T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

OPTICAL DETECTION of EARLY MI MARKERS by ABSORPTION REFLECTION SYSTEM:

TARGETED CHARECTERIZATION OF BLOOD SAMPLES CONSISTING OF TROPONIN-T

ZEYNEP GERDAN

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

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İSTANBUL, AUGUST / 2014

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FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

SOĞURMA-YANSIMA SiSTEMi TARAFINDAN ERKEN MİYOKARDİUM ENFARKTÜS BELiRTEÇLERiN OPTiK ALGILANMASI: TROPONİN-T İÇEREN KAN ÖRNEKLERİNİN HEDEFLENMİŞ KARAKTERİZASYONU

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İSTANBUL, AĞUSTOS / 2014

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Zeynep Gerdan, a **MSc** student of Fatih University **Institute of Biomedical Engineering** student ID 520112001, successfully defended the **thesis/dissertation** entitled "**OPTICAL DETECTION of EARLY MI MARKERS by ABSORPTION REFLECTION SYSTEM:TARGETED CHARECTERIZATION OF BLOOD SAMPLES CONSISTING OF TROPONIN-T**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Dedicated to my father Hasan GERDAN,

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I would thank my mother, my sister and my brothers for their continuous support, patience and encouragement. I would like to thank my supervisor Assist. Prof. Dr. Haşim Özgür TABAKOĞLU for his guidance, continous patience, support and directing me to this productive study during the course of this research

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LIST OF SYMBOLS

ʎ	Lambda factor
µ	Micro
µm	Micrometer
L	Liter

ABBREVIATIONS

Ac	: Absorbance Control Group
AC	: Alternate Current
Ap	: Absorbance Patient
ACS	: Acute Coronary Sendrome
AMI	: Acute Myocardial Infarction
AV	: Atrioventricular
Ca	: Calcium
CK	: Creatine Cinase
cTn	: Cardiac Troponin
Da	: Dalton
Hz	: Hertz
IR	: Infrared
K	: Potassium
kDa	: Kilo Dalton
kHz	: Kilohertz
MI	: Myocardial Infarction
mL	: Milliliter
Na	: Sodium
ng	: Nanogram
NIR	: Near Infrared
nm	: Nanometer
NSTEMI	: Non ST Elevation Myocardial Infarction
O_2	: Oxygen
SA	: Sinoatrial
TEKHARF	: Turkish Adult Cardiological Diseases and Risk Factors
UAP	: Unstable Angina Pectoris
UV	: Ultraviolet
WHO	: World Health Organization

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SUMMARY

OPTICAL DETECTION of EARLY MI MARKERS by ABSORPTION REFLECTION SYSTEM:

TARGETED CHARECTERIZATION OF BLOOD SAMPLES CONSISTING OF TROPONIN-T

Zeynep GERDAN

Biomedical Engineering Programme

MSc Thesis

Advisor: Assist. Prof. Dr. Haşim Özgür TABAKOĞLU

Due to blockage of coronary arteries, inability in oxygen content in short cardiac muscle result from extended ischemia that happened sudden remission of blood flow is necrosis and when cardiac muscle is died myocardial infarction that defined as irreversible damaging is the most important of death and diseases in developed western countries. According to WHO statistics in 2008 millions of people died due to cardiovascular diseases. According to TEKHARF study that applied in our country, this number is specified as 160000 persons in every year.

In this study, transmission values of blood serum, that has increased Troponin-T content belonging to MI patients, have been inspected with absorption-reflection optical system in order to scan from UV region to NIR region. Results showed that transmission values of blood serum from MI patients were significantly less than the values of control group at the following wavelenghts: for UV region; 320 nm, 370 nm, for VIS region; 514nm, 591nm, and 650 nm, for NIR region; 755 nm, 890 nm, 958 nm 1064 nm.

Keywords: Myocardial Infarction, Troponin-T, Absorption-Reflection Spectrophotometer.

ÖZET

SOĞURMA-YANSIMA SiSTEMi İLE ERKEN MİYOKARDİUM ENFARKTÜS BELiRTEÇLERiN OPTİK ALGILANMASI: TROPONİN-T İÇEREN KAN ÖRNEKLERİNİN HEDEFLENMİŞ KARAKTERİZASYONU

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Yüksek Lisans Tezi

Danışman: Yrd. Doç. Dr. Haşim Özgür TABAKOĞLU

Kalp kası nekrozu, koroner artelerde tıkanmadan dolayı oksijen miktarındaki yetersizliğin yani kan akışındaki ani azalmaya bağlı olarak meydana gelen uzamış iskeminin sonucudur ve kalp kası öldüğünde geri dönüşümsüz hasar meydana gelmesi olarak tanımlanan miyokardiyal enfarktüs gelişmiş batılı ülkelerde ölüm oranı ve hasta olma oranının en önemli sebebi olmuştur. WHO istatistiklerine gore, 2008 yılında milyonlarca insan kardiyovasküler hastalıklardan dolayı hayatını kaybetmiştir. Ülkemizde yapılan TEKHARF çalışmasına göre bu sayı her yıl 160000 kişi olarak belirlenmiştir.

Bu çalışmada, Troponin-T değerleri artmış olan MI hastalarının kanlarından elde edilmiş serum örnekleri kullanılarak, örneklerin farklı dalga boylarındaki geçirgenlik değerleri tespit edilmeye çalışıldı. Elde edilen bulgulara göre kan serumlarının geçirgenlik değerlerinin, morötesi bölgesinde (UV) 320nm, 370nm görünür ışık bölgesinde (VIS) 514nm, 591nm ve 650 nm, ve yakın kızılaltı bölgesinde (NIR) ise 755nm, 890nm, 958nm ve 1064 nm'lerde, hasta grubundan alınan örneklerinin kontrol grubundan alınan örneklere göre anlamlı bir şekilde daha az olduğu tespit edildi.

Anahtar kelimeler: Miyocardial enfarktüs, Troponin-T, Absorption- Reflection Spectrophotometer.

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

Acute myocardial infarction is a major health problem for humanity[1]. Although diagnostic and therapeutic methods for myocardial infarction is highly developed, it is leading reason both mortality and morbidity in Western countries in women who over 65 age, in men who over 45 age [2, 3].

According to WHO statistics, 17 million people died because of cardiovascular illnesses in 2008 and it's supposed to this number will be over 23 million in 2020 and %50 of these deaths occur in the first 1 hour as from the AMI beginning[4, 5].

After beginning the acute myocardial infarction, CK-MB and total CK levels increase in blood within 4-8 hours, cardiac troponin T and I within 3-12 hours and myoglobin within 1 hour. Laboratory results are needed in order to intervening patients [6, 7].

The earlier determination of biomarkers raised in the blood can accomplished by optical systems and non-invasive results can be taken without waiting 45 minutes – 1 hour for laboratory.

1.2 Motivation

The goal of this thesis is to determine optical properties, such as transmission and reflection, of blood samples which belong to patients who got Myocardial Infarction by using Zolix Omni AS absopriton –Reflection Spectrphotometer

CHAPTER 2

2.1 Circulatory System

The circulatory system includes the heart, the blood vessels and the blood[8].

The blood consists of cell and cell fragments which are in the suspension state within the plasma containing lots of proteins, metabolic wastes and food stuffs. These cells are erythrocytes, leucocytes and the cell fragment is thrombocyte. Erythrocytes are responsible for carrying the oxygen which constitutes a major part of the blood cells. Leucocytes are interested in the body's defence system, while thrombocytes are responsible for blood coagulation[9].

In the centrifuged blood, erythrocytes are placed at the bottom of the centrifuge tube, thrombocytes and leucocytes in the middle of the tube and the plasma is located at the top of the tube[9].

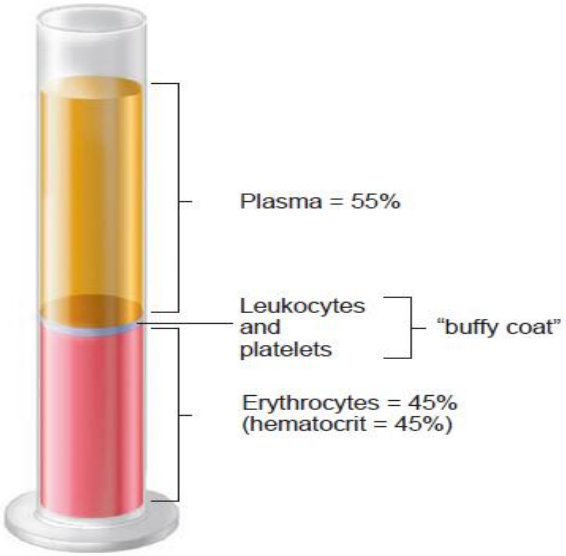

Figure 2.1.Measurement of the hematocrit by centrifugation. [9]

The Anatomy of the Heart

The heart is a conical punch-sized muscled organ between the lungs in the center of the thorax covered with serous and fibrous pericardium which is 310 gr. in men and 255 gr. in women [10, 11].

The three-layered heart's outermost layer is the epicardium, the middle layer which consists of the blood muscle cells is the myocardium, and the innermost layer is the tiny fibrosis primer endocardium which covers the inner surface of the inner walls of the blood vessels and the inside of the heart valves[8, 10].

