J Appl Biotechnol Rep. 2023 March;10(1):934-942

Journal of
Applied Biotechnology Reports

doi 10.30491/JABR.2022.333178.1506

Original Article

Implementation of the Electrochemical Impedance Method for Measuring Troponin Enzyme in the Laboratory on a Chip

Sajedeh Jafarypour [1], Mohebali Rahdar [1]*, Gholamreza Farnoosh [2]

[1] Department of Industrial Engineering, Faculty of Engineering, Sistan and Baluchestan University, Sistan and Baluchestan, Iran
[2] Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Corresponding Author: Mohebali Rahdar, PhD, Assistant Professor, Department of Industrial Engineering, Faculty of Engineering, Sistan and Baluchestan University, Sistan and Baluchestan, Iran. Tel: +98-9153410725, E-mail: m.ali.rahdar@eng.usb.ac.ir

Received March 9, 2022; **Accepted** July 3, 2022; **Online Published** March 9, 2023

Abstract

Introduction: Troponin enzyme is a gold biomarker for detecting heart attacks. If it is possible to measure blood troponin levels at the onset of heart pain or symptoms of myocardial infarction, heart damage can be determined by measuring how much it changes. Diagnostic kits are available on the market to detect the presence of enzymes in the blood, but these kits do not check the extent of enzymatic changes. Actually, these kits only check for more than a certain amount of enzyme in the blood. Many experiments can be performed with the advent of lab-on-chips.

Materials and Methods: In the proposed method, the troponin enzyme is separated from the rest of the blood by the selected aptamer and then the concentration of the troponin enzyme in the sample is measured by the electrochemical impedance method and Arduino board and coding. The Arduino software was used for coding and simulation, and electrochemical spectroscopy was performed by simulating the behavior of an electrochemical sensor.

Results: According to the findings of the present study, the initial measurement by the device showed an error of 55%, which was reduced to 13.5% in the measurement by changing the measuring factors.

Conclusions: The manufactured device has the ability to receive the sample, separate it into essential and non-essential components, extract the required information from the sample and also analyze the obtained information. However, the electronic structural factors of the device such as resistance, etc. but must be changed in order to reach 95% reliability.

Keywords: Troponin Enzyme, Lab on Chips, Enzyme Level Measurement, Myocardial Infarction, Feasibility

Citation: Jafarypour S, Rahdar M, Farnoosh G. Implementation of the Electrochemical Impedance Method for Measuring Troponin Enzyme in the Laboratory on a Chip. J Appl Biotechnol Rep. 2023;10(1):934-42. doi:10.30491/JABR.2022.333178.1506

Introduction

Troponins are proteins found in muscle, skeletal, and heart fibers, that cause muscle contraction.[1] Normally, the level of troponin enzyme in the bloodstream is extremely low. However, after damage to the heart muscles the level of the troponin enzyme of the heart increases. After about 10 to 14 days this enzyme gradually decreases in order to return to the normal level, and it has the highest stability in cardiac markers of troponin enzyme.[2,3] Since there are different levels of the troponin enzyme in different bodies (blacks and whites, men and women, athletes and non-athletes, people who have had heart surgery, or have pre-existing heart disease), it is better to measure the troponin changes over time in order to be aware of the possibility of any heart diseases by examining its changes. In other words, an increase in troponin levels, predicts cardiac events[4] Regarding to the higher specificity of troponin I enzyme compared to CK-MB and even troponin T, troponin I enzyme is called a golden biomarker for investigating cardiac problems. Studies have shown that the sensitivity of the troponin enzyme is 100% in quantitative and 90% in qualitative assays. This issue is related to the amount of base enzyme in different bodies. In quality tests, this is the only enzyme examined in bodies. If the enzyme is higher than the amount specified in the test strip, the test result declares positive. However, if the person's body is potentially high in troponin, the test result will be positive again.[5] The lab-on-chip is a device that is formed from one or more laboratory operators on a chip.[6] It actually makes several laboratory operations available on just one single chip. The laboratory chip deals with the flow of very small volumes of liquids (nanoliter to picoliter) within micron channels that are applied by the single or multiple in-chip processes for chemical analysis.[7] In fact, each part of the chip has the same function as one part of the lab. So this technology is named "lab-on-chip".[8] One of the advantages of the lab-on-chip technology is the lower manufacturing costs in production, consuming less volume of fluids for analysis, and a shorter time needed for testing and massive conclusion. Several methods have been reported for a better detection of cTnI about the using of laboratory chips to measure the amount of troponin including the enzyme-

linked Immunosorbent assay method - Electrochemiescence, fluorescence, and colorimetric methods.

