	1.3577
12	1.3571
13	1.3551
14	1.3586
15	1.3567
16	1.3571
17	1.3553
18	1.3592
20	1,3596
21	1.3595
22	1.3598
23	1.3570
24	1.3570
25	1.3570
26	1.3570

The refractive index value are changed according to the concentration of enzymes in blood samples, higher concentration of samples higher refractive index, Maximum concentration of ALT enzyme (101.9 U/L), the higher refractive index (1.3570) ,the minimum concentration of ALT enzyme (9.9 U/L),the lower refractive index(1.3567) .Maximum concentration of AST enzyme (224.5U/L), the higher refractive index (1.3570), the minimum concentration of ALT enzyme (4.3U/L), the lower refractive index (1.3545).

3.5 Statistical Result

The findings of the data analysis in tables and in figures systematically are demonstrated in the present section for detection of ALT and AST enzyme and as follows:

Testing coincidence of Intensity among Studied Parameters:

Normal Distribution Function (Goodness of Fit test):

Table (3-6) shows the descriptive analysis of the whole samples treated with different techniques using normal distribution function (Goodness of Fit test), test procedure one-sample using Kolmogorov-Smirnov test represented to match and detect collective distribution function and interpretations for studied reading with a identified theoretical distribution.

Table (3-6): Normal distribution function test due to different Enzymes treated with different Techniques in relative to (Under/Upper) cut off points of Intensity readings.

One-Sample Kolmogorov-Smirnov Test					
Techniques	Test Statistic and Comparison's Significant	Enzymes			
		ALT		AST	
		Under	Upper	Under	Upper
SMF	No.	4	20	4	19
	Kolmogorov-Smirnov Z	0.571	0.939	0.318	0.392
	Asymp. Sig. (2-tailed)	0.900	0.342	1.000	0.998
	C.S. (*)	NS	NS	NS	NS
Test distribution are Normal					
PCF	No.	4	20	4	21
	Kolmogorov-Smirnov Z	0.627	0.596	0.388	0.612
	Asymp. Sig. (2-tailed)	0.827	0.869	0.998	0.848
	C.S. (*)	NS	NS	NS	NS
Test distribution are Normal					
MMF	No.	4	20	4	19
	Kolmogorov-Smirnov Z	0.592	0.643	0.452	0.708
	Asymp. Sig. (2-tailed)	0.875	0.803	0.987	0.698
	C.S. (*)	NS	NS	NS	NS
Test distribution are Normal					

(*) NS: Non Sig. at P>0.05

Findings presented normal distribution tests for studied readings concerning different enzymes treated with different techniques in relative to (Under/Upper) cut off points distribution due to intensity readings, since P-value are accounted at (P>0.05) the significant levels, and that could be enables of applying conventional methods of statistics.

Table (3-7) represents a summary statistics such as mean values, and standard deviation of intensity readings, distributed according to different parameters, classified by different Enzymes, different Techniques in relative to (Under/Upper) cut off points of normal responds.

Table (3-7): Descriptive Statistics of Intensity readings distributed due to different parameters

Dependent Variable: Intensity					
Enzyme	Technique	Evaluation	No.	Mean	Std. Deviation
ALT	SMF	Under	4	1557.25	72.42
		Upper	20	2410.85	422.47
	PCF	Under	4	1873.25	392.31
		Upper	20	2239.05	47.12
	MMF	Under	4	1809.00	770.41
		Upper	20	2920.05	90.26
AST	SMF	Under	4	1352.25	94.76
		Upper	19	2276.58	537.14
	PCF	Under	4	1589.50	115.32
		Upper	21	2503.29	420.67
	MMF	Under	4	1896.75	314.58
		Upper	19	2604.05	145.42

Results shows due to mean values that ALT enzyme of using MMF different parameters technique has accounted the high intensity level, then followed by SM technique, and finally by PCF technique, which has recorded the low mean value in relative to upper of normal cut off point comparing with the leftover states. In addition to that, AST enzyme of using MM technique has accounted the high intensity level, followed by PCF technique and finally by SM technique, which has recorded the low mean value in relative to upper of normal cut off point comparing with the leftover states.

From the previous results, it could be summarized that ALT enzyme of using MM technique has accounted the high intensity level, while PCF technique, has recorded the lowest mean value in relative to upper of normal cut off point comparing with the leftover status.

Different enzymes with different Techniques in relative to (Under/Upper) cut off points of normal responds represented as shown in Figure (3-7) graphically plotting of bar chart regarding mean values.

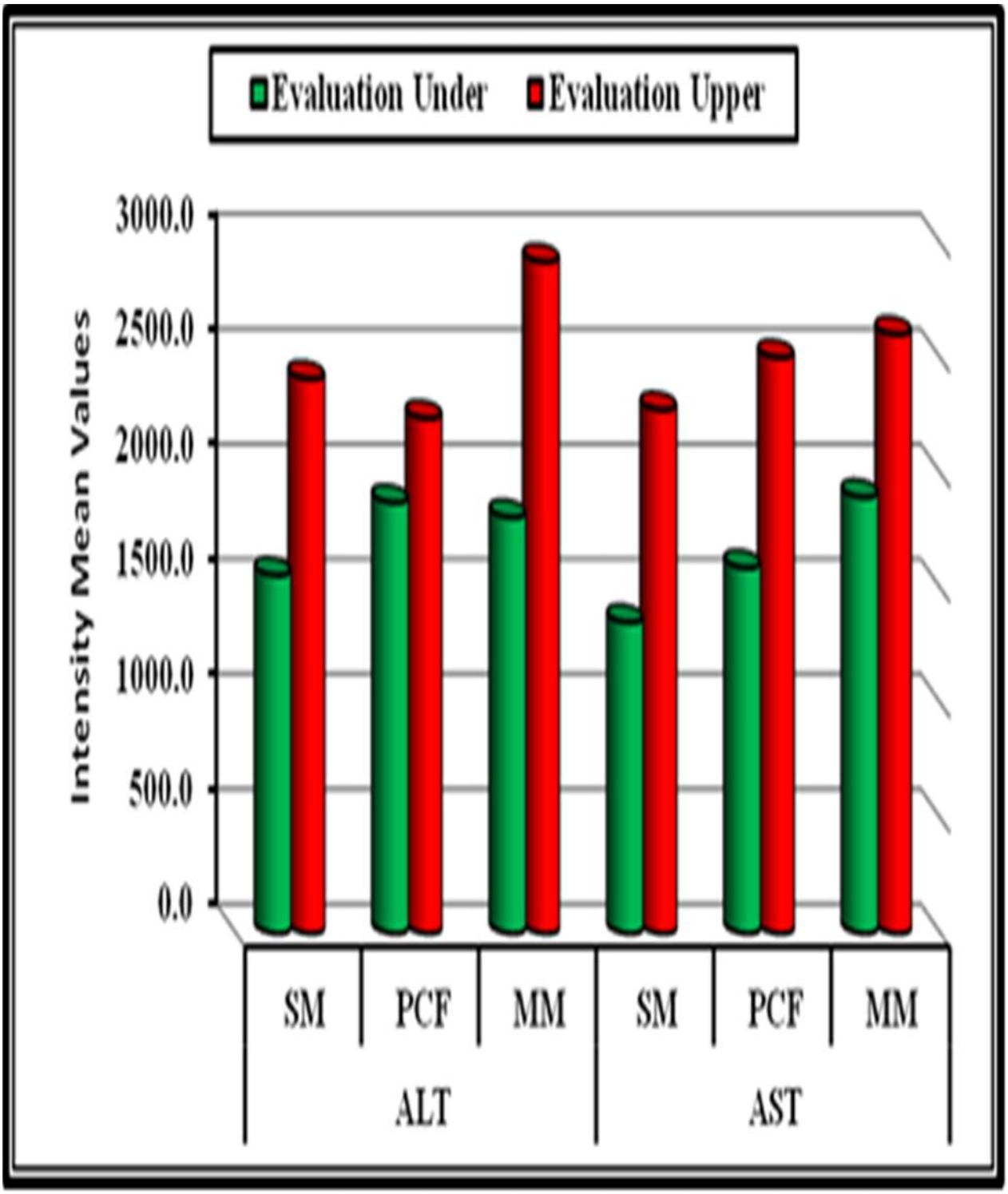


Figure (3-7): Bar Chart for Intensity Mean values concerning different of studied parameters.

From this figure we can see that MM biosensor are more efficient from the others biosensors in detection the concentration of the ALT and AST enzymes. Figure (3-8) represent graphically Stem-Leaf plot of different criteria, concerning upper cut off points of normal responds.

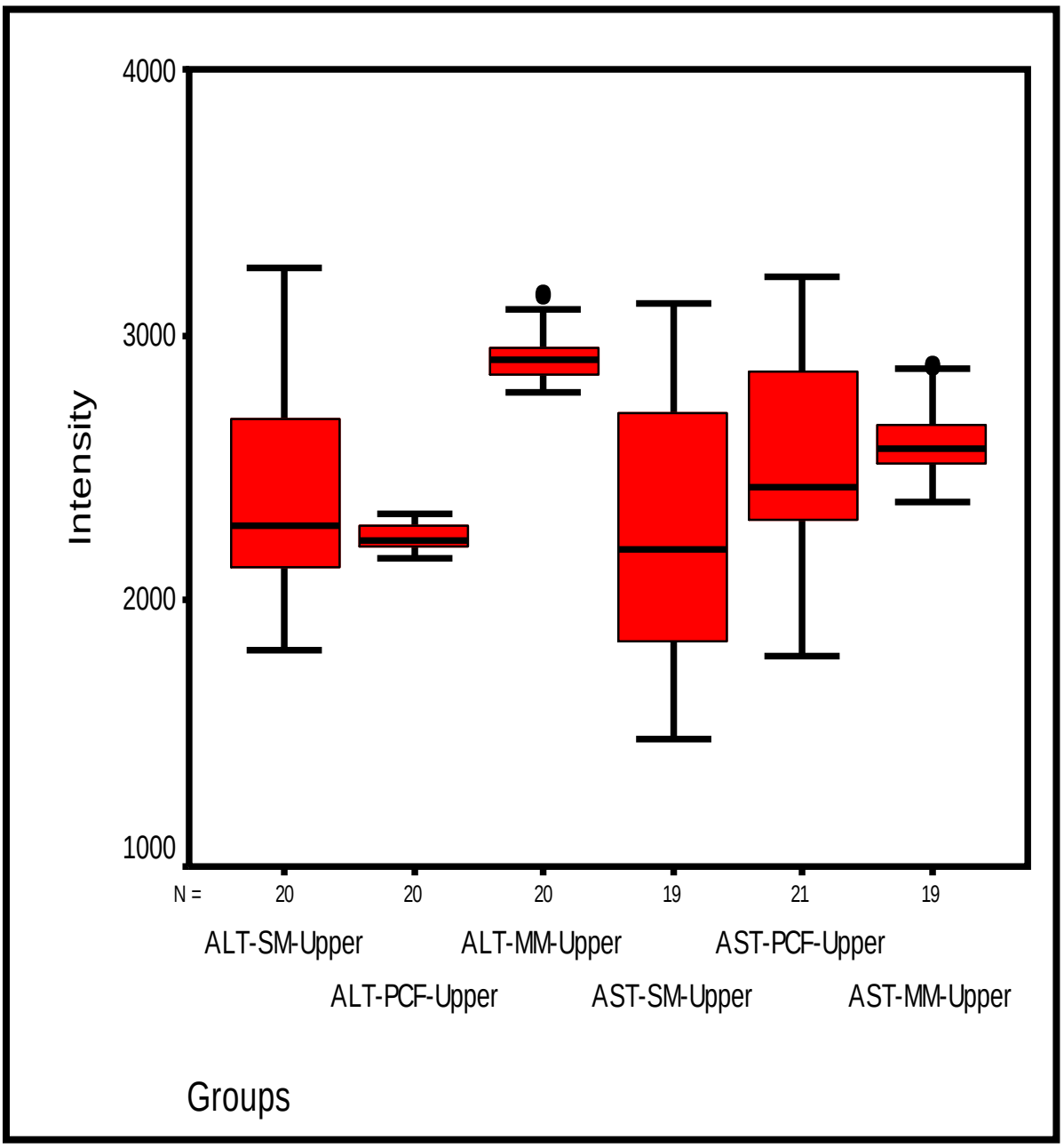


Figure (3-8): Stem-Leaf plot concerning Intensity readings by studied Parameters in light of upper cut off points of normal responds.

The studying group's concerning (**Intensity Marker**) analyses uses different enzymes, and different techniques should be proved by testing of equal variances are supposed and equal mean vales are expected through application of "Levine and one-way ANOVA" correspondingly, appeared in table (3-8)

Table (3-8): Testing equal variances and equal mean values for Intensity marker concerning studied groups.

Test	Testing Homogeneity of Variances		ANOVA- Testing equality of means	
	Levene Statistic	Sig. (*)	F-test	Sig. (*)
Toughness	10.738	0.000 (HS)	15.813	0.000 (HS)

(*) HS: Highly Significant at P< 0.01; NS: Non Sig. at P>0.05

Orientation of equal variances testing are assumed, outcomes indicated highly significant different is appeared at P<0.01 among variances group's concerning all examining groups. As well as, highly significant different are obtained for testing of equal mean values among studied groups at P<0.01. and accordance to apparent results, it essentials to be continuing testing of alternative statistical hypothesis which says that at least two groups has a different, and that should be achieved through using (Games Howell-GH) test, supposing variances are assumed among groups not equal in order to assigns in which compromised pairs wised of groups that (**Intensity marker**) would be different as shown in table (3-9).