The heart is divided into four chambers; upper right and left atria and lower right and left ventricles. [8] Between the right atrium and the right ventricle there is the tricuspid valve and between the left atrium and the left ventricle there is the mitral valve[9].

The opening and the closing of the valves happen due to pressure differences and they prevent the blood from flowing backwards.

However, travelling to the pulmonary trunk from the right ventricle and to the aorta from the left ventricle is forced. Between the pulmonary trunk and the left ventricles there are pulmonary valves, and between the aorta and the left ventricle there is aortic ventricle.

The right atrium located on the upper side of the heart receives the blood by three veins. It takes venous blood in the superior vena cava from the lower regions of the body, with the inferior vena cava from the upper region of the body and with the coronary sinus from the myocardium.

The right ventricle pumps the blood required for the pulmonary circulation to the lungs. Therefore, its walls are thick and it is abundantly muscled[10, 12].

After the change of gas in the capillaries the lungs, it transfers the O_2 rich incoming blood to the left atrium by means of two left and two right pulmonary veins [8, 10].

The left ventricle forms the apex of the heart. The cavity of the left ventricle is oval-shaped. The papillary muscles in the left ventricle are considerably large strong and thick[10].

2.3 Physiology of the Heart

The conduction system of the heart constitutes it is physiological activity.

The conduction system is responsible for protecting the cardiac stretching rhythm which makes the heart chambers be filled and emptied[11, 12]**.**

The components of the conduction system are the, sinoatrial node (SA node), atrioventricular node (AV node), atrioventricular bundles (bundle of His) and conduction myofibers (Purkinje fibers).

The SA node which is called also as pacemaker is located in the entry of the superior vena cava on the posterior wall of the right atrium; it starts and regulates the heartbeat. It sends every impulse in 0.85 seconds[10, 12].

AV node receives the action potential created by the SA node. The purkinje fibers are full scale fibers which provides the stretch by delivering the impulse to the ventricle muscles[10, 12].

The unstimulated state of the blood cell is called as resting potential and there are negative proteins and nucleic acid in the cell. There is a balance between the inner part of the cell K and the outer part of the cell Na. When this balance changes, the cell becomes polarized. The change of this balance happens in series.

These series are:

1 with quick-opening of Na channel into the cardiac muscle cell caused by the insertion of Na ion rapid depolarization.The Na^+ channels rapidly close.

2. A plateau phase Ca muscle cells enter in the cytosol and connect with the troponin. By means of this connection, the cross-bridge happens and the actin filaments shift to the myosine and the stretch happens. When the Ca channel is opened, the transmissivity to the K increases and this incident affects the transmission of the ions and the depolarization resulted from the membrane potential happens.

3. In the repolarization, K is spread out to the cell by opening the channels.

4. In the refractory period, K and Na return back to their balanced states and to the resting potential.

2.3.1 The Electrocardiogram (ECG)

The electrical activity generated by the re- and depolarization of the blood muscle fibers are recorded on a graph-paper as graphically by the electrodes located on the arms and the breast wall. It is measured by the 12-electrode electrocardiograph [8, 12, 13].

P Wave

Depolarization of the atrial fibers of the SA node produces the P wave P wave is a small wave[8, 12].

The first half on the P wave corresponds to the depolarization of the right atria, the second half corresponds to the left atrial depolarization[13].

The QRS Complex

It represents the electrical powers generated by the ventricular depolarization[14].

The PR Interval

The time period in which the AV node sensory branches activating the electrical signal coming from the atrium arrive at right and left bundle branches and ultimately at the Purkinje fibers[13].

The ST Segment

It represents the start and completion period of the ventricular depolarization and the repolarization[8].

T Wave

The repolarization of the ventricle is represented by T wave[13].

2.4 Anatomy of the Blood Vessels

There are three kinds of blood vessels: arteries, veins, and capillaries[10].

The Arteries and the Arterioles

The arteries are 3-layered strong, thick walled vessels which carry the blood from the heart to the body.

The *tunica interna* is an endothelium layer with a basement membrane. The *tunica media* is a thick middle layer of smooth muscle and elastic fibers. The *tunica externa* is an outer connective tissue layer composed principally of elastic and collagen fibers[10].

The Capillaries

About %5 of the circulation blood is carried by these vessels and it is this 5 percent that is performing the ultimate function of the entire cardiovascular system the exchange of nutrients, metabolic and products, and cell secretions. They are microscopic and tiny walled[9, 12].

The Veins and the Venules

The blood is carried to the hearts by the veins. It flows into the microscopic blood venules.

The average pressure of the veins is only 2mmHg, while it is about 100mmHg in the arteries[8].

2.5 Acute Myocardial Infarction

The Definition:

As a result of the lack of the oxygen resulting from the clogging of the coroner arteries or in other words as a result of the long ischemia determined by a sharp decrease in the blood flow, it is a heart muscle necrosis and when the heart muscle is dead, there happens an irreversible defect and Acute Myocardial Infarction (AMI) happens [15, 16].

According to WHO, criteria of the diagnosis of AMI [5]

- Ischemic breast pain which lasts longer than 20 minutes
- Pathological changes in the ECG
- Increase or decrease in the specific enzymes and markers for the heart

In the world population, it is still accepted as an important cause of the mortality and the morbidity in spite of the improvements in the AMI diagnosis and methods. Thousands of people pass away in America every year[5, 17].

According to WHO statistics; the AMI is the first to affect the human health among the cardiovascular diseases. In 2008, 17 million people died because of cardiovascular disease in the middle and lower income countries. It is expected that in 2030, the number will pass 23 million[2, 4].

In the European countries it is a cause of death over the age of 65 in women and over the age of 45 in men[5].

According to TEKHARF (Turkish Adult Cardiological Diseases and Risk Factors), which is carried out in our country, there is coroner artery disease in 2 million people. The frequency of this disease in men is %5.1 and in women %3.3 [1].

The 2000 year study datum of TEKHARF show the fact that the number of coroner artery disease people in Turkey happens to be 2 million after 2000 and in our country, about 160.000 people are dying due to coroner artery disease every year[18].

2.5.1 Acute Coroner Syndromes

ACS is a clinical incident when the patients consult to the emergency services for breast pain[19].

In 2009, more than 600.000 people were discharged from the hospital after the treatment from the ACS[20].

According to 12 lead ECG, ACS is examined by 3 categories[15].

1. ST elevation MI

2. ST depression MI

3. Unstable angina pectoris

The major cause of ACS is the erosion or rupture of atherome plaque. If the atherome plaque or rupture is defected, the endothelium shroud tears and after contact, the thrombocytes are activated and clustered together. The structure of the thrombocytes changes and becomes stick. The activated thrombocyte factors start the coagulation and create thrombin and thrombus. These thromboses start to block the lumen. This blockage leads to either complete occlusion or partial vessel blockage. Some pathological changes are observed from the ischemia till the necrosis in either the of myocard transmural or subendocardial region according to the blockage time and level and to the existence of the collateral vessels and AMI happens [1, 6, 16].

Atherosclerosis happens in the big and medium sized vessels of the coroner artery. If the coroner occlusion continues more than 15 or 20 minutes, it will lead to irreversible cell defects. In the interventions carried out for about 4-6 hours, the necrosis area is small[15, 21].

Table 2.1 Risk Factors Effective in Developing Atherosclerosis [16, 22]

I.Unconvertible
1. Age
2. Sex
3. Genetic (family history)
II. Convertible
A. Major
1. Hyperlipidemia
2. Cigarette
3. Hypertension
4. Diabetes mellitus

5. Obesity
6. Sedantary life
B Minor
1. Excess alcohol consumption
2. Stress
3. A type personality
4. Increased fibrinogen
5. Increased homocysteine when muscle fatigue occurs,

Table 2.1 (cont)

The Characteristics of the Atheroma Plaque

Thin fibrosis capsule

High lipid content

Increased macrophage activity [23]

Factors Effective To Plaque Stability [22]

a) extrinsic factors:

I.blood pressure

II.heart rate

III.intracoronary diastolic pressure

b) intrinsic factors

I.thickness of fibrous capsule of plaque

II.amount of collagen of fibrous structure

III.degree of intracoronary tightness

IV.size of stone rich from lipid

V.activity degree of inflammatory in plaque.

Non ST Elevation MI

If the thrombus generated in the coroner vessels is not a total blocker or if it is opened spontaneously after reperfusion, in that there is no permanent blockage in the vessels, it leads to the ST depression in the ECG.

In the NSTEMI, myocardium necrosis is not transmural. In other words, it is limited in the subendocardial region. The way for distinguishing the NSTEMI from the unstable angina is to measure the cardiac biomarkers [16, 24].

ST Elevation MI

STEMI is totally different from the NSTEMI which is distinguished by biomarkers.

STEMI is a type of transmural infarction in which the necrosis is developed almost totally, if the thrombus leading to a total or partial blockage in the coroner vessels is created[24].

If there are no rich collateral vessels in these patients, in the subsequent derivations in the ECG, more than 1 mm ST segment elevations are observed. There are current increases in the serum cardiac and ischemic breast pain [16, 24].