The aim of the present study was to provide a model for measuring troponin enzyme that is portable and implemented on silicon by study troponin test methods that can be performed in the laboratory. The measurement results must be very accurate and the device should be user friendly, and most importantly the result time must be as soon as possible in order to achieve the goal of building this laboratory on the chip. All this process is done in order to eventually be able to prevent a heart attack.

Materials and Methods

Programming and Designing a Measurement System

To measure the concentration of troponin enzyme in a blood sample, we must be able to separate the enzyme from the other components of the blood. There are different methods for separating blood components from each other. Using aptamer is the method used in this experiment. It works by reacting with the enzyme troponin in a blood sample to separate it from other blood components. The selected aptamer is in accordance with the sequence: 5′-CTAGTG GTCCTAAACATTTCACTTT-3′. The aptamer must first be coated on a substrate so that the troponin enzymes in the blood sample that are exposed to the substrate must react with the coated aptamers on the substrate to separate from the rest of the blood. In the next step, the amount of troponin enzyme isolated from the rest of the blood components must be measured, so that the amount of isolated enzyme is converted into measurable amounts such as light, scatter, electric current, etc. in order to be measurable. The method selected in this research is the electrochemical impedance measurement method and its operating method is that an electrochemical cell containing three conductive electrodes is exposed to alternating voltage and with a constant value when the output electronic current can be measured. Any factor in the electrochemical cell that changes the current flowing through the circuit causes a change in the resistance (impedance) of the electronic circuit.[9]

In order to be able to use the electrochemical impedance method to measure the concentration of troponin, the isolated troponin enzyme must be placed in the electrochemical cell to change the current flowing through the circuit. Nano-substrates are very suitable for coating by aptamers due to their high conductivity, so a carbon nanotube electrode for the substrate is a good option. 12 ng/ml-12×10^5 ng/ml with the lowest possible detection limit of 0.043 ng/ml Therefore, the use of the aforementioned aptamer on the carbon nanotube in the electrochemical method enables the measuring range of troponin enzyme between 12ng/ml-12×10^5 ng/ml with the lowest detection limit of 0.043 ng/ml. The results show a 95% confidence level.

Each electrochemical cell consists of at least two electrodes, one of which is the reference electrode, which is made of silver or silver chloride, but the other electrode, which is a working electrode, can be made of carbon nanotubes, which are very suitable substrates for reacting with aptamers. Carbon nanotubes can be used as a working electrode. The nanotubes are first functionalized by functional groups and chemical methods and then coated with aptamers until the working electrode is exposed to a blood sample. The troponin enzyme in the blood sample reacts with the aptamer and the other components are removed. Since the purity of the electrode affects its flow, we first place the carbon nanotube when it is coated with aptamers and not exposed to the enzyme troponin, and measure its output impedance when the electrode is exposed to the blood sample. When the electrode is exposed to the blood sample, it reacts with troponin enzyme and as a result the current passing through the electrode changes. As shown in a previous study,[10] a linear relationship between the logarithm of the enzyme troponin concentration and the output impedance is established when in several blood samples. If the concentration of troponin enzyme is known, the output impedance of the test is calculated and the line equation is determined. The selected board must be programmed to let us meet the desired results. Electrochemical sensor acts as the device input sensor and is the brain board of the device. This brain follows the rules of the program written on the microchip. The program should be written as follows:

After turning on the device and adding analyte to the substrate, the device applies a specified voltage in a specified range and for a specified time according to the recorded repetition to the electrodes and finally records and analyzes their output. The electronic hardware of the laboratory device was simulated in proteus software version 8.1.13 and finally the impedance output was calculated. Electrochemical cell simulation in Proteus software can be done by using capacitors, resistors and diodes to show the function of an electrode and finally electrochemical cell, which shows a behavior similar to electrochemical cell.[11,12]

Results

An overview of the laboratory on the chip is shown in Figure 1, which shows how to obtain a sample, the operation of the electrodes and the device schematically. The blood sample is placed on the test bed, which is the electrochemical cell. Aptamers are located inside this electrochemical cell that reacts with the enzyme troponin in the blood and separates them from the blood and the enzymes adhere to the surface of the nanotube. This test bed enters the device to apply voltage to the electrochemical cell.