Table (3-9): Levels of significant for probable pair wised comparisons using (GH) test among studied groups for (Intensity marker).

(*) HS: Highly Significant at P< 0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05

	ALT-SMF-Under	ALT-SMF-Upper	ALT-PCF-Under	ALT-PCF-Upper	ALT-MMF-Under	ALT-MMF-Upper	AST-SMF-Under	AST-SMF-Upper	AST-PCF-Under	AST-PCF-Upper	AST-MMF-Under
ALT-SMF-Upper	0.000										
ALT-PCF-Under	0.849	0.496									
ALT-PCF-Upper	0.001	0.796	0.747								
ALT-MMF-Under	0.999	0.873	1.000	0.968							
ALT-MMF-Upper	0.000	0.002	0.102	0.000	0.410						
AST-SMF-Under	0.197	0.000	0.484	0.002	0.958	0.000					
AST-SMF-Upper	0.001	0.999	0.804	1.000	0.966	0.002	0.000				
AST-PCF-Under	1.000	0.000	0.913	0.011	1.000	0.001	0.248	0.002			
AST-PCF-Upper	0.000	1.000	0.353	0.226	0.789	0.009	0.000	0.937	0.000		
AST-MMF-Under	0.654	0.361	1.000	0.632	1.000	0.059	0.288	0.736	0.759	0.226	
AST-MMF-Upper	0.000	0.732	0.244	0.000	0.674	0.000	0.000	0.357	0.000	0.995	0.153

Results shows that with respect to subject of studied (ALT-SM-Upper) group was accounted significant different compared with the (ALT-MM-Upper) group at least at P<0.01, while no significant differences are accounted with the rest of other groups in light of upper cut off points. Regarding to (ALT-PCF-Upper) group, results accounted significant differences compared with the (ALT-MM-Upper, and AST-MM-Upper) groups at P<0.01, while no significant differences are accounted with the rest of other groups in light of upper cut off points.

With respect to (ALT-MM-Upper) group, results were accounted significant differences compared with (AST-SM-Upper, AST-PCF-Upper, and AST-MM-Upper) groups at P<0.01.

On the subject of (AST-SM-Upper, AST-PCF-Upper, and AST-MM-Upper) groups, the obtained results illustrate that p value at P>0.05, means no significant differences are illustrated compared with each other's,

The obtained results show that, (ALT-MM-Upper) group has scored the highest result concerning intensity marker, since highly levels of significant at P<0.01 were represented with the rest of others upper to cut off points group.

3.5.1 Intensity reading's test in compact form

Table (3-10) illustrate the statistics summary , like mean values and standard error, 95% confidence interval of mean of studied groups by measuring parameter (Intensity reading's test), resulted by different parameters, such that (Enzymes, Techniques, and Redistribution under/upper cut off points due to normal responds) in compact form.

Table (3-10): Summary Statistics of Intensity Marker in compact form distributed by different Parameters.

Parameters in compact form	Groups	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Enzymes	ALT	2134.91	54.08	2027.93	2241.89
	AST	2037.07	54.16	1929.92	2144.22
Techniques	SMF	1899.23	66.38	1767.93	2030.54
	PCF	2051.27	66.10	1920.51	2182.03
	MM	2307.46	66.38	2176.16	2438.77
Cutoff points	Under	1679.67	69.81	1541.56	1817.77
	Upper	2492.31	31.37	2430.25	2554.37

Results showed that enzyme ALT are accounted the highest level of intensity marker by marginal mean value, in addition to that and with respect to subject of using techniques, results showed that MM was accounted the highest level of intensity marker by marginal mean value, as well as high gap reported with reference of redistribution under/upper cut off points due to normal responds.

Figure (3-9) represent bar chart regarding marginal mean values of studied factors, which classified by different Enzymes, different Techniques in relative to (Under/Upper) cut off points of normal responds.

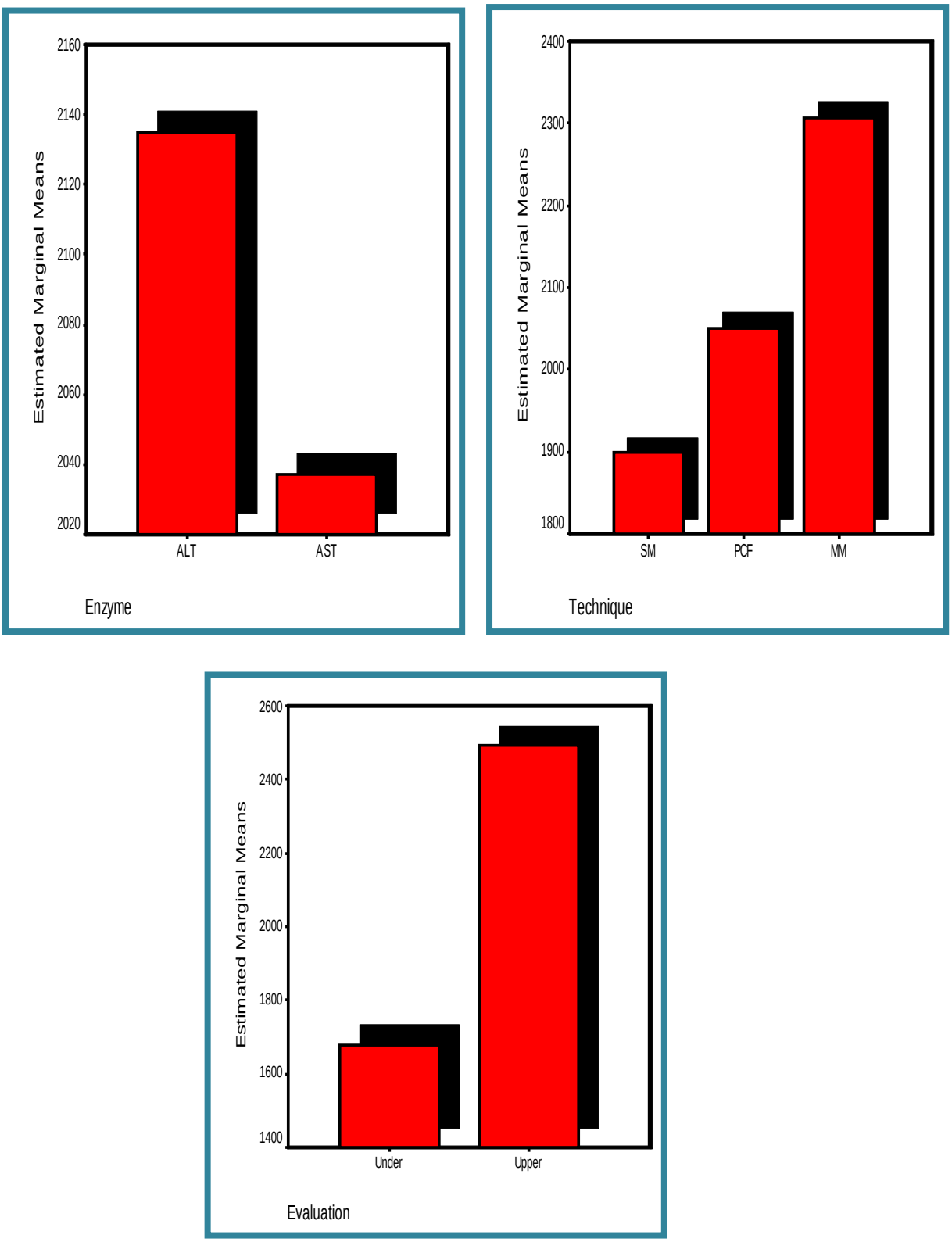


Figure (3-9): Bar Charts for Marginal Mean values concerning Intensity Marker distributed by different of studied Parameters

Table (3-11) shows the most common statistical method of testing and analyzing studied parameters in light of (**Intensity Marker**) associated with different sources of variations, such as: (Enzymes, Techniques, and redistribution in relative (Under/Upper) cut off points of normal responds, as well as an interaction factors that represented by preceding sources of variations through applying general linear model (G.L.M.) with fixed interaction effects complete design, as well as testing effectiveness of others source of variations that not including in studied model (i.e. the Intercept).

Table (3-11): G.L.M. of Fixed Effects Model with interaction for testing of Marginal mean values for different (S.O.V.) effects in compact form

Dependent Variable: Roughness test						
Source of Variation (S.O.V.)	**Type III Sum of Squares**	**d.f.**	**Mean Square**	**F**	**Sig.**	**C.S. (*)**
Corrected Model	**20345982.0**	**11**	**1849634.7**	**15.81**	**0.000**	**HS**
Intercept	**347551480.8**	**1**	**347551480.8**	**2971.3**	**0.000**	**HS**
Enzymes	**191142.5**	**1**	**191142.5**	**1.63**	**0.203**	**NS**
Techniques	**2260599.3**	**2**	**1130299.6**	**9.66**	**0.000**	**HS**
Evaluation	**13186708.4**	**1**	**13186708.4**	**112.74**	**0.000**	**HS**
Enzymes * Techniques	**87902.7**	**2**	**43951.4**	**0.38**	**0.688**	**NS**
Enzymes * Evaluation	**25631.7**	**1**	**25631.7**	**0.22**	**0.640**	**NS**
Techniques * Evaluation	**300486.9**	**2**	**150243.4**	**1.28**	**0.280**	**NS**
Enzymes * Techniques * Evaluation	**754684.9**	**2**	**377342.5**	**3.23**	**0.043**	**S**
Error	**34.25**	**54**	**0.634**			
Total	**669.48**	**60**				
R - Squared = 0.570						

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05

Results show highly significant effect were obtained at P<0.01for studied (Techniques, and redistribution in relative (Under/Upper) cut off points of normal responds), at P<0.01, a s well as interaction among assignable factors at P<0.05 and that should be adding as a hidden factor for interpretation of the variations among the studied intensity marker's readings, while no significant different concerning enzyme parameter at P<0.05. In addition that, an intercept (other sources of variations not included in the studied equation) has recorded highly significant effect at P<0.01.

Finally, determination coefficient (i.e. the R-Square) was recorded 57% which represent percent value of studied (S.O.V.) that interpretation the amount of variations among intensity markers readings.

With respect to preceding results, (Least Significant Difference-LSD) test applied toward recognize real significant levels in all probable pair wised of intensity marker's readings in light of different techniques in compact form, and as shown in **table (3-12).**

Table (3-12): Pair's wised Comparisons by (LSD) test among studied groups concerning Intensity Marker in compact form

Dependent Variable: Roughness				
(I) Group	**(J) Group**	**Mean Difference (I-J)**	**Sig.**	**C.S. (*)**
MS	**PCF**	**-75.58**	**0.281**	**NS**
	MMF	**-416.83**	**0.000**	**HS**
PCF	**MMF**	**-341.25**	**0.000**	**HS**

(*) HS: Highly Sig. at P<0.01;; NS: Non Sig. at P>0.05

The present results exhibited that, significant different at P<0.01 were reported between SM and MM groups, and between PCF and MM groups, while the rest of the other comparison appeared with no significant different at P>0.05.

For studying influence of concentration on intensity marker's readings in light of ALT enzyme, a several functions are supposed such as: (Linear, Quadratic, Cubic, Power, Compound, S-Shape Logarithmic, Inverse, Exponential , Growth, , and Logistic) in order to be certain that a selected model has the best and an optimal one, which represented their outcomes in **appendix 5.**

Results shows that nonlinear regression of "S-Shape, Cubic, and Linear" models(in statistical analysis) has been registered, the one of the finest mathematical models for learning influence of concentration in relation to the intensity marker for different techniques, SM, PCF, and MM respectively.

Table (3-13). Results show strong and too highly significant correlation ships are accounted at $P<0.01$ concentration on intensity marker's readings.

Others source of variations are not included in the studied models, (i.e. constant term), shows that a meaningful of an initial value that not include in the regression equation.

Table (3-13): Regression outcomes of Influence Intensity Marker in ALT enzyme using different Techniques.

Influence of Concentration on Intensity Marker using SM Technique					
Simple Correlation Coefficient	0.97133	Non Linear Regression Tested in two tailed alternative Statistical hypothesis			
Determination Coefficient R-Square	0.94348				
F (Statistic)	367.208	Sig. Level	0.0000 (HS) (*)		
Variables in the Equation					
Parameters	B	SE.B	Beta	t-test	Sig. of (t)
Slope (B1)	9.387410	0.489880	0.971326	19.163	0.0000
(Constant) : B0	7.251296	0.026062	-	278.236	0.0000
Predicted equation is: [illegible]					
Influence of Concentration on Intensity Marker using PCF Technique					
Simple Correlation Coefficient	0.99601	Non Linear Regression Tested in two tailed alternative Statistical hypothesis			
Determination Coefficient R-Square	0.99203				
F (Statistic)	830.253	Sig. Level	0.0000 (HS) (*)		
Variables in the Equation					
Parameters	B	SE.B	Beta	t-test	Sig. of (t)
Slope (B1) X^1	-30.393306	2.03306	-3.575058	-14.875	0.0000
Slope (B1) X^2	0.716675	0.045438	9.038444	15.772	0.0000
Slope (B1) X^3	-0.005305	0.000287	-6.531954	-18.513	0.0000
(Constant) : B0	2584.584	24.692137	-	104.672	0.0000
Predicted equation is: [illegible]					
Influence of Concentration on Intensity Marker using MM Technique					
Simple Correlation Coefficient	0.94982	Non Linear Regression Tested in two tailed alternative Statistical hypothesis			
Determination Coefficient R-Square	0.90216				
F (Statistic)	202.854	Sig. Level	0.0000 (HS) (*)		
Variables in the Equation					
Parameters	B	SE.B	Beta	t-test	Sig. of (t)
Slope (B1)	-20.42056	1.433759	0.94982-	-14.243	0.0000
(Constant) : B0	3341.876	54.19835	-	61.660	0.0000
Predicted equation is: [illegible]					

(*) HS: Highly Significant at P<0.01

Figure (3-10) shows long term trends of "S-Shape, Cubic, and Linear" models for studying the influence of concentration on the intensity marker for different techniques, SM, PCF, and MM respectively in ALT enzyme.