The ST segment increase in the right coroner arteries (RCA) happens generally with immediate or total blockage. The %30-35 of the patients die during the attack and the risk of having an infraction in the survivals from these attacks is 8 times more than normal[15, 25, 26].

Unstable Angina Pectoris

It is a clinical incident associated with the ischemia occurring by non-blocker thrombus agglomeration which is in between %70-%100 in the arteries as a result of the erosion of the atheroma plaque or rupture.

Besides the fact that the angina pectoris patients cannot determine the place of the ache correctly, the ache occurs generally in the arms and the breast. This disease is not associated with the emotional stress or extreme exercises. This ache can be cured by either taking sublingual pills or by resting. The attacks are strong and long term[15, 24, 27].

Anamnesis is important for the doctors to diagnose the disease accurately.

Myocardium ischemia consists of several mechanisms. The first one is lack of oxygen necessity as a result of the insufficient blood flow to the myocardium when there is a higher level of blockage. The other one is the coroner artery spasm and thrombocyte aggregation as a result of the endothelial dysfunction. Because of the fact that the blood flow is decreased as a result of thrombocyte aggregation and the coroner artery spasm, subendocardial or transmural ischemia occurs [21].

2.6 AMI Symptoms and Biomarkers

The ache strongly gives pressure on the breast and the arms and it spreads towards the back. This strong ache continues more than 20 minutes and it does not go away by resting.

The ache which starts from the breast spreads through the arms, the chin, the back, the shoulders and the wrists[24].

In the %50 of the patients who are exposed to other symptoms of AMI, there happens nausea, vomiting, weakness, faintness, cold sweating and fear of death[28].

The enzymes used for the AMI diagnosis are both important for diagnosing and prognosis. Intercellular molecules can be detected in the peripheral circulatory as a result of the defects in the unity of the myocardium cell during myocardial ischemia.

2.6.1 Creatin Kinase and Isoenzymes

It is a cytosolic enzyme which has molecular weight from CK 39000 to 42000 Da. It consists of 2 subunits: B and M. It has 3 isoforms: CK-MM, CK-MB, CK-BB [6, 29].

MM: in the skeleton muscle and the myocardium.

MB: in the myocardium.

BB: in the brain and kidney.

CK-MB: has 86000 Da molecular weight, constitutes %15 of total CK activity.

CK/ CK-MB: It it is more than %2.5, it is sufficient for the AMI diagnosis. Because this proportion is rooted from the myocardium not from the striped muscle and it shows the myosit defect[5, 6].

The level of CK-MB is used for differentiating the UAP and the NSTEMI. An increase in the CK-MB level is observed from the death of the developing cells associated with the ischemia necrosis in the myocardium cells. If there is happen a myocardial defect, it will increase in the blood within 4-8 hours and will reach its peak value in 24 hours and in 36-72 hours, the emulsification occurs and it turns back its normal state[24].

It provides 98 % sensitivity within 36 - 72 hours after the breast ache has started.

2.6.2 Myoglobine

When there is a myoglobine cell defect, it is an oxygen connecting protein consisting of a light heme group weighing 18 kDa which is exhausted in the skeleton and heart muscle cell cytoplasms[7, 17].

Because it is light, it is given to the circulatory after 2-3 hours from the start of AMI and reaches its top boundary and after 9-12 hours ir reaches its maximum concentration[17].

The myoglobine is sensitive to the AMI but not specific. The proportion of its liar positivity is %50. In order not to increase its quantity in the blood, it excludes the AMI.

The increased myglobine in the blood is filtered by the kidneys and then it is given out by the urine [1, 16].

2.6.3 Cardiac Troponin

This protein which has 3 subunits, cTnT , cTnl and cTnC which are responsible in the contraction associated with the actine and myosin Ca in the striped muscles is located in the contractile aparatus and cytosolic pool in the myocyte[5, 30].

Troponin T: Connected to the thropomyocyne[5]

Troponin I: Connected to the actine and prevents the relation of actine and myosin.

Troponin C: Connects Ca.

Troponins are used for both assay and diagnose the prognosis in the cardiac insufficiency and the ACS diseases[6].

The Troponin increase in the incidents such as pulmonary embolism, hypotension and kidney insufficiency other than myocard defects as a result of ischemia[16].

The Troponin quantity is %13-15 more than CK-MB. cTn sensitivity is more than CK-MB in early periods. That's why it is more sensitive and specific than CK-MB in the myocard defect[31, 32].

In healthy individuals cTn is nearly non-existent in the blood plasma. It can reach measurable levels due to the fact that it is exhausted from the cytosolic wall in the early stage of tissue defect less than 1 gr. In later periods, because of the fact that it continues to be exhausted from the contractile aparatus, it can stay in higher quantities in the blood in long-term[30, 33].

Troponin T and I are located at the striped muscle and the heart muscle. However, the Troponin I is a specific marker for the heart and for the myocardial cell defects.

Troponin T and I starts increasing within 3-12 hours after the AMI has started and because of the fact that it continues to be exhausted from the contractile aparatus, it reaches its peak within 24 hours and returns back its normal state within 5-14 days.[1, 6, 30].

The Troponin in the cardiac, T and I are different. Because, they are encode by different genes [30].

The Troponin T is a cardiocyte protein which determines the minimal increases' subclinical defects, which decreases the myocardium necrotic defects when its blood

concentration is over 1.5 ng/mL, which stays higher within 3-12 hours after the AMI starts, whose normal values are under 0.6 ng/mL, which has sensitivity and specificity to the AMI exhausted by the death of myocardial cell or defects and which weighs 39.7 kDa[2, 34, 35].

Because of the fact that it is specific to myocard defect, it is called as "golden standard'' marker test. When compared to other biomarkers which are used in diagnosing the AMI, it has a perfect sensitivity and specificity. It is reliable and important for risk evaluation and for the treatment of cardiovascular disease [32, 36].

2.7 Optical Properties

The basic optical properties of absorption, scattering and reflection and refraction characterize photon propagation in biological tissue. These properties govern the numbers of photons that are transmitted amidst spot on the surface of tissue[37].

Refraction

Incident photon came toward interface between two medias refracts when it passes into second platform and this occurrence is arise from speed of light in different platforms. Speed of light can be occurred cause of characteristic of first and second

platform[37, 38].

The simple mathematical relation governing refraction is known as snell's law[37].

$$\text{Sin Q/ sin Q''= v/ v'} \qquad (2.1)$$

Q" is the angle of refraction

v speeds of light in the media before the reflecting surface

v′ speeds of light in the media after the reflecting surface

Reflection

Electromagnetic radiation returned back from interface between two platforms have same characteristics called as surfaces. Wave front cannot be interacted with surface[37, 38].

The reflection angle θ equals the gradient of incidence θ as indicate in Fig. 2.3 and stated by

$\theta = \theta^{1}$

The angles θ and θ are steady amidst the surface normal and the incident and reflected beams, in turn in order[39].

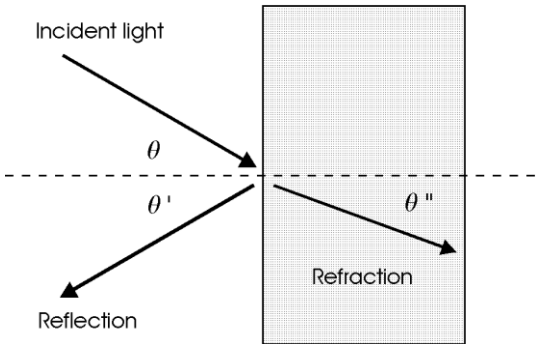

Figure 2.2 Geometry of specular reflection and refraction[37]

Absorption

Intensity of electromagnetic wave that came to dispersive surface is declination when it's passing over the platform or destroying the photonics energy completely.

The proportion of absorbed and incident intensities marked out by the absorbance of a medium[38].

Scattering

It is interaction between molecules and electromagnetic radiation and it is a physical process[38, 40].

It is change of light direction that came over the material.

Light scattering in tissue depends on wavelength of light, various refractive incidents change and a lot of variables like tissue, components as cell organelles [40].

Rayleigh scattering is known as elastic scattering is debilitation the light beam by changing initial ways of photons without electromagnetic radiation energy loss over molecules that smaller than scattering wavelength[38, 40].

Brillouin scattering –inelastic scattering-arises from acoustic waves which propagating through a medium.Another name of Brillouin scattering is inelastic scattering which homogeneities of the refractive index induced with.The reason of the fact that scattering particles are moving away from the light source, with Brillouin scattering higher frequencies occurs[38].

2.7.1 Measurement of optical tissue properties

Two of the methods for the measurement of optical tissue properties are first integrating sphere and the second one is double integrating sphere system[41].

Single integrating sphere system

Transmitted light and reflected that samples placed to input and output port is evaluated by detectors. Evaluating samples are placed among glass plates in general so surface effect is minimized and mechanical support is provided. Transmitted light and reflected can be evaluated by this effect.

Determined power by the help of detector varies from detector size and the total light rest in ball [42].

Double integrating sphere system

This system is a system that includes plates for placing the samples from two spheres and among these balls for evaluating the tissue features as in vitro.

Geometries of sphere and surface reflectance can be differed[42].At the first sphere, all radiation integrates either scattered or reflected which originated from the specimen.On the other hand, in the second sphere transmitted and guiding sattered radiation is absorbed too.thus. With three detectors can be fulfilled as it is demanded at the same time[37].