Figure 2 shows the instruments needed in the laboratory on a chip that are used to measure the concentration of the enzyme troponin by changing the output impedance of the electrodes. In this laboratory, the microchip and Arduino board

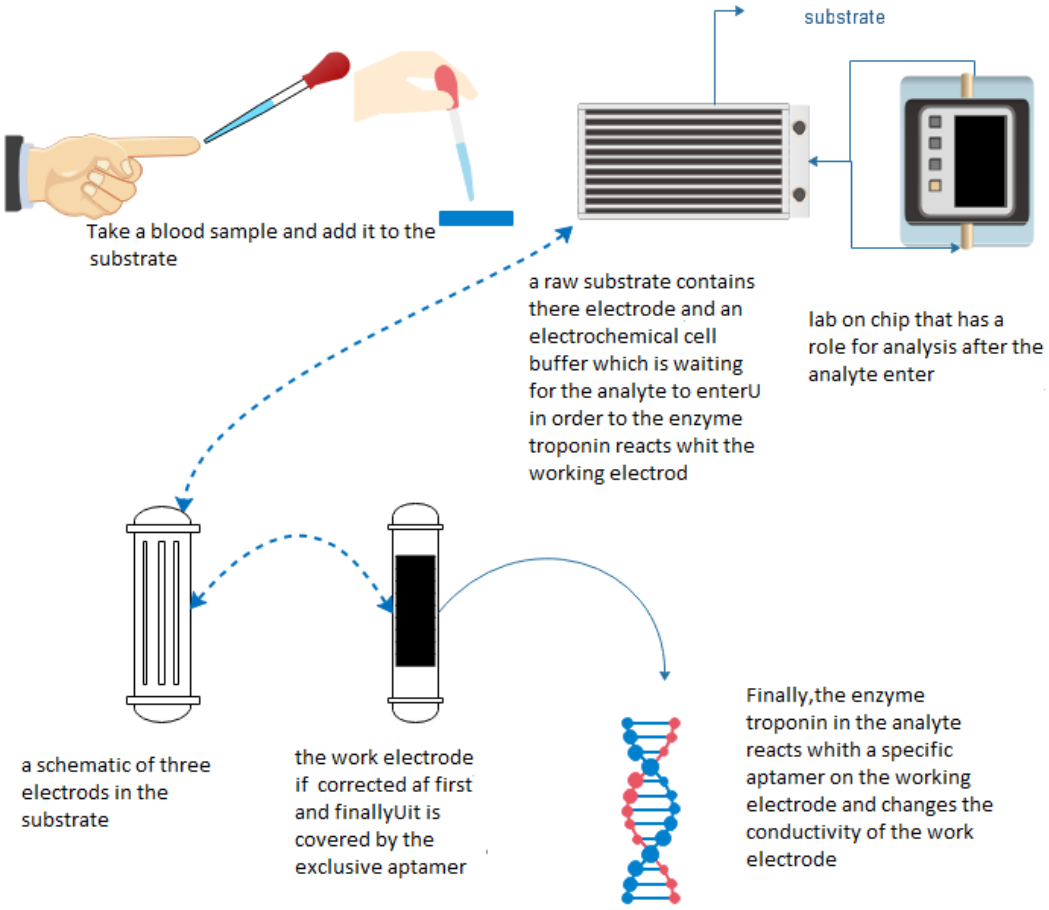

Figure 1. Schematic of Working Electrode Function.