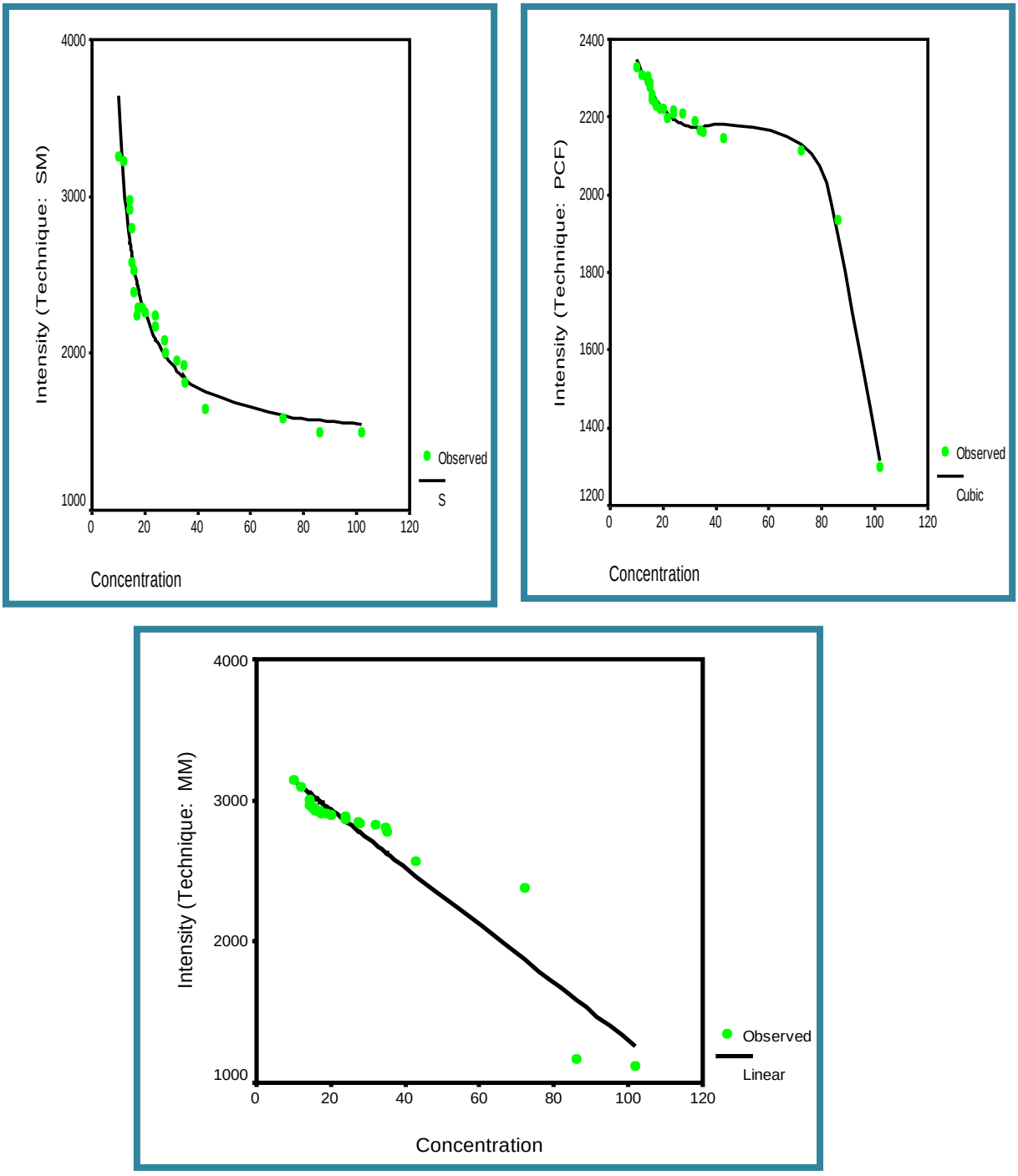


Figure (3-10) Long Term Trend relationship of Influence Concentration on the Intensity Marker's readings in ALT enzyme using different Techniques

For studying influence of concentration on intensity marker's readings in light of AST enzyme, a several functions are supposed such as: (Logarithmic, Linear, Cubic, Power , Inverse, Quadratic, , Compound, S-Shape, , Exponential, Growth and Logistic) in order to be certain that a selected model has the best and an optimal one, which represented their outcomes in(appendix 6) .

Results shows that "Power-Shape, Power, and Logarithmic" models has been registered the one of the greatest mathematical models with nonlinear regression for studying the influence of concentration on the intensity marker for different techniques, SM, PCF, and MM respectively.

Table (3-14) Results shows strong and too highly significant correlation ships are accounted at $P<0.01$ concentration on intensity marker's readings.

Others source of variations are not included in the studied models, (i.e. constant term), shows that a meaningful of an initial value that not include in the regression equation.

As seen in Table (3-14), the results shows strong and too highly significant correlation ships are accounted at $P<0.01$ concentration on intensity marker's readings.

Table (3-14): Regression outcomes of Influence Concentration on the Intensity Marker in AST enzyme using different Techniques.

Influence of Concentration on Intensity Marker using SM Technique					
Simple Correlation Coefficient	0.90412	Non Linear Regression Tested in two tailed alternative Statistical hypothesis			
Determination Coefficient R-Square	0.81744				
F (Statistic)	94.028	Sig. Level	0.0000 (HS) (*)		
Variables in the Equation					
Parameters	B	SE.B	Beta	t-test	Sig. of (t)
Slope (B1)	-0.290088	0.029916	0.904122-	-9.697	0.0000
(Constant) : B0	5041.856	.491.465	-	10.259	0.0000
Predicted equation is: [illegible]					
Influence of Concentration on Intensity Marker using PCF Technique					
Simple Correlation Coefficient	0.93995	Non Linear Regression Tested in two tailed alternative Statistical hypothesis			
Determination Coefficient R-Square	0.88351				
F (Statistic)	174.4499	Sig. Level	0.0000 (HS) (*)		
Variables in the Equation					
Parameters	B	SE.B	Beta	t-test	Sig. of (t)
Slope (B1)	-0.225661	0.017085	-0.939955	-13.208	0.0000
(Constant) : B0	4560.503	247.1715	-	18.451	0.0000
Predicted equation is: [illegible]					
Influence of Concentration on Intensity Marker using MM Technique					
Simple Correlation Coefficient	0.96553	Non Linear Regression Tested in two tailed alternative Statistical hypothesis			
Determination Coefficient R-Square	0.93225				
F (Statistic)	288.982	Sig. Level	0.0000 (HS) (*)		
Variables in the Equation					
Parameters	B	SE.B	Beta	t-test	Sig. of (t)
Slope (B1)	-343.0888	20.18233	0.96553-	-16.999	0.0000
(Constant) : B0	3555.878	65.76181	-	54.072	0.0000
Predicted equation is: [illegible]					

(*) HS: Highly Significant at P<0.01

Figure (3-11) shows long term trends of "Power-Shape, Power-Shape, and Logarithmic" models for studying the influence of concentration on intensity marker for different techniques, SM, PCF, and MM respectively in AST enzyme.

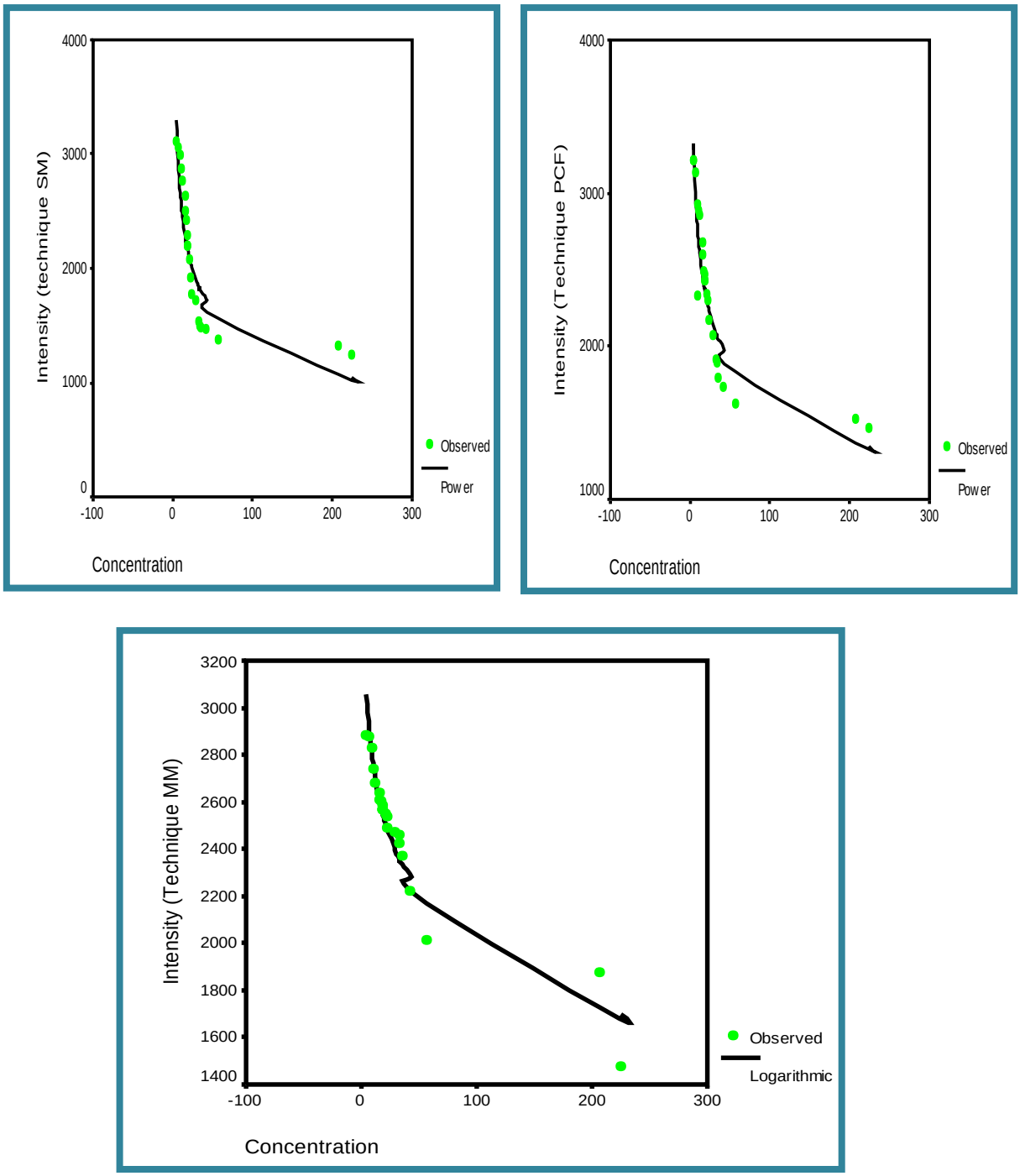


Figure (3-11) Long Term Trend relationship of Influence Concentration on the Intensity Marker in AST enzyme using different Technique

3.5.2 ROC Curve for Enzymes indicators of (ALT by AST)

Table (3-15) illustrate estimation area of trade - off between complement probability level of a specificity rate and sensitivity rate by plotting sensitivity against (1- specificity) to study that trade - off, which is called "Receiver Operating Characteristic" ROC - curve for testing a diseased indicators in light of studied controlled responses along different markers as state variables, as well as significant levels for testing area under fifty percentage, with 95% confidence interval of area indicator are illustrated.

Table (3-15): ROC Curve for Enzymes indicators of (ALT by AST) in light of different techniques.

Statistics		Cutoff Point	Sen.	Spec.	Area	Std. Error	Asy. Sig. (*)	Asy. 95% C.I.	
								L.b.	U.b.
Techniques	SMF	1496.5	1.000	0.261	0.591	0.085	0.283	0.425	0.758
	PCF	1926.0	0.958	0.280	0.341	0.090	0.056	0.163	0.518
	MMF	2760.5	0.833	0.870	0.835	0.066	0.000	0.705	0.965

(*) **HS: Highly Sig. at P<0.01; Non Sig. at P>0.05; the positive actual state is Positive.**

Results shows meaning differentiated was assigned within "MM" technique, since positive highly significant area are calculated at P<0.01 in light of subjective ALT enzyme in contrast of AST enzyme, as well as meaning reversed differentiated was assigned concerning "PCF" technique in light of subjective ALT enzyme in contrast of AST enzyme, rather than significant level was not reached, Robert [98].