CHAPTER 3

MATERIAL AND METHOD

3.1 Absorption Reflection Spectrophotometer

It's supplied to evaluating as transmission reflectance and absorption in high quality from solid and liquid samples in too large spectral range from UV to IR by ZLX-AS system. Large range evaluation is made by proper light source monochromator grating and detector selection. Sample chamber is completely modular. This device diffuse reflectance measurement can be used with integration of optimal integrating system if it's required [43].

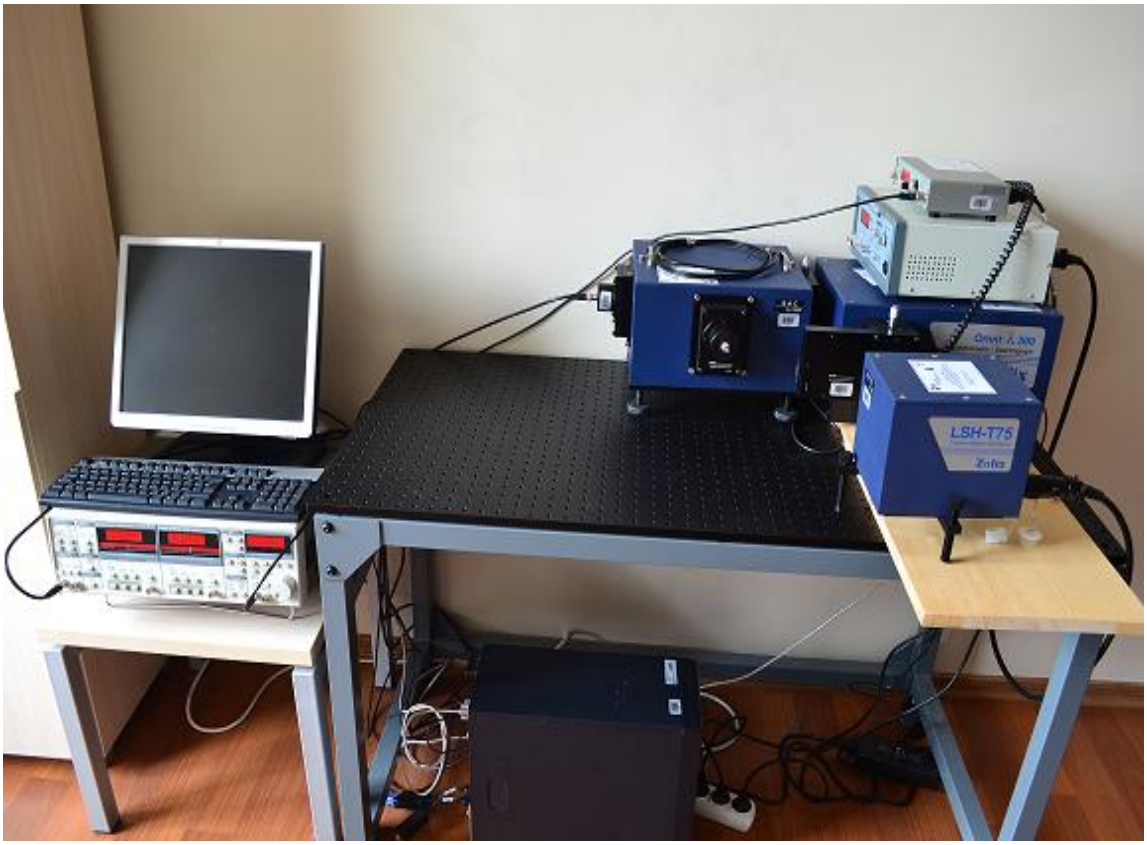

Figure 3.1 Absorption - Reflection Spectrophotometer

3.1.1 Monochromator

Multi-purpose full automatic focal length 300 nm is very sensitive system. It's an ideal and multi-purpose system that supplies large spectral resolution application by low leak electric levels and excellent optic performance from UV to IR with flexible grating options.

Omni ʎ 300 that has strong high performance has flexible input output point options and it's arranged with two dimensions detector, monochromator or spectrograph system.

To change wavelength and to switch grating, stepping motor drive that supplied 0, 1 nm reproducibility is used and min. step size is 0,005 nm. It's connected to computer by USB 2.00 interface [44].

Figure 3.2 Zolix Omni ʎ 300 Monochromator [44]

3.1.2 Sample Chamber

SAC 3 port is configured to evaluations of sample chamber transmission reflectance absorbance.

Each quartz lens unity are designed as moving to supply parallel or focused beam and give opportunity of 360^{o} turning properly to requirements of sample and application. It's compatible with SAC Omni ʎ300 and spectrographs [45].

Figure 3.3 Sample Chamber [45]

3.1.3 DSi Silicon Detectors

It's a sensitive detector that spectrum range sensitive to UV between 200-1100 nm.[46].

Figure 3.4 DSi Silicon Dedectors [46]

3.1.4 DInGaAs Detector

This detector that worked in NIR spectral range from 0,8 µm to 1,7µm supplies high performance at room temperature[47].

Figure 3.5 DInGaAs Dedector [47]

3.1.5 Tungsten-Halogen Light Source

Light source that spectral range is 300-2500 nm is durable about 4000 hours [48].

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Figure 3.6 Tungsten-Halogen Light Source [48]

3.1.6 Lock in Amplifier

It serves to evaluate true very small AC signals and use the techniques of a definite reference frequency and phase to phase sensitive detection.

Reference frequency is required for evaluating and it produces this reference signal. This inner reference signal is produced by phase-locked-loop. It refuses the noise signals in frequencies except the reference frequency and evaluation is not affected[49].

Figure 3.7 Lock in Amplifier [49]

3.1.7 Optical Chopper SR540

For SR540 chopper optical chopping evaluations, there are four-digit frequency screen, front panel frequency control, analog voltage frequency control, optional operation modes and two reference outputs. It's ideal for dual beam and intermosulation tests.

It supplies 5/6 slot blade for 400 Hz frequency and 25/30 slot blade for 3,7 kHz frequency[50].

Figure 3.8 Optical Chopper SR540 [50]

3.3 Preparing Blood Samples

Control group preparation: The venous blood that taken from the patients in proper hygiene conditions is waited at room temperature about 20 minutes after putting into red capped tubes. At the end of 20 minutes, it's placed into centrifuge device and it's centrifuged in 1500 speed about 10 minutes. Red blood cell is bottom of the tube; plasma is top of the tube.

The plasma part is taken from the top of the tube by a dropping glass and it's transferred into quartz cuvette that hold and examination.

In this study, zolix absorption reflection system was used to understanding that CK-MB Troponin T and Myoglobin values that started to increase in the blood as a result of cardiac muscle damage and heart attack are in which wavelength in transmission.

Experiments preparation

With chest pain or a heart attack admitted to hospital with suspected blood samples from patients were isolated in the laboratory of biochemistry. The patient has a bar code affixed to each tube This isolated serum samples were stored in a cooling temperature of -20 c.

To reach the required number of special containers and transported by serum samples were taken from the hospital. These samples were allowed to dissolve storing for one day +4 c.

Experiment

Serum samples in the laboratory of the institute after arriving sufficient flowability were investigated.

Each patient serum samples were sterile quartz tubs. And quartz sample chamber was placed in the tub. The light detector to the focal point of attention was paid to come.

Sample chamber on the cover to see the light kapatıldı.zolix basic scan program was set to take the necessary measures.

Grid 1 is set to 300-500 nm, interval is set to 1 nm, grid 2 is set to 500-800 nm, interval 1nm and grid 3 is set to 800-1100 nm interval 1 is set to so different wavelengths are set to different grids.

Light source power is set to 6,45 A optical chooper is set to 233 hz.

All these adjustments came after the system is ready wavelenght scan was initiated.

5 female and 3 male patients during the study group and the control group were 1 male and 1 female.