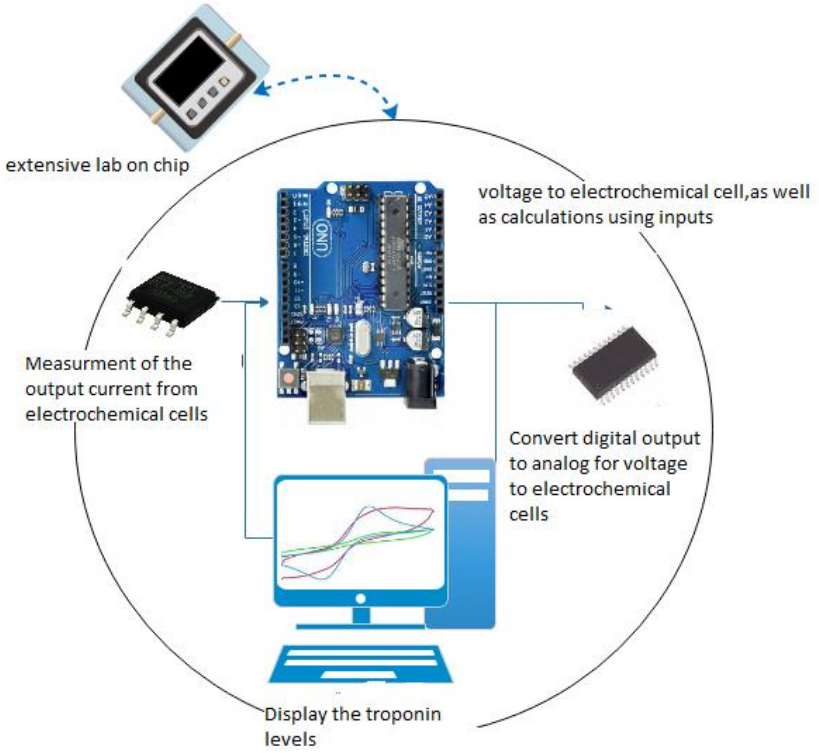

Figure 2. Extensive Schematic of Lab-on-Chip.

are used to change the voltage from direct voltage of battery to alternating voltage and apply it to electrochemical cells. By measuring the output current from the circuit and performing programmed calculations, the output impedance is first calculated and then based on the calculated impedance, the amount of troponin enzyme present in the sample is calculated. The last step is to display the concentration of the troponin enzyme.

Validation and Accuracy of Measurement System

Given that the accuracy and precision of the number obtained from the experiment is very important for us, so, the accuracy of the results must be ensured. Therefore, lab-on-chip experiments should be performed in the presence of analytes with different concentrations of troponin enzyme, without the presence and with the presence of troponin enzyme, with or without the presence of irritants such as the enzyme troponin type c, troponin type T, myoglobin, and etc, to understand the accuracy of the system by comparing the resulting numbers and real numbers. In addition, its repeatability should be measured which shows the accuracy of the system by repeating the experiment many times. The lab on chip device is built according to the above and its performance is tested.

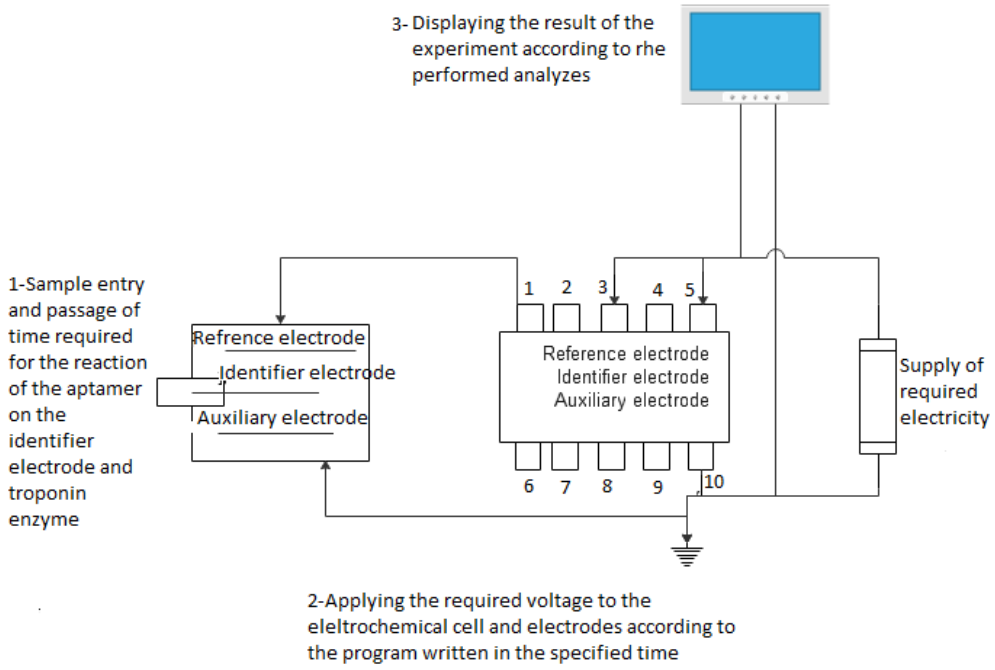

Figure 3. The Schematic of Device Making.