Figure (3-12) shows ROC curve plots for studied marker concerning for Enzymes indicators of (ALT by AST) in light of different techniques.

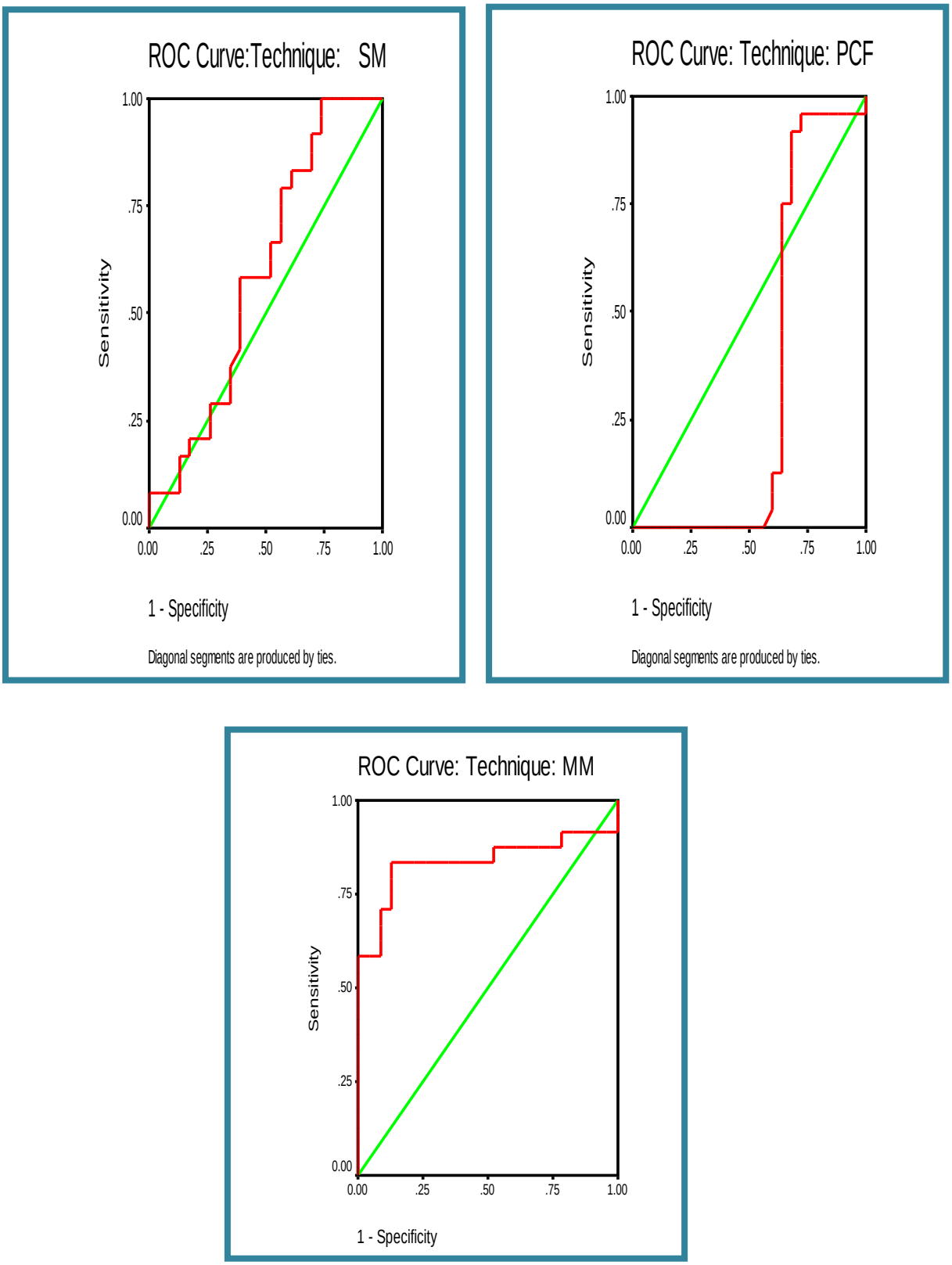


Figure (3-12): ROC Curve plots for Enzymes indicators of (ALT by AST) in light of different technique

3.6 Discussion

Typical biosensor is sensitive device that cnvert the biological change into a noticeable sign, later is then converted into a numerical signal consequence.

Enzymes are included as the sensitive substances provide the biosensor selectivity to the molecule. Regarding optical biosensors are considered as the greatest widespread because of high sensitivity and fasting time response. [99,100]

The absorbance can be observed by directing a beam of radiation at the sample and noticing the intensity of emission that originates across it.

The obtaining results demonstrated that the absorption of laser light by highly concentrated samples are higher and inversely proportional to the intensity of light, means the intensity of light are high when the concentration of enzymes are low. This phenomenon could be explained as, the higher absorption of light by samples (ALT and AST enzymes) due to the selection of suitable laser which depends on the absorption of the samples to the wavelength of the laser. The refractive index regulates how much the route of light is resolved, or refracted, when arriving a material.

The present result established that the refractive index value are changed according to the concentration of enzymes in blood samples, higher concentration of samples means higher refractive index, Maximum concentration of ALT enzyme (101.9 U/L), the higher refractive index (1.3570) ,the minimum concentration of ALT enzyme (9.9 U/L),the lower refractive index(1.3567) . Maximum concentration of AST enzyme (224.5U/L), the higher refractive index (1.3570), the minimum concentration of ALT enzyme (4.3U/L), the lower refractive index (1.3545).

From the findings of the present results the constructed biosensor are fastest response ,stable, more accurate , fair sensitivity and inexpensive when used in the measurement of the concentration of ALT and AST liver enzyme, multimode (MM) biosensor considered the more effective and sensing than the other types (SM and PCF) fibers biosensors because the output intensity of multimode fiber have a large number of modes compared with single mode and photonic crystal fiber which have limitation number of modes due to their basic structure.

The present study are in agreement with other many studies using biosensor to detect the level of ALT and AST enzymes in human blood sample. ALT biosensor built on platinum wire microelectrode has been demonstrated with a convenient, fast, and best selectivity in contradiction of both negatively and positively charged interferents, this biosensor has good detection and reasonable sensitivity. [101] Thuy and Seng (2016)

Detection on paper-based micro analytical devices (PADs) are used in general biochemistry, using the proposed PADs for detection the concentration detection of AST and ALT in human serum samples are originate to be in good agreement with those achieved using spectrophotometric a traditional type in detection technique.[102]

A study had been done by Hsueh et al in 2011 established the procedure of a two-step enzymatic reaction mechanism by an extremely sensitive Ir/C nano-catalyst for single use only, screen-printing biosensor for detection ALT enzyme. This detection was approved by counting the enzymatically-produced H_2O_2 . The constructed biosensor showed an excellent results and could be very useful for clinical applications in detection the level of ALT enzyme. [103]

In the present study Photonic crystal biosensor were constructed using single mode fiber (SMF-28) as a standard and the (PCF-LMA-10) prepared

optical fiber by cleaving and splicing, solid core are used which contain four circles of air holes hexagonal in shape, Outer cladding diameter has (125 ± 2 µm), diameter of core are (10.1 ± 0.5) µm, air hole diameter 3.1µm and the space between hole and hole are 6.6µm. The procedure of PCF biosnsor constuction are in agreement with other many studies, photonic crystal fiber interferometer based in dew detection that functions in mode reflection. Cleaving and fusion splicing are used for building the sensor head. The sensor illustrations good sensitivity through a large wavelength peak. The device's reaction to temperature and ambient humidity. [104]

Rawaa and Hanan 2015, they constructed a sensor chemical in type invented it by cutting and splicing photonic crystal fiber (PCF LMA-10) and single-mode fibers (SMF-28) at different length. Collapsed region in the PCF are examined under microscope and consider the key component for stimulating and recombination of two core modes, they decided that interferometers demonstrate regular interference patterns which change remarkable when the spaces of the fiber are penetrated with molecules of instable compounds and Acceptance of the system with a fast sensing information. [105]

Using Lasers with different wavelengths have been used for different biological applications such as blood and urine tests, based on Mach-Zehnder interferometer laser with (532nm) as a laser source with input power of (12.2 nw). A laser biosensor is designed with different lengths of solid core photonic crystal fibers (LMA-10) (1.5cm, 1cm and 0.5cm) to be used for the detection of different types of anemia such as iron deficiency and aplastic anemia ,the device with the hole collapsed, the LMA-10 photonic crystal fiber was spliced by fusion splicer type (FSM 60 S) to the SMF-28. Collapsing technique can be

Implemented in this sensor. PCF sensor used with length 1.5cm because it proved to be the most sensitive sensor for changing the refractive index of the biological .The light green source laser been used in this experiment with λ=532nm and output power=12.2nw [106].

The manufacture of photonic crystal fiber modal interferometers is approved with different post-processing techniques such as tapering, cleaving, and splicing. Photonic crystal fiber interferometers display low thermal sensitivity, applications range from sensing temperature or strain to refractive index and organic compounds (volatile). [107]

3.7 Conclusions

The present study highlights the following conclusions in reference to the results obtained.

1- The ALT enzyme sensing in wavelength 531.62 nm and the AST enzyme sensing in wavelength 531.16 nm in 3 setup.

2- The refractive index value are changed according to the concentration of enzymes in blood samples, higher concentration of samples higher refractive index,

3- The best type of laser biosensor is multimode biosensor for detection the concentration of both enzymes in the sample in addition it is highly sensitive in transmission of signal light intensity.

4- The intensity of light are high when the concentration of enzymes are low means the absorption of laser light by highly concentrated samples are higher compared with lower emitted light (intensity).

5- Biosensor are most a currate, with a rapid diagnosis ,more costley method than the traditional method to avoid any biological changes in blood sample that lead to changes optical charastetic (refractive index and absorption) of blood sample.

3.8 Suggestions

1- Detection other types of liver enzymes such as Gamma-glutamyltransferase (GGT), Bilirubin and Alkaline phosphatase (ALP) or another types of enzymes in human body.

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Appendix 1

RANDOX CE

ALT

Alanine Aminotransferase
Manual

INTENDED USE

For the quantitative *in vitro* determination of Alanine Aminotransferase (ALT) in serum. This product is suitable for Manual use.

Cat. No.

AL 146	R1.	Buffer	1 x 100 ml
200 tests	R2.	2,4-Dinitrophenylhydrazine	1 x 100 ml
	R3.	Sodium Hydroxide	1 x 100 ml
	CAL.	Pyruvate Standard	1 x 10 ml

GTIN: 05055273200188

Colorimetric method (Reitman and Frankel) for determination of serum alanine aminotransferase.

PRINCIPLE

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{ALT} \text{L-glutamate} + \text{pyruvate}$$

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine.

SAMPLE MATERIAL

Serum.

REAGENT COMPOSITION

Contents		Initial Concentration of Solutions
R1.	**Buffer**	
	Phosphate buffer	100 mmol/l, pH7.4
	L-alanine	200 mmol/l
	α-oxoglutarate	2.0 mmol/l
R2.	**2,4-dinitrophenylhydrazine**	2.0 mmol/l
R3.	**Sodium Hydroxide**	4.0 mol/l
CAL.	**Pyruvate Standard**	See lot specific insert

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Buffer
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

R2. 2,4-dinitrophenyl-hydrazine
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

R3. Sodium Hydroxide
Make 1 vial of sodium hydroxide 3 up to **1000 ml** with redistilled water in a volumetric flask. Stable up to expiry date when stored at +2 to +8°C.

CAL. Pyruvate Standard
Contents ready for use. Stable up to expiry date when stored at +2 to +8°C. To construct a calibration curve for GPT, use the Pyruvate Standard undiluted.

MATERIALS PROVIDED

Buffer
2,4-Dinitrophenylhydrazine
Sodium Hydroxide
Pyruvate Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Human Assayed Multi-Sera Control Level 2 (Cat. No. HN1530).

PROCEDURE NOTE

Transaminase activities in some sera are stimulated by high concentrations of aldehydes, ketones, or oxo acids. Measurement against a sample blank (Procedure 2) instead of a reagent blank (Procedure 1) avoids the risk of finding such artifacts.

PROCEDURE

Wavelength:	Hg 546 nm (530 - 550 nm)
Cuvette:	1cm light path
Incubation Temperature:	37°C

1. Measurement against Reagent Blank

Pipette into test tubes:

	Reagent Blank	Sample
Sample	---	0.1 ml
Buffer (R1)	0.5 ml	0.5 ml
Distilled Water	0.1 ml	---
Mix, incubate for exactly 30 min. at 37°C		
2,4-DNP (R2)	0.5 ml	0.5 ml
Mix, allow to stand for exactly 20 min. at 20 to 25°C		
Sodium Hydroxide (R3)	5.0 ml	5.0 ml

Mix, read the absorbance of sample (A_{sample}) against the reagent blank after 5 minutes.

2. Measurement against Sample Blank

Pipette into test tubes:

	Sample Blank	Sample
Sample	---	0.1 ml
Buffer (R1)	0.5 ml	0.5 ml
Mix, incubate for exactly 30 min. at 37°C		
2,4-DNP (R2)	0.5 ml	0.5 ml
Sample	0.1 ml	---
Mix, allow to stand for exactly 20 min. at 20 to 25°C		
Sodium Hydroxide (R3)	5.0 ml	5.0 ml

Mix, read the absorbance of the sample (A_{sample}) against the sample blank after 5 minutes.