CHAPTER 4

RESULTS

The results of the analysis of blood samples taken from the patients and the control group are shown below

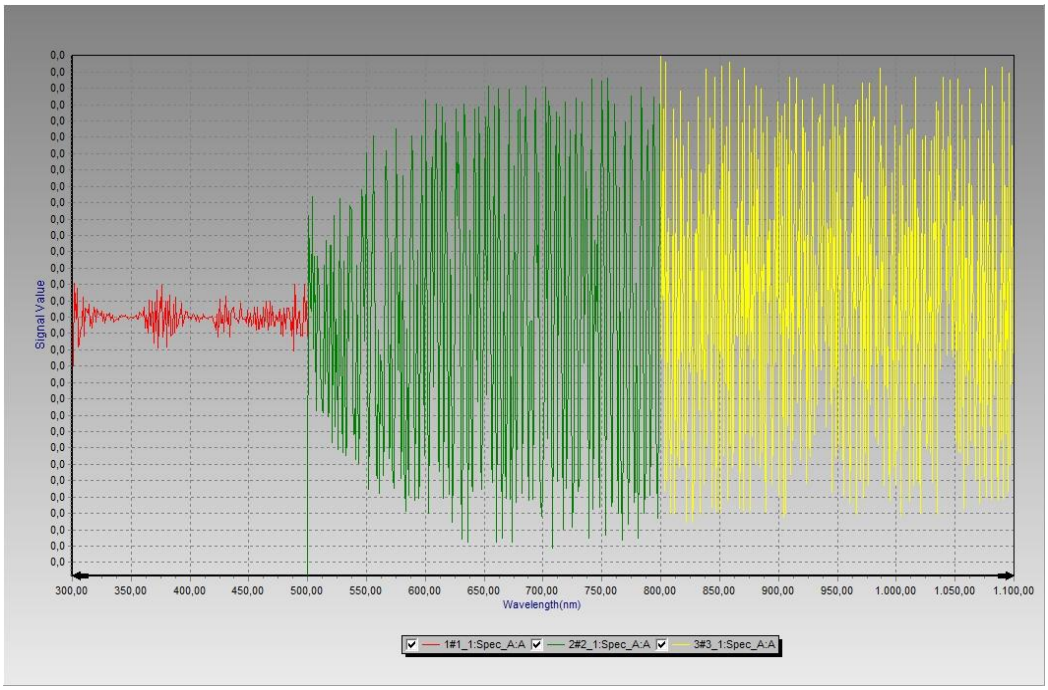

Figure 4.1 First patient's measurement results

Age-sex	78-female
Biochemistry results	0,100ng/ml
Patient history	Emergency patients from
Measurement time	0^{th} hour

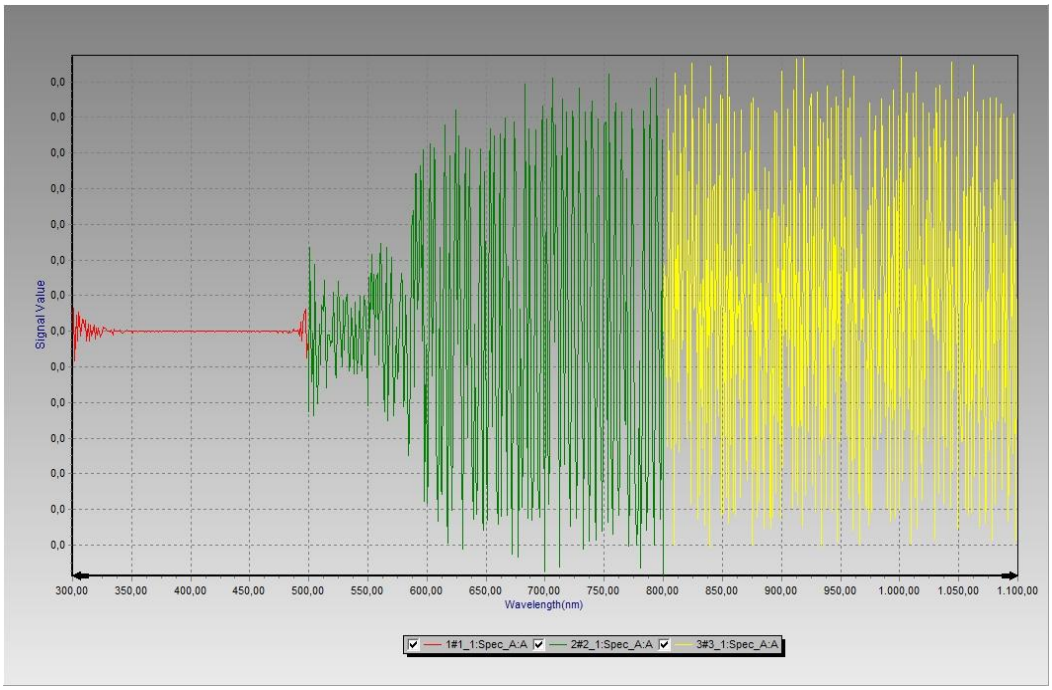

Figure 4.2 Second patient's measurement results

Age-sex	82-female
Biochemistry results	0,136ng/ml
Patient history	Emergency patients from
Measurement time	0^{th} hour

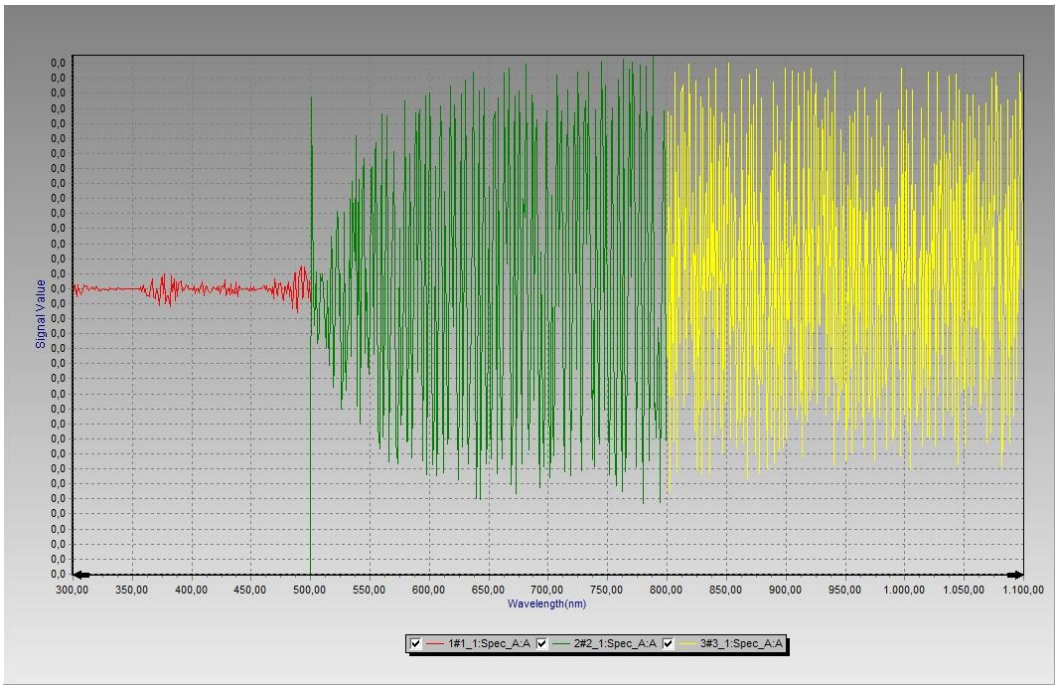

Figure 4.3 Third patient's measurement results

Age-sex	61-female
Biochemistry results	-
Patient history	Emergency patients from
Measurement time	0^{th} hour

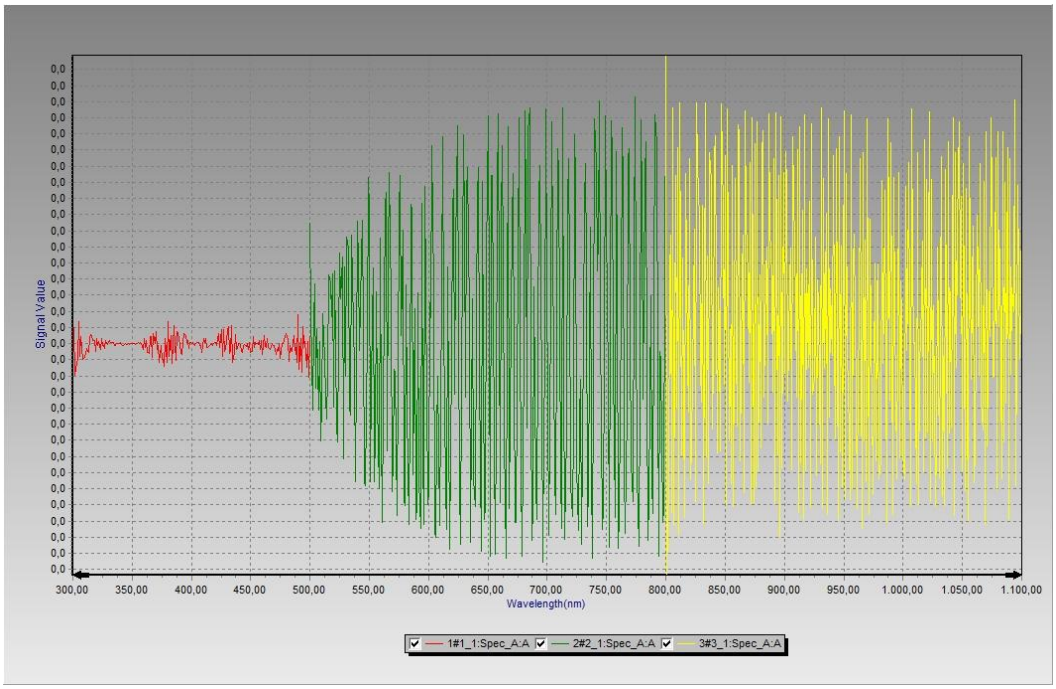

Figure 4.4 Fourth patient's measurement results

Age-sex	73-male
Biochemistry results	-
Patient history	Emergency patients from
Measurement time	0^{th} hour

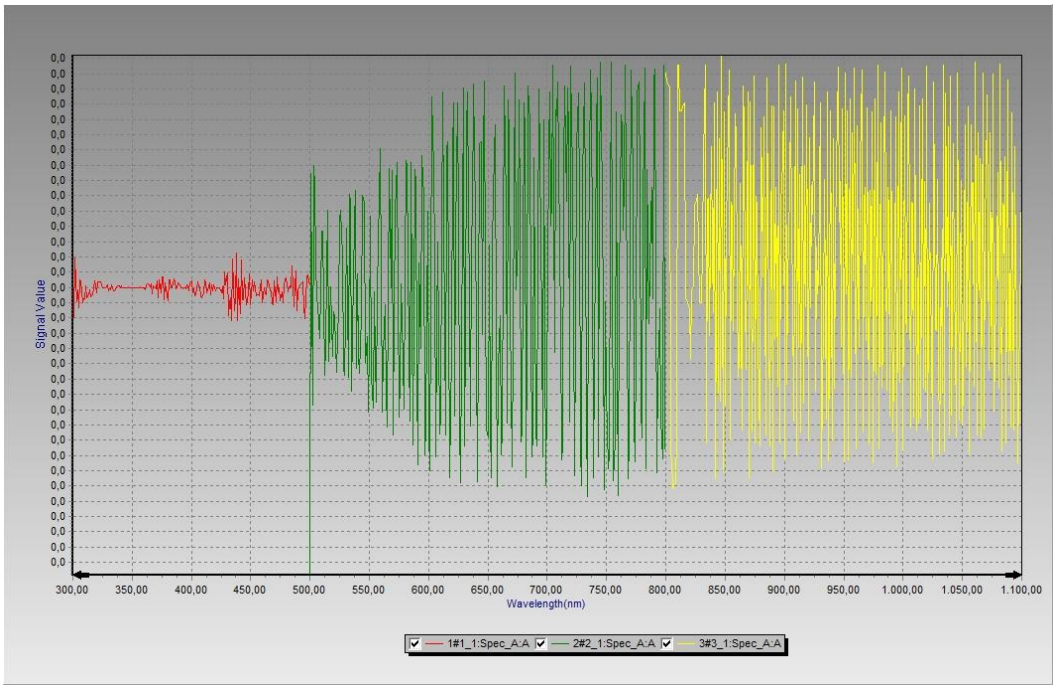

Figure 4.5 Fifth patient's measurement results

Age-sex	78-female
Biochemistry results	0,159 ng/ml
Patient history	inpatient
Measurement time	4^{th} day

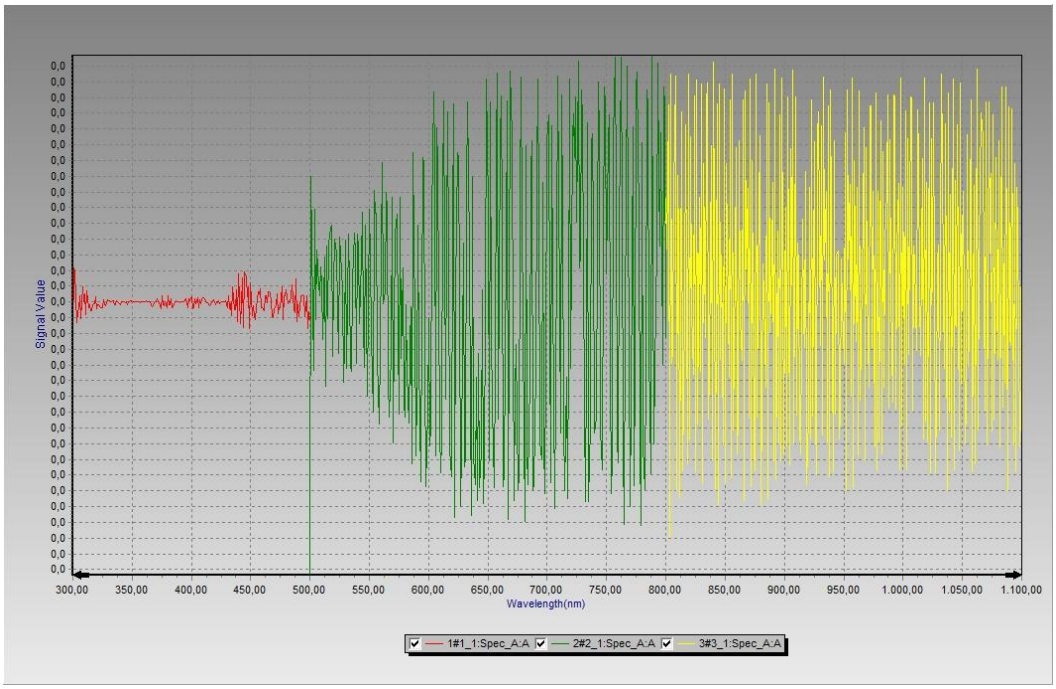

Figure 4.6 Sixth patient's measurement results

Age-sex	61-male
Biochemistry results	1.26ng/ml
Patient history	inpatient
Measurement time	-

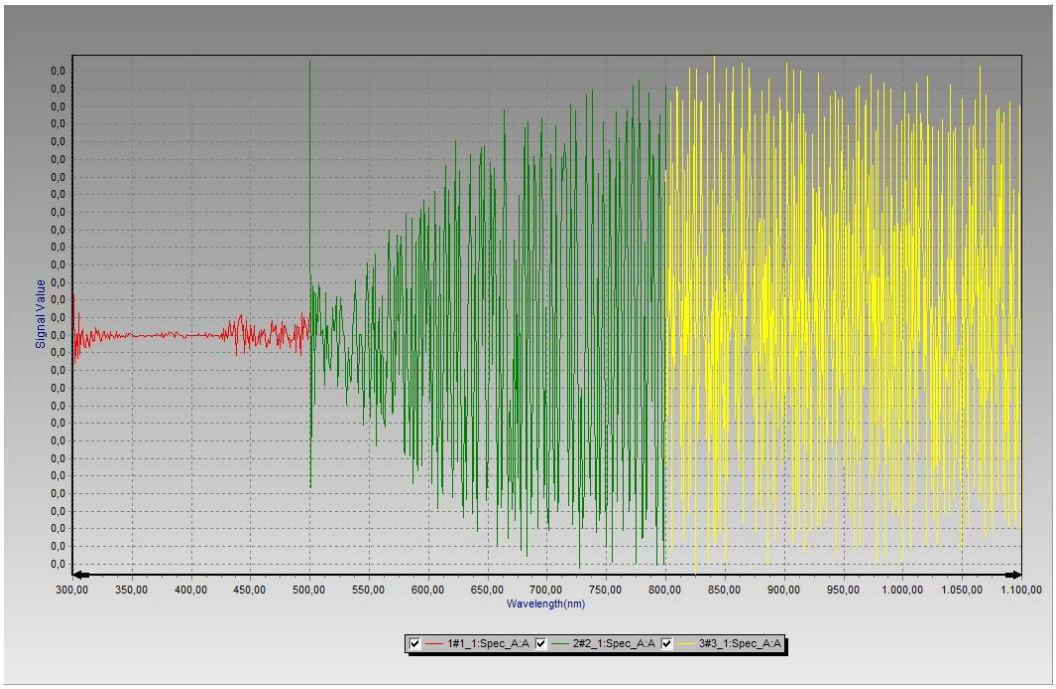

Figure 4.7 Seventh patient's measurement results

Age-sex	82-male
Biochemistry results	0,104 ng/ml
Patient history	Emergency patients from
Measurement time	0^{th} hour

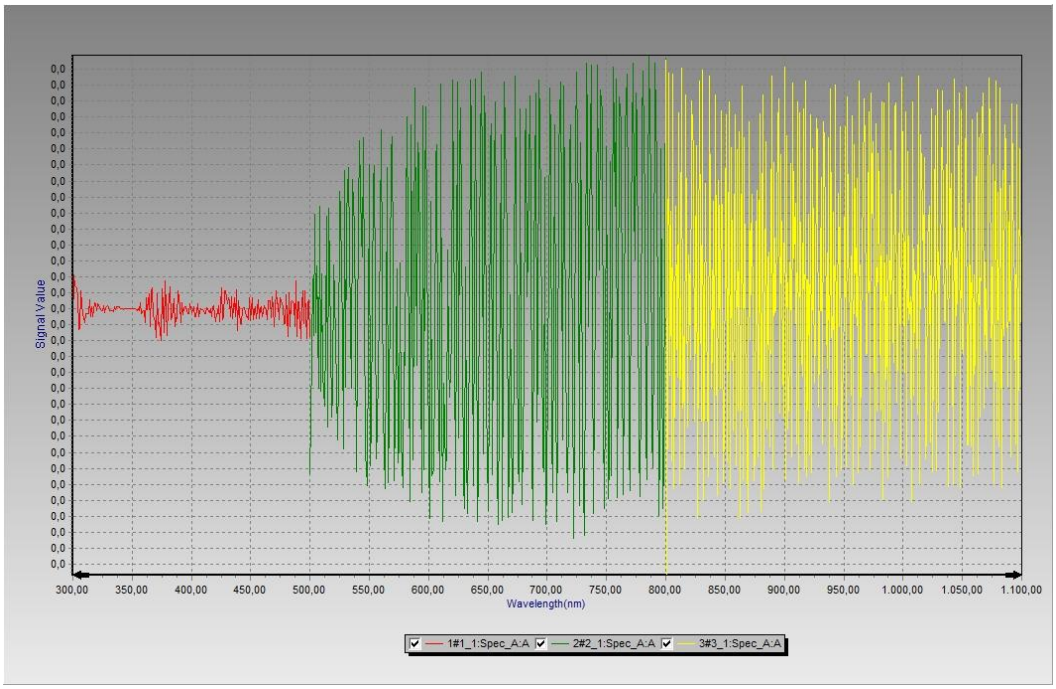

Figure 4.8 Eight patient's measurement results

Age-sex	63-female
Biochemistry results	0,531ng/ml
Patient history	inpatient
Measurement time	1st day