PROCEDURE

Wavelength	490-560 nm
Cuvette	1 cm light path
Temperature	20 - 25°C

Pipette into test tubes:

Tube No	Pyruvate Standard (ml)	Redistilled Water (ml)	Buffer Solution (ml)
1	0.00	0.2	1.00
2	0.05	0.2	0.95
3	0.10	0.2	0.90
4	0.15	0.2	0.85
5	0.20	0.2	0.80
6	0.25	0.2	0.75
7	0.30	0.2	0.70
8	0.35	0.2	0.65
9	0.40	0.2	0.60
10	0.45	0.2	0.55

Mix and pipette into each tube **1.0 ml** of Reagent Solution R2. Mix and incubate for 20 min at 20 to 25°C. Add **10 ml** of Sodium Hydroxide soln to each tube. Mix and read absorbance against blank (tube no 1) after 5 mins.

The absorbances of the increasing amounts of pyruvate (0.05 - 0.45 ml Pyruvate Standard) correspond to the following transaminase activities in U/l.

Tube No.	GPT U/l
2	9
3	18
4	27
5	37
6	46
7	56
8	67
9	77
10	87

The calibration curve is obtained by plotting the measured absorbances against the transaminase activities in U/l.

ordinate = absorbance
abscissca = activity in U/l.

CALIBRATION USING STANDARD

Pyruvate Standard is used to construct a calibration curve when readings cannot be taken at 546 nm. Increasing amounts of pyruvate are allowed to react with 2,4-dinitrophenylhydrazine. The concentration of hydrazone formed is approximately proportional to the amount of pyruvate reacted.

QUALITY CONTROL

Randox Human Assayed Multi –Sera Control Level 2 is recommended for daily quality control. The value obtained should fall within a specified range. If the value falls outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water Contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

CALCULATION

Obtain the activity of ALT in the serum from the table:

Absorbance	U/l	Absorbance	U/l
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

INTERFERENCE

Haemolysis interferes with the assay.

NORMAL VALUES

Serum up to 12 U/l

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

LINEARITY

If the absorbance exceeds 0.5 dilute 0.1 ml of sample with 0.9 ml of 0.9% NaCl solution and reassay. Multiply the result by 10.

REFERENCE

Reitman, S., and Frankel, S., Amer. J. Clin. Path., 1957; **28**: 56.

Revised 26 Apr 16 bi
Rev. 002

Randox Laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom
T +44 (0) 28 9442 2412

Appendix 2

RANDOX

CE

AST

Aspartate Aminotransferase
Manual

INTENDED USE

For the quantitative *in vitro* determination of Aspartate Aminotransferase (AST) in serum. This product is suitable for Manual use.

Cat. No.			
AS 147	R1.	Buffer	1 x 100 ml
200 tests	R2.	2,4-Dinitrophenylhydrazine	1 x 100 ml
	R3.	Sodium Hydroxide	1 x 100 ml
	CAL.	Pyruvate Standard	1 x 10 ml

GTIN: 05055273200447

Colorimeteric method (Reitman and Frankel) for determination of serum aspartate aminotransferase.

PRINCIPLE

$$\alpha\text{-oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{GOT}} \text{L-glutamate} + \text{Oxaloacetate}$$

Aspartate Aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine.

SAMPLE MATERIAL

Serum.

REAGENT COMPOSITION

Contents		Initial Concentration of Solutions
R1.	**Buffer**	
	Phosphate buffer	100 mmol/l, pH 7.4
	L-aspartate	100 mmol/l
	α-oxoglutarate	2 mmol/l
R2.	**2,4-dinitrophenylhydrazine**	2.0 mmol/l
R3.	**Sodium Hydroxide**	
CAL.	**Pyruvate Standard**	
	Pyruvate	See lot specific insert

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Buffer (R1) and 2,4-dinitrophenylhydrazine (R2) contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Buffer
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R2. 2,4-dinitrophenyl-hydrazine
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R3. Sodium Hydroxide
Make 1 vial of Sodium Hydroxide R3 up to **1000 ml** with redistilled water in a volumetric flask. Stable up to expiry date when stored at +2 to +8°C.

CAL. Pyruvate Standard
Contents ready for use. Stable up to expiry date when stored at +2 to +8°C. To construct a calibration curve for AST, dilute **1.5 ml** of Pyruvate Standard (CAL) with **4.5 ml** of Buffer (R1) immediately before measurement.

MATERIALS PROVIDED

Buffer
2,4-dinitrophenylhydrazine
Sodium Hydroxide
Pyruvate Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Human Assayed Multi-Sera Control Level 2 (Cat. No. HN1530).

PROCEDURE NOTE

Transaminase activities in some sera are stimulated by high concentrations of aldehydes, ketones, or oxo acids. Measurement against a serum blank (Procedure 2) instead of a reagent blank (Procedure 1) avoids the risk of finding such artifacts.

AST

PROCEDURE

Wavelength:	Hg 546 nm (530 - 550 nm)
Cuvette:	1cm light path
Incubation Temperature:	37°C

1. Measurement against Reagent Blank

Pipette into test tubes:

	Reagent Blank	Sample
Sample	---	0.1 ml
Buffer (R1)	0.5 ml	0.5 ml
Distilled Water	0.1 ml	---
Mix, incubate for exactly 30 min. at 37°C		
2,4-dinitrophenylhydrazine (R2)	0.5 ml	0.5 ml
Mix, allow to stand for exactly 20 min. at 20 to 25°C		
Sodium Hydroxide (R3)	5.0 ml	5.0 ml

Mix, read the absorbance of sample (A_{sample}) against the reagent blank after 5 minutes.

2. Measurement against Sample Blank

Pipette into test tubes:

	Sample Blank	Sample
Sample	---	0.1 ml
Buffer (R1)	0.5 ml	0.5 ml
Mix, incubate for exactly 30 min. at 37°C		
2,4-dinitrophenylhydrazine (R2)	0.5 ml	0.5 ml
Sample	0.1 ml	---
Mix allow to stand for exactly 20 min. at 20 to 25°C		
Sodium Hydroxide (R3)	5.0 ml	5.0 ml

Mix, read the absorbance of the sample (A_{sample}) against the sample blank after 5 minutes.

PROCEDURE

AST

Wavelength:	(490-560 nm)
Cuvette:	1 cm light path
Temperature:	20 - 25°C

Pipette into test tubes:

Tube No	Diluted Pyruvate Standard(ml)	Redistilled water (ml)	Buffer (ml)
1	0.00	0.2	1.00
2	0.05	0.2	0.95
3	0.10	0.2	0.90
4	0.15	0.2	0.85
5	0.20	0.2	0.80
6	0.25	0.2	0.75
7	0.30	0.2	0.70
8	0.35	0.2	0.65
9	0.40	0.2	0.60
10	0.45	0.2	0.55

Mix and pipette into each tube **1.0 ml** of 2,4-dinitrophenylhydrazine. Mix and incubate for 20 min at 20 to 25°C. Add **10 ml** of Sodium Hydroxide soln to each tube. Mix and read absorbance against blank (tube no 1) after 5 mins.

The absorbances of the increasing amounts of pyruvate (0.05 - 0.45 ml Pyruvate Standard) correspond to the following transaminase activities in U/l.

Tube No.	AST U/l
2	6
3	11
4	16
5	20
6	25
7	31
8	37
9	44
10	52

The Standard curve is obtained by plotting the measured absorbances against the transaminase activities in U/l.

ordinate = absorbance
abscissca = activity in U/l.

CALCULATION

Obtain the activity of AST in the serum from the table:

Absorbance	U/l	Absorbance	U/l
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

CALIBRATION USING STANDARD

Pyruvate Standard is used to construct a calibration curve when readings cannot be taken at 546 nm. Increasing amounts of pyruvate are allowed to react with 2,4-dinitrophenylhydrazine. The concentration of hydrazone formed is approximately proportional to the amount of pyruvate reacted.

QUALITY CONTROL

Randox Human Assayed Multi-Sera Control Level 2 is recommended for daily quality control. The value obtained should fall within a specified range. If the value falls outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water Contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

INTERFERENCE

Haemolysis interferes with the assay.

NORMAL VALUES

Serum up to 12 U/l

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

LINEARITY

If the absorbance exceeds 0.170 dilute 0.1 ml of sample with 0.9 ml of 0.9% NaCl solution and reassay. Multiply the result by 10.

REFERENCE

Reitman, S., and Frankel, S., Amer. J. Clin. Path., 1957; **28**: 56.

Revised 26 Apr 16 bi
Rev. 002

Appendix 3

Corning® Single-Mode Optical Fiber

SMF-28™ Fiber

Product Information

CORNING

PI1036
Issued: 01/00
Supercedes: 10/99
ISO 9001 Registered

The Standard For Performance

Corning® SMF-28™ single-mode fiber has set the standard for value and performance for telephony, cable television, submarine, and utility network applications. Widely used in the transmission of voice, data, and/or video services, SMF-28 fiber is manufactured to the most demanding specifications in the industry.

Taking advantage of today's high capacity, low cost transmission components developed for the 1310 nm window, SMF-28 fiber features low dispersion and is optimized for use in the 1310 nm wavelength region. SMF-28 fiber also can be used effectively with TDM and WDM systems operating in the 1550 nm wavelength region.

Protection And Versatility

SMF-28 fiber is protected for long-term performance and reliability by the CPC coating system. Corning's enhanced, dual acrylate CPC coatings provide excellent fiber protection and are easy to work with. CPC coatings are designed to be mechanically stripped and have an outside diameter of 245 µm. They are optimized for use in many single- and multi-fiber cable designs including loose tube, ribbon, slotted core, and tight buffer cables.

Patented Quality Process

SMF-28 fiber is manufactured using the Outside Vapor Deposition (OVD) process, which produces a totally synthetic ultra-pure fiber. As a result, Corning SMF-28 fiber has consistent geometric properties, high strength, and low attenuation. Corning SMF-28 fiber can be counted on to deliver excellent performance and high reliability, reel after reel. Measurement methods comply with ITU recommendations G.650, IEC 60793-1, and Bellcore GR-20-CORE.

Features And Benefits

- Versatility in 1310 nm and 1550 nm applications.
- Outstanding geometrical properties for low splice loss and high splice yields.
- OVD manufacturing reliability and product consistency.
- Optimized for use in loose tube, ribbon, and other common cable designs.

CORNING Fiber

Optical Specifications

Attenuation

Standard Attenuation Cells

Wavelength (nm)	Attenuation Cells (dB/km)	
	Premium *	Standard
1310	≤0.35	≤0.40
1550	≤0.25	≤0.30

** Lower attenuation available in limited quantities.*

Point Discontinuity
No point discontinuity greater than 0.10 dB at either 1310 nm or 1550 nm.

Attenuation at the Water Peak
The attenuation at 1383 ± 3 nm shall not exceed 2.1 dB/km.

Attenuation vs. Wavelength

Range (nm)	Ref. λ (nm)	Max. α Difference (dB/km)
1285 - 1330	1310	0.05
1525 - 1575	1550	0.05

The attenuation in a given wavelength range does not exceed the attenuation of the reference wavelength (λ) by more than the value α.

Attenuation with Bending

Mandrel Diameter (mm)	Number of Turns	Wavelength (nm)	Induced Attenuation* (dB)
32	1	1550	≤0.50
50	100	1310	≤0.05
50	100	1550	≤0.10

*The induced attenuation due to fiber wrapped around a mandrel of a specified diameter.

Cable Cutoff Wavelength (λ_{cc})

$\lambda_{cc} \leq 1260$ nm

Mode-Field Diameter

9.2 ± .4 µm at 1310 nm
10.4 ± .8 µm at 1550 nm

Dispersion

Zero Dispersion Wavelength (λ_0):
1301.5 nm ≤ λ_0 ≤ 1321.5 nm

Zero Dispersion Slope (S_0):
≤ 0.092 ps/(nm²•km)

$$\text{Dispersion} = D(\lambda) \approx \frac{S_0}{4}\left[\lambda - \frac{\lambda_0^4}{\lambda^3}\right] \text{ps/(nm•km)},$$

for 1200 nm ≤ λ ≤ 1600 nm

λ = Operating Wavelength

Polarization Mode Dispersion

Fiber Polarization Mode Dispersion (PMD)

	Value (ps/√km)
PMD Link Value	≤ 0.1*
Maximum Individual Fiber	≤ 0.2

** Complies with IEC SC 86A/WG1, Method 1, September 1997.*

The PMD link value is a term used to describe the PMD of concatenated lengths of fiber (also known as the link quadrature average). This value is used to determine a statistical upper limit for system PMD performance.

Individual PMD values may change when cabled. Corning's fiber specification supports emerging network design requirements for a 0.5 ps/√km maximum PMD.

Environmental Specifications

Environmental Test Condition	Induced Attenuation (dB/km)	
	1310 nm	1550 nm
Temperature Dependence -60°C to +85°C*	≤0.05	≤0.05
Temperature-Humidity Cycling -10°C to +85°C*, up to 98% RH	≤0.05	≤0.05
Water Immersion, 23°± 2°C*	≤0.05	≤0.05
Heat Aging, 85°± 2°C*	≤0.05	≤0.05

**Reference temperature = +23°C*
Operating Temperature Range -60°C to +85°C

Dimensional Specifications

Standard Length (km/reel): 2.2 - 25.2*

** Longer spliced lengths available at a premium.*

Glass Geometry

Fiber Curl: ≥ 4.0 m radius of curvature
Cladding Diameter: 125.0 ± 1.0 µm
Core-Clad Concentricity: ≤ 0.5 µm
Cladding Non-Circularity: ≤ 1.0%

Defined as: $\left[1-\frac{\text{Min. Cladding Diameter}}{\text{Max. Cladding Diameter}}\right] \times 100$

Coating Geometry

Coating Diameter: 245 ± 5 µm
Coating-Cladding Concentricity: < 12 µm

Mechanical Specifications

Proof Test

The entire fiber length is subjected to a tensile proof stress ≥ 100 kpsi (0.7 GN/m²)*.

** Higher proof test levels available at a premium.*

Performance Characterizations

Characterized parameters are typical values.

Core Diameter: 8.2 µm

Numerical Aperture: 0.14
NA is measured at the one percent power level of a one-dimensional far-field scan at 1310 nm.

Zero Dispersion Wavelength (λ_0): 1312 nm

Zero Dispersion Slope (S_0): 0.090 ps/(nm²•km)

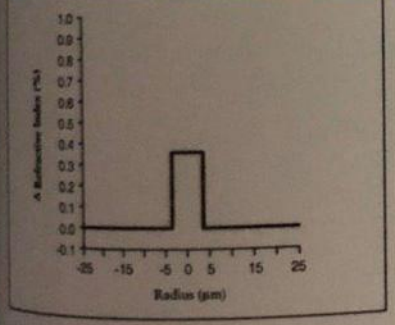
Refractive Index Difference: 0.36%

Effective Group Index of Refraction (N_{eff}):
1.4677 at 1310 nm
1.4682 at 1550 nm

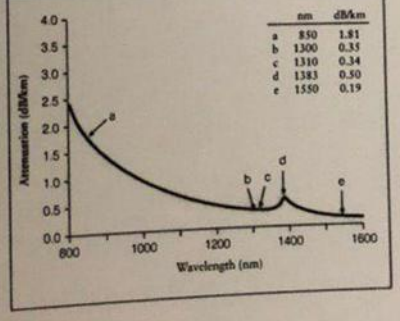
Fatigue Resistance Parameter (n_d): 20

Coating Strip Force:
Dry: 0.6 lbs (2.7N)
Wet, 14-day room temperature: 0.6 lbs (2.7N)

Refractive Index Profile (typical fiber)



Spectral Attenuation (typical fiber)



Ordering Information

To order Corning® SMF-28™ optical fiber, contact your sales representative, or call the Telecommunications Products Division Customer Service Department at **910-395-7659** (North America) and **+1 607-974-7174** (International). Please specify the following parameters when ordering.

Fiber Type: Corning® SMF-28™

Fiber Attenuation Cell: dB/km

Fiber Quantity: km

Other: (Requested ship date, etc.)

Corning Incorporated
Telecommunications Products Division
Corning, NY 14831

Tel: 800-525-2524 (North America)
Tel: +1 607-786-8125 (International)

Fax: 800-539-3632 (North America)
Fax: +1 607-786-8344 (International)

E-mail: info@corningfiber.com
Internet: www.corningfiber.com

APPEDIX 4

NKT Photonics
the power of light

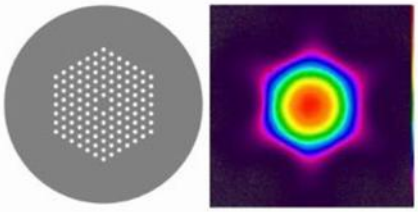
Crystal Fibre • *aero*LASE • Koheras • SuperK

LMA-10

Single-mode 10 µm core fiber

Single Mode | Large Mode-area

- Low fiber loss from 500 to 1700 nm
- Single-mode at all wavelengths
- Radiation hard pure silica fiber
- Wavelength independent MFD



This single-mode photonic crystal fiber is optimized to exhibit low loss across the widest possible wavelength region from 500 nm to above 1700 nm while keeping an almost constant mode field diameter.

The fiber is endlessly single-mode with no higher order mode cut-off and delivers excellent mode quality at all wavelengths.

The fiber has a standard 125 µm outer diameter and is compatible with all common fiber tools.

This product is also available in a polarization-maintaining version as the LMA-PM-10.

Applications
- Single-mode high power delivery
- Mode filtering
- Single-mode pigtailing

Optical properties	
Single mode cut-off wavelength*	None
Attenuation @ 532 nm**	< 40 dB/km
Attenuation @ 632 nm**	< 20 dB/km
Attenuation @ 1064 nm**	< 5 dB/km
Mode field diameter @ 532 nm (1/e²)	8.4 ± 1.0 µm
Mode field diameter @ 1064 nm (1/e²)	8.8 ± 1.0 µm
NA @ 1064 nm (5%)	0.11 ± 0.02
Physical properties	
Core diameter	10.1 ± 0.5 µm
Outer cladding diameter, OD	125 ± 2 µm
Coating diameter	245 ± 10 µm
Core and cladding material	Pure silica
Coating material, single layer	Acrylate
Coating-Cladding concent. error	< 10 µm
Proof test level	0.5 %

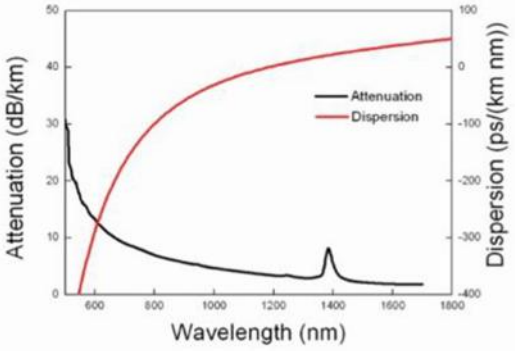
Standard interfacing options	
FC/PC connector	0.0 ± 0.5 deg angle
FC/APC connector	8.0 ± 0.5 deg angle
Collapse and cleave	0.0 ± 0.5 deg angle

All interfaces are provided with a 150 ± 25 µm sealing length of the PCF structure.

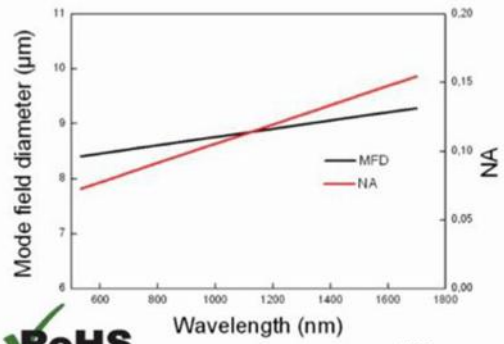
Please contact us for other custom interfacing options.

* TIA-455-80-C standard ** 16 cm bend diameter

Typical spectral attenuation and dispersion



Typical MFD and NA



RoHS 2002/95/EC

LMA-10-v1--131104

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Appendix 5

Regression outcomes for Influence of Concentration on the Intensity Marker using SM Technique in ALT enzyme

Dep.	Independent: Saliva Ferritin (ng/ml)								
	Math. Model	R sq.	d.f.	F	Sig. F	b0	b1	b2	b3
Intensity Marker	Linear	0.602	22	33.3	0.000	2755.5	-16.38		
	Logarithmic	0.831	22	107.8	0.000	4658.5	-751.31		
	Inverse	0.939	22	340.4	0.000	1253.2	21094		
	Quadratic	0.845	21	57.2	0.000	3511.7	-63.404	0.449	
	Cubic	0.905	20	63.8	0.000	4119.8	-119.81	1.8023	-0.0087
	Compound	0.704	22	52.4	0.000	2799.0	0.9922		
	Power	0.902	22	201.8	0.000	6693.6	-0.3476		
	S - Shape	0.943	22	367.2	0.000	7.3	9.3874		
	Growth	0.704	22	52.4	0.000	7.9	-0.0079		
	Exponential	0.704	22	52.4	0.000	2799.0	-0.0079		
	Logistic	0.704	22	52.4	0.000	0.0	1.0079		

Shaded model reported the best fitness Equation.

Regression outcomes for Influence of Concentration on the Intensity Marker using PCF Technique in ALT enzyme

Dep.	Independent: Saliva Ferritin (ng/ml)								
	Math. Model	R sq.	d.f.	F	Sig. F	b0	b1	b2	b3
Intensity Marker	Linear	0.764	22	71.13	0.000	2396.89	-7.4297		
	Logarithmic	0.617	22	35.49	0.000	3007.63	-261.68		
	Inverse	0.426	22	16.36	0.001	1897.67	5772.76		
	Quadratic	0.856	21	62.18	0.000	2207.91	4.4219	-0.1131	
	Cubic	0.992	20	830.25	0.000	2584.58	-30.393	0.7167	-0.0053
	Compound	0.71	22	53.95	0.000	2440.46	0.996		
	Power	0.55	22	26.91	0.000	3371.81	-0.1396		
	S - Shape	0.362	22	12.5	0.002	7.5347	3.0068		
	Growth	0.71	22	53.95	0.000	7.7999	-0.004		
	Exponential	0.71	22	53.95	0.000	2440.46	-0.004		
	Logistic	0.71	22	53.95	0.000	0.0004	1.0041		

Shaded model reported the best fitness Equation.

Regression outcomes for Influence of Concentration on the Intensity Marker using MM Technique in ALT enzyme

Dep.	Independent: Saliva Ferritin (ng/ml)								
	Math. Model	R sq.	d.f.	F	Sig. F	b0	b1	b2	b3
Intensity Marker	Linear	0.902	22	202.9	0.000	3341.9	-20.4		
	Logarithmic	0.739	22	62.2	0.000	5029.8	-721.4		
	Inverse	0.494	22	21.5	0.000	1984.9	15581.0		
	Quadratic	0.938	21	160.0	0.000	3044.5	-1.9	-0.1766	
	Cubic	0.939	20	102.7	0.000	3107.5	-7.8	-0.0365	-0.0009
	Compound	0.854	22	128.5	0.000	3616.0	1.0		
	Power	0.662	22	43.0	0.000	8207.0	-0.4		
	S - Shape	0.414	22	15.5	0.001	7.5	7.4		
	Growth	0.854	22	128.5	0.000	8.2	0.0		
	Exponential	0.854	22	128.5	0.000	3616.0	0.0		
	Logistic	0.854	22	128.5	0.000	0.0	1.0		

Shaded model reported the best fitness Equation.

Regression outcomes for Influence of Concentration on the Intensity Marker using SM Technique in AST enzyme

Dep.	Independent: Saliva Ferritin (ng/ml)								
	Math. Model	R sq.	d.f.	F	Sig. F	b0	b1	b2	b3
Intensity Marker	Linear	0.356	21	11.62	0.003	2359.76	-6.31		
	Logarithmic	0.779	21	74.23	0.000	3941.64	-582.80		
	Inverse	0.719	21	53.65	0.000	1495.14	10316.80		
	Quadratic	0.867	20	65.1	0.000	3197.32	-51.52	0.1969	
	Cubic	0.936	19	92.79	0.000	3461.46	-75.37	0.6569	-0.0016
	Compound	0.428	21	15.71	0.001	2313.91	1.00		
	Power	0.817	21	94.03	0.000	5041.86	-0.29		
	S - Shape	0.658	21	40.32	0.000	7.33	4.80		
	Growth	0.428	21	15.71	0.001	7.75	0.00		
	Exponential	0.428	21	15.71	0.001	2313.91	0.00		
	Logistic	0.428	21	15.71	0.001	0.00	1.00		

Shaded model reported the best fitness Equation.

Regression outcomes for Influence of Concentration on the Intensity Marker using PCF Technique in AST enzyme

Dep.	Independent: Saliva Ferritin (ng/ml)								
	Math. Model	R sq.	d.f.	F	Sig. F	b0	b1	b2	b3
Intensity Marker	Linear	0.441	23	18.14	0.000	2580.3	-6.17		
	Logarithmic	0.855	23	135.76	0.000	3877.6	-501.43		
	Inverse	0.725	23	60.53	0.000	1846.9	7426.95		
	Quadratic	0.891	22	89.78	0.000	3194.3	-41.43	0.155	
	Cubic	0.934	21	99.25	0.000	3352.2	-56.92	0.4675	-0.0011
	Compound	0.523	23	25.24	0.000	2562.0	1.00		
	Power	0.884	23	174.45	0.000	4560.5	-0.23		
	S - Shape	0.651	23	42.93	0.000	7.5	3.12		
	Growth	0.523	23	25.24	0.000	7.8	0.00		
	Exponential	0.523	23	25.24	0.000	2562.0	0.00		
	Logistic	0.523	23	25.24	0.000	0.0	1.00		

Shaded model reported the best fitness Equation.