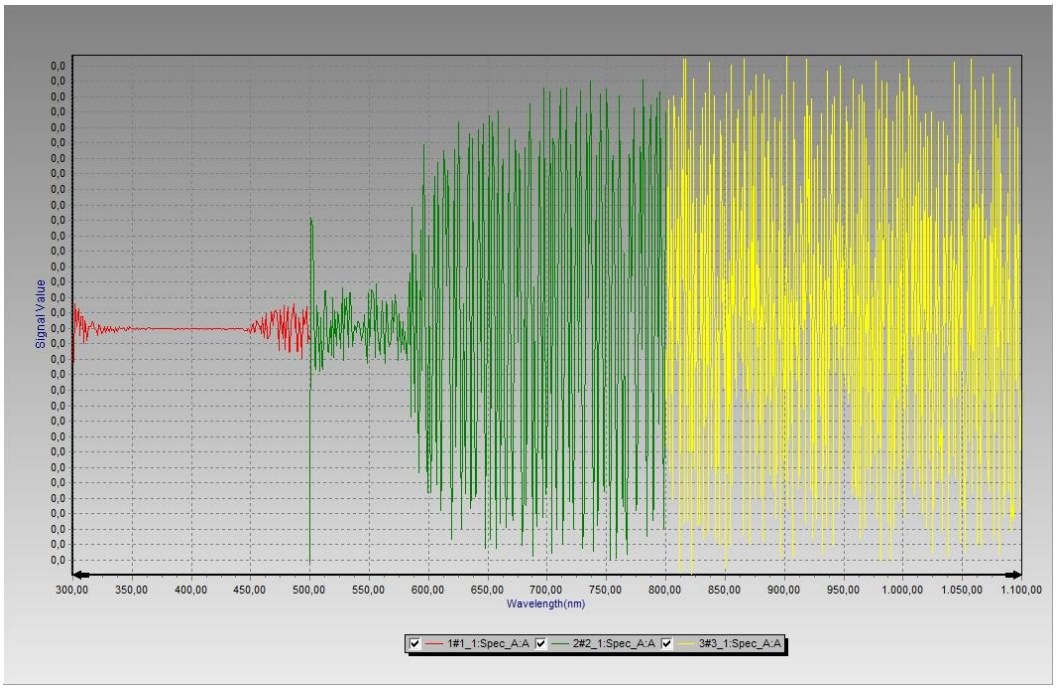

Figure 4.9 Ninth patient's measurement results

Age-sex	72-female
Biochemistry results	0.208 ng/ml
Patient history	inpatient
Measurement time	0^{th} hour

4.1 CONTROL GRUP (Non Troponin-T)

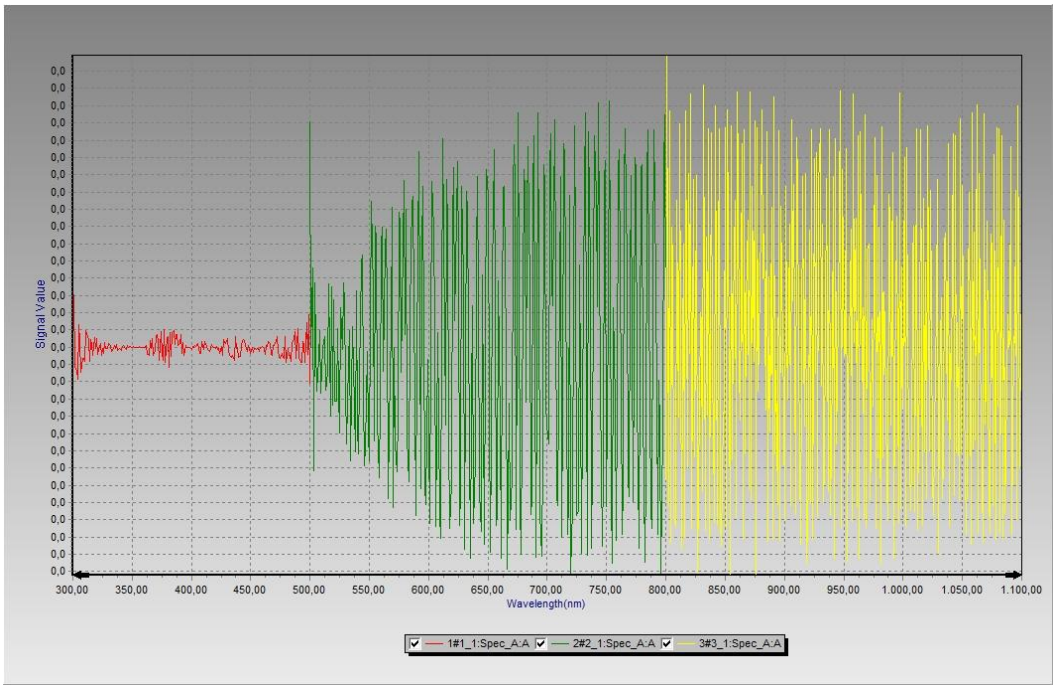

Figure 4.10 First group's measurement results

Age-sex	29-female

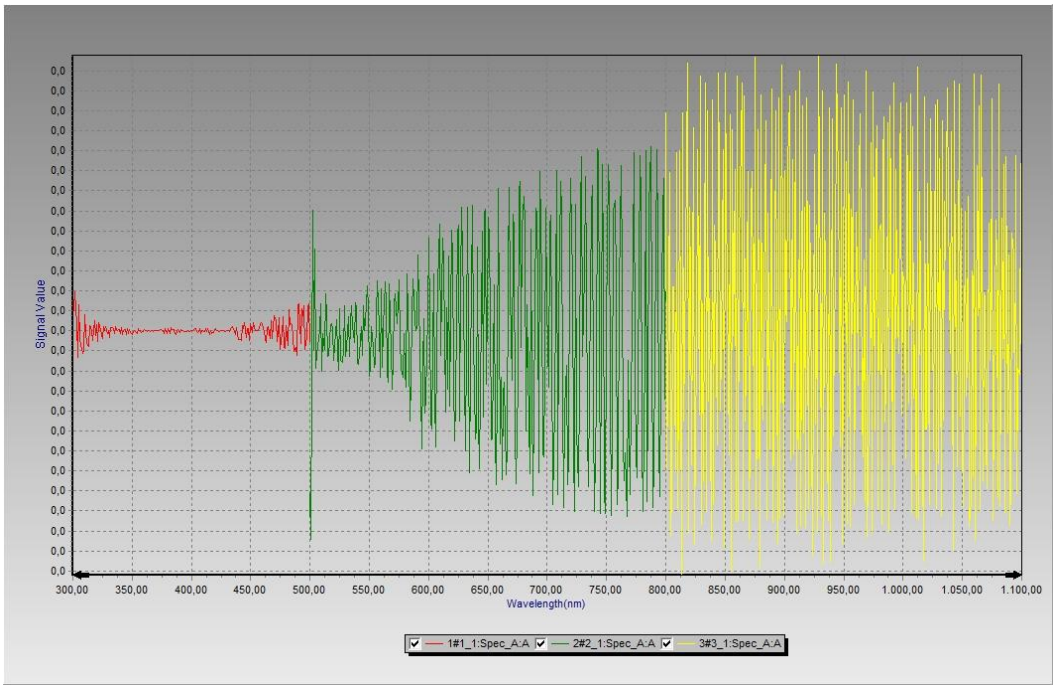

Figure 4.11 Second group's measurement results

Age-sex	27-male

DISCUSSION

It is observed that, measurement in the **UV region** between 300-400 nm wavelengths, absorbance of blood serum samples of the control and patient groups was very high (Figure 4.12)

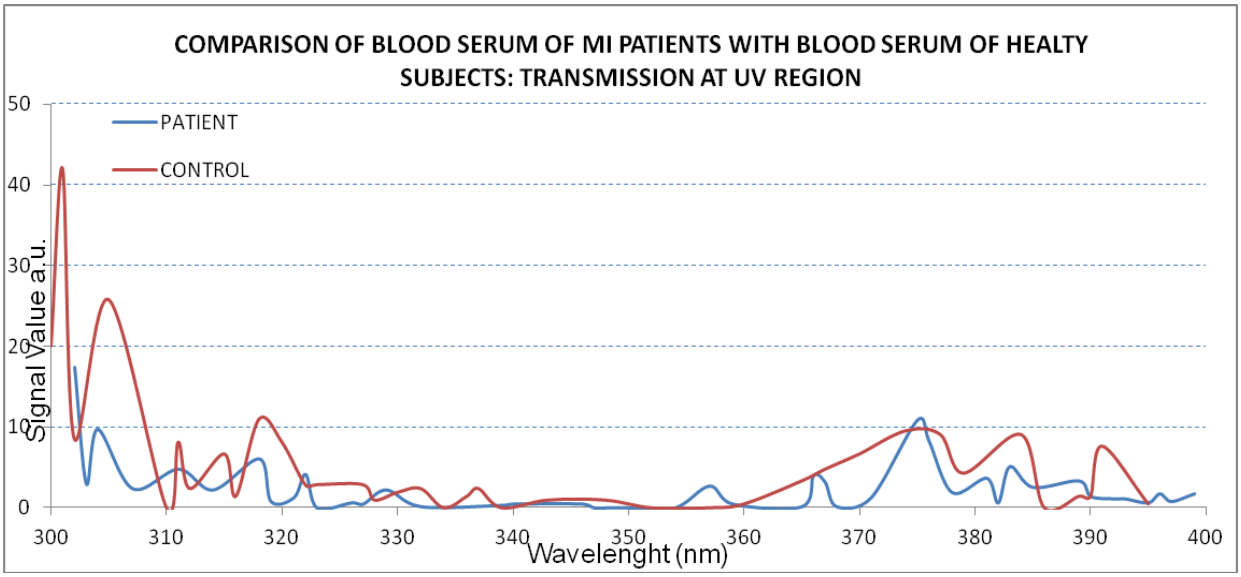

Figure 4.12. Comparison of bloodserum of MI patient with blood serum of healty subjects: transmission at UV region

In the VIS region: From 400 nm to 500 nm high absorbance regime progression has been observed as observed in the UV region. From 500 nm up to 700 nm, transmittance has been gradually increased from 50 a.u. to 150 a.u. (Figure 4.13)

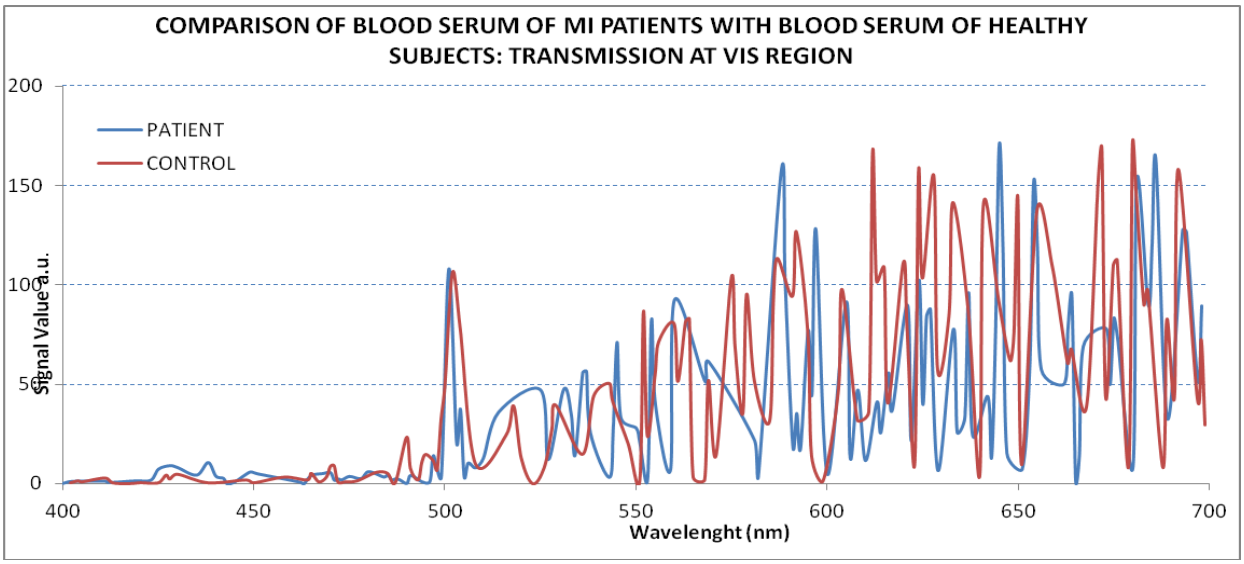

Figure 4.13 Comparison of bloodserum of MI patient with blood serum of healty subjects: transmission at VIS region

In the NIR region: From 700 nm to 1100 nm, signal values flactuates between 100 a.u. and 200 a.u. Increase in transmission at the NIR region is consistent with the tissue optical characterstics. It is expected that protein absorption in the UV region is higher than that of NIR region because of the therapeutic window. Actually this findings supports that measurements have been conducted accurately.(Figure 4.14)

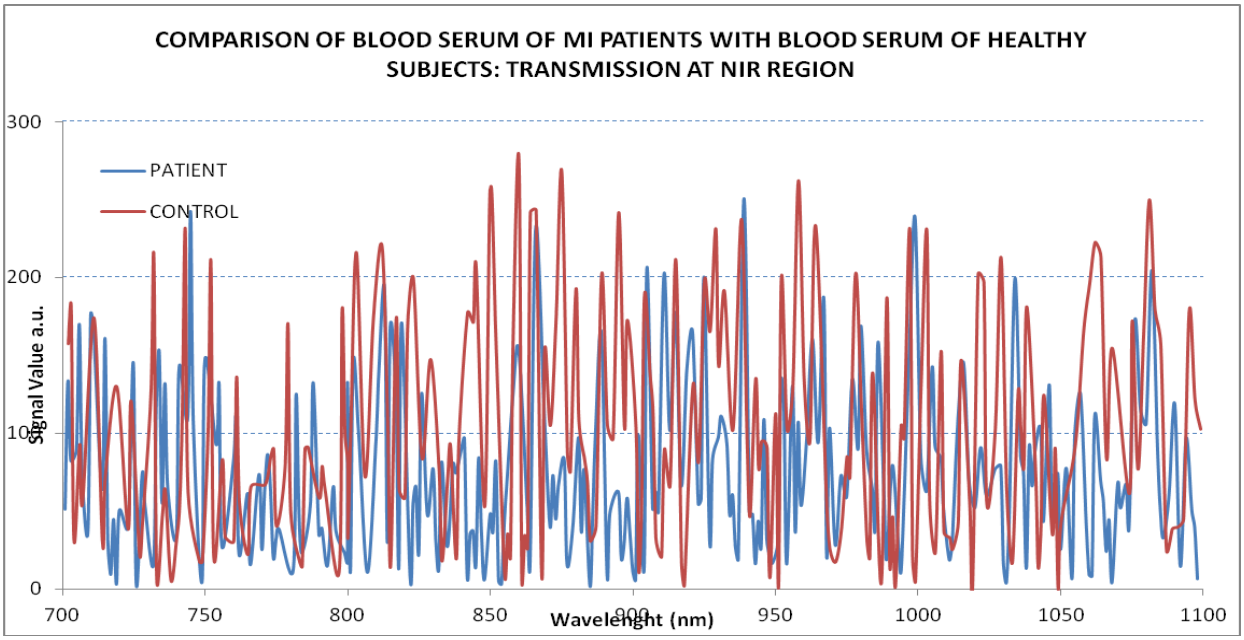

Figure 4.14 Comparison of bloodserum of MI patient with blood serum of healty subjects: transmission at NIR region

The differences in the UV region: 303 nm, 320 nm, 370 nm, 391 nm wavelengths absorbance of control group significantly higher than patient group.

The differences in the VIS region: For VIS region significant differences have been observed for wavelengths indicated at Table 4.1,

Table 4.1 Comparision wave length, A_C AND A_P

Wavelenght	Comparision	P value
514 nm	Ac>Ap	P<0,05
539 nm	Ap>Ac	P<0,05
559 nm	Ap>Ac	P<0,05
591 nm	Ap>Ac	P<0,05
613 nm	Ap>Ac	P<0,05
627 nm	Ap>Ac	P<0,05
634 nm	Ap>Ac	P<0,05
650 nm	Ap>Ac	P<0,05
686 nm	Ac>Ap	P<0,05

The differences in the NIR region: For NIR region significant differences have been observed for wavelengths indicated at Table 4.2.

Table 4.2 Comparision wave lengt, A_C AND A_P

Wavelenght	Comparision	P value
719	Ap>Ac	P<0,05
732	Ap>Ac	P<0,05
755	Ac>Ap	P<0,05
822-825	Ap>Ac	P<0,05
842-845	Ap>Ac	P<0,05
873-875	Ap>Ac	P<0,05
891-901	Ap>Ac	P<0,05
927-937	Ap>Ac	P<0,05
958	Ap>Ac	P<0,05
999	Ac>Ap	P<0,05
1003	Ap>Ac	P<0,05
1022	Ap>Ac	P<0,05
1038	Ap>Ac	P<0,05
1061-1066	Ap>Ac	P<0,05

CONCLUSIONS AND RECOMMENDATIONS

The values above show the possible wavelengths to determine troponin T. Upon the analysis conducted on these wavelengths optical devices can be developed without taking samples from the organs such as skin vein eye ear only by using beam.

For the future studies of other MI marker concentrations such as CK-MB, myoglobin should be correlated and their transmission and absorbance will be tried to observe.

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