Regression outcomes for Influence of Concentration on the Intensity Marker using MM Technique in AST enzyme

Dep.	Independent: Saliva Ferritin (ng/ml)								
	Math. Model	R sq.	d.f.	F	Sig. F	b0	b1	b2	b3
Intensity Marker	Linear	0.797	21	82.58	0.000	2677.5	-5.1		
	Logarithmic	0.932	21	288.98	0.000	3555.9	-343.1		
	Inverse	0.518	21	22.53	0.000	2197.5	4712.8		
	Quadratic	0.927	20	126.5	0.000	2904.5	-17.3	0.0534	
	Cubic	0.978	19	286.96	0.000	3027.3	-28.4	0.2672	-0.0008
	Compound	0.84	21	110.38	0.000	2696.8	0.998		
	Power	0.892	21	172.98	0.000	3994.3	-0.155		
	S - Shape	0.438	21	16.39	0.001	7.686	2.005		
	Growth	0.84	21	110.38	0.000	7.900	-0.002		
	Exponential	0.84	21	110.38	0.000	2696.8	-0.002		
	Logistic	0.84	21	110.38	0.000	0.0004	1.002		

Shaded model reported the best fitness Equation.

APPENIX 6

محافظة بغداد

دائرة صحة بغداد الرصافة

مستشفى ابن النفيس التعليمي

Baghdad Governorate

استمارة نتائج الفحوص المختبرية للكيمياء الحياتية

Patient name : ستار عبد الجبار Gender : Age :

Ward : Bed :

Test	Results	Normal Value	Conversion Factor
S. Glucose		4.0 -6.4 mmol/L	X 18 = mg/dl
S. Urea		2.5 – 7.5 mmol/L	X 6 = mg/dl
S. Creatinine		Up to 124 umol/L	/ 88.4 = mg/dl
S. Sodium		136 – 155 mmol/L	X 1.0 = meq/L
S. Potassium		3.5 – 5.3 mmol/L	X 1.0 = meq/L
S. Chloride		95 – 105 mmol/L	X 1.0 = meq/L
S. Calcium		2.1 – 2.6 mmol/L	X 1.0 = meq/L
S. Uric Acid		180 – 420 umol/L	/ 60 = mg/dl
S. Cholesterol		3.9 – 6.2 mmol/L	X 38.6 = mg/dl
S. Triglyceride		0.9 – 2.25 mmol/L	X 88.5 = mg/dl
S. HDL-Cholesterol		>1.04 mmol/L	X 38.6 = mg/dl
S. LDL-Cholesterol		<3.37 mmol/L	X 38.6 = mg/dl
S. Total Protein		60 – 80 g/L	/ 10 = g/dl
S. Albumin		36 – 52 g/L	/ 10 = g/dl
S. Globulin		24 – 37 g/L	/ 10 = g/dl
S. Total Bilirubin		5 – 17 umol/L	/ 17.1 = mg/dl
S. Direct Bilirubin		< 5 umol/L	/ 17.1 = mg/dl
S. Indirect Bilirubin		< 12 umol/L	/ 17.1 = mg/dl
S. Alkaline Phosphatase		21 – 120 u/L	/ 7.1 = KAU/dl
S. ALT (GPT)	١٩	Up to 40 u/L	X 1.0 = u/L
S. AST (GOT)		Up to 40 u/L	X 1.0 = u/L
S. LDH		80 – 190 u/L	X 1.0 = u/L
S. CK		24 – 195 Male 24 – 170 female	X 1.0 = u/L
S. Troponin			

Examiner :

محافظة بغداد

دائرة صحة بغداد الرصافة

مستشفى ابن النفيس التعليمي

Baghdad Governorate

استمارة نتائج الفحوص المختبرية للكيمياء الحياتية

Patient name : وسام كرهش Gender : Age :

Ward : Bed :

Test	Results	Normal Value	Conversion Factor
S. Glucose		4.0 -6.4 mmol/L	X 18 = mg/dl
S. Urea		2.5 – 7.5 mmol/L	X 6 = mg/dl
S. Creatinine		Up to 124 umol/L	/ 88.4 = mg/dl
S. Sodium		136 – 155 mmol/L	X 1.0 = meq/L
S. Potassium		3.5 – 5.3 mmol/L	X 1.0 = meq/L
S. Chloride		95 – 105 mmol/L	X 1.0 = meq/L
S. Calcium		2.1 – 2.6 mmol/L	X 1.0 = meq/L
S. Uric Acid		180 – 420 umol/L	/ 60 = mg/dl
S. Cholesterol		3.9 – 6.2 mmol/L	X 38.6 = mg/dl
S. Triglyceride		0.9 – 2.25 mmol/L	X 88.5 = mg/dl
S. HDL-Cholesterol		>1.04 mmol/L	X 38.6 = mg/dl
S. LDL-Cholesterol		<3.37 mmol/L	X 38.6 = mg/dl
S. Total Protein		60 – 80 g/L	/ 10 = g/dl
S. Albumin		36 – 52 g/L	/ 10 = g/dl
S. Globulin		24 – 37 g/L	/ 10 = g/dl
S. Total Bilirubin		5 – 17 umol/L	/ 17.1 = mg/dl
S. Direct Bilirubin		< 5 umol/L	/ 17.1 = mg/dl
S. Indirect Bilirubin		< 12 umol/L	/ 17.1 = mg/dl
S. Alkaline Phosphatase	80	21 – 120 u/L	/ 7.1 = KAU/dl
S. ALT (GPT)	86	Up to 40 u/L	X 1.0 = u/L
S. AST (GOT)	18	Up to 40 u/L	X 1.0 = u/L
S. LDH		80 – 190 u/L	X 1.0 = u/L
S. CK		24 – 195 Male 24 – 170 female	X 1.0 = u/L
S. Troponin			

Examiner :

محافظة بغداد

دائرة صحة بغداد الرصافة

مستشفى ابن النفيس التعليمي

Baghdad Governorate

استمارة نتائج الفحوص المختبرية للكيمياء الحياتية

Patient name : هديل	Gender :	Age :
	Ward :	Bed :

Test	Results	Normal Value	Conversion Factor
S. Glucose		4.0 -6.4 mmol/L	X 18 = mg/dl
S. Urea	4.1	2.5 – 7.5 mmol/L	X 6 = mg/dl
S. Creatinine	59	Up to 124 umol/L	/ 88.4 = mg/dl
S. Sodium		136 – 155 mmol/L	X 1.0 = meq/L
S. Potassium		3.5 – 5.3 mmol/L	X 1.0 = meq/L
S. Chloride		95 – 105 mmol/L	X 1.0 = meq/L
S. Calcium		2.1 – 2.6 mmol/L	X 1.0 = meq/L
S. Uric Acid		180 – 420 umol/L	/ 60 = mg/dl
S. Cholesterol	5.5	3.9 – 6.2 mmol/L	X 38.6 = mg/dl
S. Triglyceride	1.9	0.9 – 2.25 mmol/L	X 88.5 = mg/dl
S. HDL-Cholesterol	1.9	>1.04 mmol/L	X 38.6 = mg/dl
S. LDL-Cholesterol	3.47	<3.37 mmol/L	X 38.6 = mg/dl
S. Total Protein		60 – 80 g/L	/ 10 = g/dl
S. Albumin		36 – 52 g/L	/ 10 = g/dl
S. Globulin		24 – 37 g/L	/ 10 = g/dl
S. Total Bilirubin	7.2	5 – 17 umol/L	/ 17.1 = mg/dl
S. Direct Bilirubin		< 5 umol/L	/ 17.1 = mg/dl
S. Indirect Bilirubin		< 12 umol/L	/ 17.1 = mg/dl
S. Alkaline Phosphatase	79	21 – 120 u/L	/ 7.1 = KAU/dl
S. ALT (GPT)	16	Up to 40 u/L	X 1.0 = u/L
S. AST (GOT)	14	Up to 40 u/L	X 1.0 = u/L
S. LDH		80 – 190 u/L	X 1.0 = u/L
S. CK		24 – 195 Male 24 – 170 female	X 1.0 = u/L
S. Troponin			

Examiner :

محافظة بغداد

دائرة صحة بغداد الرصافة

مستشفى ابن النفيس التعليمي

Baghdad Governorate

استمارة نتائج الفحوص المختبرية للكيمياء الحياتية

Patient name : خميس توفيق	Gender :	Age :
	Ward :	Bed :

Test	Results	Normal Value	Conversion Factor
S. Glucose		4.0 -6.4 mmol/L	X 18 = mg/dl
S. Urea		2.5 – 7.5 mmol/L	X 6 = mg/dl
S. Creatinine		Up to 124 umol/L	/ 88.4 = mg/dl
S. Sodium		136 – 155 mmol/L	X 1.0 = meq/L
S. Potassium		3.5 – 5.3 mmol/L	X 1.0 = meq/L
S. Chloride		95 – 105 mmol/L	X 1.0 = meq/L
S. Calcium		2.1 – 2.6 mmol/L	X 1.0 = meq/L
S. Uric Acid		180 – 420 umol/L	/ 60 = mg/dl
S. Cholesterol		3.9 – 6.2 mmol/L	X 38.6 = mg/dl
S. Triglyceride		0.9 – 2.25 mmol/L	X 88.5 = mg/dl
S. HDL-Cholesterol		>1.04 mmol/L	X 38.6 = mg/dl
S. LDL-Cholesterol		<3.37 mmol/L	X 38.6 = mg/dl
S. Total Protein		60 – 80 g/L	/ 10 = g/dl
S. Albumin		36 – 52 g/L	/ 10 = g/dl
S. Globulin		24 – 37 g/L	/ 10 = g/dl
S. Total Bilirubin		5 – 17 umol/L	/ 17.1 = mg/dl
S. Direct Bilirubin		< 5 umol/L	/ 17.1 = mg/dl
S. Indirect Bilirubin		< 12 umol/L	/ 17.1 = mg/dl
S. Alkaline Phosphatase	108	21 – 120 u/L	/ 7.1 = KAU/dl
S. ALT (GPT)	43	Up to 40 u/L	X 1.0 = u/L
S. AST (GOT)	18	Up to 40 u/L	X 1.0 = u/L
S. LDH		80 – 190 u/L	X 1.0 = u/L
S. CK		24 – 195 Male 24 – 170 female	X 1.0 = u/L
S. Troponin			

Examiner :

دائرة صحة بغداد الرصافة

مستشفى ابن النفيس التعليمي

Baghdad Governorate

استمارة نتائج الفحوص المختبرية للكيمياء الحياتية

Patient name : عبد المجيد جاسم Gender : Age :

Ward : Bed :

Test	Results	Normal Value	Conversion Factor
S. Glucose		4.0 -6.4 mmol/L	X 18 = mg/dl
S. Urea		2.5 – 7.5 mmol/L	X 6 = mg/dl
S. Creatinine		Up to 124 umol/L	/ 88.4 = mg/dl
S. Sodium		136 – 155 mmol/L	X 1.0 = meq/L
S. Potassium		3.5 – 5.3 mmol/L	X 1.0 = meq/L
S. Chloride		95 – 105 mmol/L	X 1.0 = meq/L
S. Calcium		2.1 – 2.6 mmol/L	X 1.0 = meq/L
S. Uric Acid		180 – 420 umol/L	/ 60 = mg/dl
S. Cholesterol		3.9 – 6.2 mmol/L	X 38.6 = mg/dl
S. Triglyceride		0.9 – 2.25 mmol/L	X 88.5 = mg/dl
S. HDL-Cholesterol		>1.04 mmol/L	X 38.6 = mg/dl
S. LDL-Cholesterol		<3.37 mmol/L	X 38.6 = mg/dl
S. Total Protein		60 – 80 g/L	/ 10 = g/dl
S. Albumin		36 – 52 g/L	/ 10 = g/dl
S. Globulin		24 – 37 g/L	/ 10 = g/dl
S. Total Bilirubin	15	5 – 17 umol/L	/ 17.1 = mg/dl
S. Direct Bilirubin		< 5 umol/L	/ 17.1 = mg/dl
S. Indirect Bilirubin		< 12 umol/L	/ 17.1 = mg/dl
S. Alkaline Phosphatase	70	21 – 120 u/L	/ 7.1 = KAU/dl
S. ALT (GPT)	16	Up to 40 u/L	X 1.0 = u/L
S. AST (GOT)	7	Up to 40 u/L	X 1.0 = u/L
S. LDH		80 – 190 u/L	X 1.0 = u/L
S. CK		24 – 195 Male 24 – 170 female	X 1.0 = u/L
S. Troponin			

Examiner :

Baghdad Governorate

دائرة صحة بغداد الرصافة

مستشفى ابن النفيس التعليمي

استمارة نتائج الفحوص المختبرية للكيمياء الحياتية

Patient name : نرجس علي راشد Gender : Age :

Ward : Bed :

Test	Results	Normal Value	Conversion Factor
S. Glucose		4.0 -6.4 mmol/L	X 18 = mg/dl
S. Urea		2.5 – 7.5 mmol/L	X 6 = mg/dl
S. Creatinine		Up to 124 umol/L	/ 88.4 = mg/dl
S. Sodium		136 – 155 mmol/L	X 1.0 = meq/L
S. Potassium		3.5 – 5.3 mmol/L	X 1.0 = meq/L
S. Chloride		95 – 105 mmol/L	X 1.0 = meq/L
S. Calcium		2.1 – 2.6 mmol/L	X 1.0 = meq/L
S. Uric Acid		180 – 420 umol/L	/ 60 = mg/dl
S. Cholesterol		3.9 – 6.2 mmol/L	X 38.6 = mg/dl
S. Triglyceride		0.9 – 2.25 mmol/L	X 88.5 = mg/dl
S. HDL-Cholesterol		>1.04 mmol/L	X 38.6 = mg/dl
S. LDL-Cholesterol		<3.37 mmol/L	X 38.6 = mg/dl
S. Total Protein		60 – 80 g/L	/ 10 = g/dl
S. Albumin		36 – 52 g/L	/ 10 = g/dl
S. Globulin		24 – 37 g/L	/ 10 = g/dl
S. Total Bilirubin		5 – 17 umol/L	/ 17.1 = mg/dl
S. Direct Bilirubin		< 5 umol/L	/ 17.1 = mg/dl
S. Indirect Bilirubin		< 12 umol/L	/ 17.1 = mg/dl
S. Alkaline Phosphatase	98	21 – 120 u/L	/ 7.1 = KAU/dl
S. ALT (GPT)	17	Up to 40 u/L	X 1.0 = u/L
S. AST (GOT)	18	Up to 40 u/L	X 1.0 = u/L
S. LDH		80 – 190 u/L	X 1.0 = u/L
S. CK		24 – 195 Male 24 – 170 female	X 1.0 = u/L
S. Troponin			

Examiner :

APPENDIX7

وزارة العلوم والتكنولوجيا/دائرة بحوث المواد
مركز بحوث النفط والبتروكيمياويات

رقم الاستمارة : ١١٠
التاريـــخ : ١٧/٢/٢٠١٩

استمارة نتائج فحص

البيانات الاولية :

الجهة المستفيدة	اسم المستفيد	رقم وتاريخ الطلب	تاريخ استلام الطلب
وزارة التعليم العالي والبحث العلمي جامعة بغداد معهد الليزر للدراسات العليا	طالبة الدراسات العليا (هديل سلام محمد)	العدد: 331 التاريخ: 2019/2/11	2018/3/14

طبيعة النموذج	عدد النماذج	رقم النموذج	تقنية الفحص المطلوبة
سائل	3	1-3	معامل الانكسار 1) (بدرجة 25م^0)

نتائج الفحص :

ت	رقم النموذج	النتائج	كلفة الفحص	الملاحظات
1	النموذج رقم (1) ALT	1) 1.3460	20000 عشرون الف دينار	
2	النموذج رقم (2) AST	1.3462	20000 عشرون الف دينار	
3	النموذج رقم (2)	1.3434	20000 عشرون الف دينار	

المبلغ المطلوب بالدينار : 60000 ستون الف دينار عراقي فقط لاغير .

هذه الشهادة صادرة وموثقة في:

المختبر	الشعبة	القسم	المركز
توقيع الفاحص:	توقيع مسؤول الشعبة:	توقيع مدير القسم:	توقيع مدير المركز:
التاريـــخ ١٧/٢/٢٠١٩	التاريـــخ ١٧/٢/٢٠١٩	التاريـــخ ١٧/٢/٢٠١٩	التاريـــخ ٢٠١٩/٣/٤

وزارة العلوم والتكنولوجيا/دائرة بحوث المواد
مركز بحوث النفط والبتروكيمياويات

رقم الاستمارة : ١٥٨
التاريـــخ : ٢٠١٩/٤/١٥

استمارة نتائج فحص

البيانات الاولية :

الجهة المستفيدة	اسم المستفيد	رقم وتاريخ الطلب	تاريخ استلام الطلب
وزارة التعليم العالي والبحث العلمي جامعة بغداد معهد الليزر للدراسات العليا	طالبة الدراسات العليا (هديل سلام)	العدد: 331 التاريخ: 11/ 2/ 2019 (تم اضافة النماذج تباعا للكتاب اعلاه بتاريخ 2019/4/4.)	2019/3/14

طبيعة النموذج	عدد النماذج	رقم النموذج	تقنية الفحص المطلوبة
سائل	10	1-10	1) معامل الانكسار (بدرجة $25\text{-}28C^{\circ}$)

نتائج الفحص :

ت	رقم النموذج	النتائج معامل الانكسار	كلفة الفحص	الملاحظات
1	نموذج رقم(1)	1.3564	140000 مائة واربعون الف	تم خصم 30% لكونها طالبة دراسات.
2	نموذج رقم(2)	1.3547		
3	نموذج رقم(3)	1.3562		
4	نموذج رقم(4)	1.3580		
5	نموذج رقم(5)	1.3540		
6	نموذج رقم(6)	1.3545		
7	نموذج رقم(7)	1.3576		
8	نموذج رقم(8)	1.3559		
9	نموذج رقم(9)	1.3591		
10	نموذج رقم(28) (AST) Standard	1.3470		

المبلغ المطلوب بالدينار : 140000 مائة واربعون الف دينار عراقي فقط لاغير .

هذه الشهادة صادرة وموثقة في:

المختبر	الشعبة	القسم	المركز
توقيع الفاحص:	توقيع مسؤول الشعبة:	توقيع مدير القسم:	توقيع مدير المركز:
التاريـــخ: ٢٠١٩/٤/١٤	التاريـــخ ٢٠١٩/٤/١٤	٢٠١٩/٤/١٤ التاريـــخ	التاريـــخ ٢٠١٩/٤/١٤

وزارة العلوم والتكنولوجيا/دائرة بحوث المواد
مركز بحوث النفط والبتروكيمياويات

رقم الاستمارة : ١٠٨
التاريـــــخ : ٢٠١٩/٤/١٥

استمارة نتائج فحص

البيانات الاولية :

الجهة المستفيدة	اسم المستفيد	رقم وتاريخ الطلب	تاريخ استلام الطلب
وزارة التعليم العالي والبحث العلمي جامعة بغداد معهد الليزر للدراسات العليا	طالبة الماجستير (هديل سلام)	العدد: 331 التاريخ:11 /2019/2 (تم اضافة النماذج تباعا للكتاب اعلاه بتاريخ 2019/4/4)	2019/3/14

طبيعة النموذج	عدد النماذج	رقم النموذج	نقاوة الفحص المطلوبة
سائل	28	1-28	1) معامل الانكسار (بدرجة C° 25-28)

نتائج الفحص :

ت	رقم النموذج	النتائج		الملاحظات
		معامل الانكسار		
1	نموذج رقم(1)	1.3564		
2	نموذج رقم(2)	1.3547		
3	نموذج رقم(3)	1.3562		
4	نموذج رقم(4)	1.3580		
5	نموذج رقم(5)	1.3540		
6	نموذج رقم(6)	1.3545		
7	نموذج رقم(7)	1.3576		
8	نموذج رقم(8)	1.3559		
9	نموذج رقم(9)	1.3591		
10	نموذج رقم(10)	1.3572		
11	نموذج رقم(11)	1.3577		
12	نموذج رقم(12)	1.3571		
13	نموذج رقم(13)	1.3511		
14	نموذج رقم(14)	1.3586		
15	نموذج رقم(15)	1.3567		
16	نموذج رقم(16)	1.3571		
17	نموذج رقم(17)	1.3553		
18	نموذج رقم(18)	1.3592		
19	نموذج رقم(19)	1.3581		
20	نموذج رقم(20)	1.3591		
21	نموذج رقم(21)	1.3595		
22	نموذج رقم(22)	1.3598		
23	نموذج رقم(23)	1.3570		
24	نموذج رقم(24)	1.3570		
25	نموذج رقم(25)	---		
26	نموذج رقم(26)	1.3570		
27	نموذج رقم(27)	1.3570		
28	نموذج رقم(28)	1.3470		
	(AST) Standard			

الخلاصة

الخلفية:

تُعرَّف المتحسسات البيولوجية بأنها اجهزة تحليلية تشتمل على مجموعة من عناصر المتحسسات البيولوجي مثل نظام الاستشعار ومحول طاقة ، وهي تجمع عنصرًا نشطًا بيولوجيًا مع محول طاقة فيزيائي مناسب لإنتاج إشارة قابلة للقياس تتناسب مع تركيز المواد الكيميائية في أي نوع من العينات. تعرض مستشعرات الإنزيم الحيوية قيمة تطبيق ممتازة بسبب تطور التكنولوجيا الحديثة والخصائص المتميزة.

الهدف من الدراسة:

الكشف عن مستوى إنزيمات الكبد الآلنين الترانس امينيز (ALT) وأنزيمات الكبد الأمينات الناقلة الأسبارتية (AST) وتركيزها باستخدام مجموعة مختلفة من المتحسسات الليزرية كطريقة جديدة

المواد و طرق العمل :

جمعت أربع وعشرون عينة دم من المرضى ذكور وإناث اللذين تتراوح أعمارهم بين (١٥-٧٢) سنة في مستشفى ابن النفيس ، يشكون من أمراض القلب والكبد. من كل مريض ، تم أخذ ١٠ مل من الدم معاملاً بالطرد المركزي وتم تقسيمة إلى قسمين ، (٥ مل) يستخدم للكشف عن تركيز إنزيمات ALT و ASTالتي تم فحصها يدويًا في المستشفى باستخدام طقم RANDOX ومقياس الطيف الضوئي في الطول الموجي (٥٤٦) نانومتر. ٥ mlالأخرى المستخدمة للكشف عن تركيز الانزيمات باستخدام ثلاثة انواع من اجهزة المتحسسات البيولوجية الليزرية المصنعة. وفقًا للخصائص البصرية لكل من الطول الموجي القياسي للإنزيمين ALT و AST (٥٣٠-٥٥٠ نانومتر) كما هو مدرج في المجموعة ومؤشر الانكسار لمعياري للانزيمين ALT و AST باستخدام مقياس إنكسار آبي ، ١.٣٤٧٠ au ، ١.٣٤٧٠ auعلى التوالي باختيار نوع الليزر الاخضر دايود اليزر (أشباه الموصلات) المناسب في الطول الموجي ٥٣٢ نانومتر ، الطاقة <١٠٠٠ ملى واط. مؤشر الانكسار بواسطة مقياس إنكسار آبي القياسي (عادي). تُستخدم ثلاثة أنواع من الألياف الضوئية لإنشاء المتحسسات حيوية للكشف عن تركيز إنزيمات ALTو AST في عينات المصل:

١. وضع متحسس الألياف واحد (SMF-٢٨) التحسس في ٥٣١. ٦٢ نانومتر و ٥٣١.١٦ نانومتر على التوالي.

٢. متحسس الألياف المتعددة (MM) في ٥٣١.١٦ نانومتر لكل من الانزيمات.

٣. PCF Machzender متحسس تداخل الألياف البلورية الضوئية (PCF - LMA-١٠) الاستشعار في ٥٣١.١٦ نانومتر لكلا الانزيمات. يتم إنشاء ثلاثة أنواع من المستشعرات الحيوية ، وهي

الألياف ذات الوضع الأحادي (SMF) ، والوضع المتعدد الوسائط (MMF) والمستشعر الحيوي البلوري الضوئي .(PCF) تتصل المتحسسات الحيوية المصنعة (SM) ، MM و (PC بالليزر ديود الضوء الأخضر المصدر بالليزر ٥٣٢ نانومتر وفقًا للخصائص البصرية لمعيار ALT و AST ، على الجزء المعد من الألياف (٢ سم) لكل الإعداد ، تم وضع ١ مل من المعايير (لجميع العينات) و الاتصال مع محلل الطيف الضوئي الحساس (HR-٢٠٠٠) لإظهار إشارة كثافة.

النتائج :

إن امتصاص ضوء الليزر بواسطة عينات شديدة التركيز يكون أعلى ويتناسب عكسيا مع شدة الضوء ، مما يعني أن شدة الضوء تكون عالية عندما يكون تركيز الإنزيمات منخفضًا. يمكن تفسير هذه الظاهرة على أنها الامتصاص العالي للضوء بواسطة عينات) إنزيمات ALT و (AST بسبب اختيار ليزر مناسب يعتمد على امتصاص العينات لطول موجة الليزر. يعتبر المستشعر الحيوي متعدد الوسائط (MM)أكثر فعالية لأن إشارة كثافة الألياف متعددة الوسائط لها عدد كبير من الاشارات مقارنة مع النمط الفردي والألياف البلورية الضوئية التي لها عدد محدود من الأوضاع بسبب بنيتها الأساسية.

الخاتمة :

تم تصنيع ثلاثة أنواع من المتحسسات البصرية وضع أحادي ، متعدد الألياف وألياف الكريستال الضوئية باستخدام ليزر ديود ضوئي أخضر ٥٣٢ nmللكشف عن مستوى إنزيمات كبد ALT و AST في عينات الدم ، يعتبر مستشعر الليزر المتعدد الوسائط أفضل مستشعر بيولوجي للكشف عن تركيز كل من الإنزيمات في العينة بالإضافة إلى أنها حساسة للغاية في انتقال شدة ضوء الإشارة. جهاز الاستشعار البيولوجي هو الأكثر تواترا ، مع تشخيص سريع ، طريقة أكثر تكلفة من الطريقة التقليدية لتجنب أي تغييرات بيولوجية في عينة الدم تؤدي إلى تغيرات التباين البصري (مؤشر الانكسار وامتصاص) من عينة الدم.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد
معهد الليزر للدراسات العليا

تقييم أنزيمات ALT و AST بواسطة المتحسسات البصرية

رسالة مقدمة الى

معهد الليزر للدراسات العليا /جامعة بغداد /لاستكمال متطلبات نيل شهادة

ماجستير علوم في الليزر/ علوم الحياة

مقدمه من قبل

هديل سلام محمد العبد

بكالوريوس علوم الحياة - ٢٠٠٨

بإشراف

المدرس الدكتور ليلى محمد حسن العامري

بغداد – العراق

٢٠١٩م ١٤٤١هـ