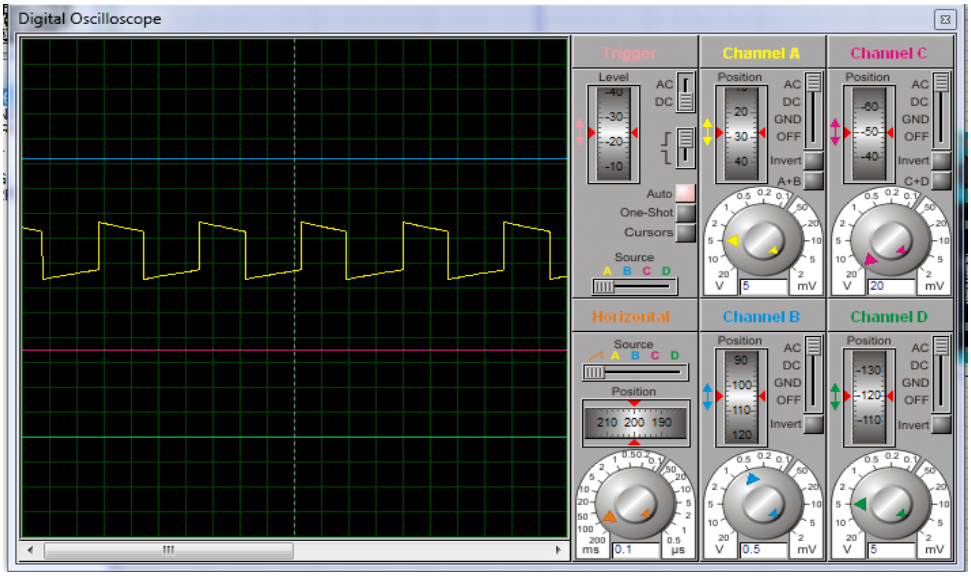

Figure 4. Oscilloscope Output.

The schematic of making the device in general is shown in Figure 3, in which the electrodes are placed on the test bed and the test bed is placed inside the device. By applying voltage to the electrochemical cell, the current output is measured by the electronic board and calculated by the program. The recorded troponin concentration is calculated and displayed on the display.

As shown in Figure 4, by an oscilloscope in which our input voltage is converted from direct voltage to square or quasi-sine voltage, in the selected method of this research AC voltage is required and because this measuring device is portable and it is mobile, we do not have access to AC voltage. However, we can use a direct voltage battery and programming the Arduino board to convert the voltage output from the Arduino board to AC voltage according to the desired voltage and frequency.

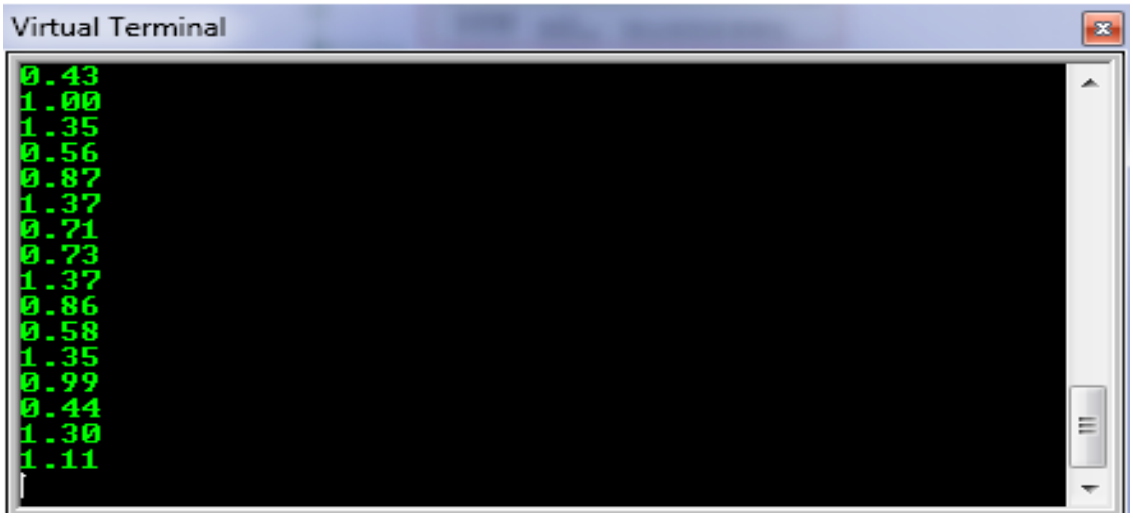

Figure 5. Impedance Calculation Cycle.

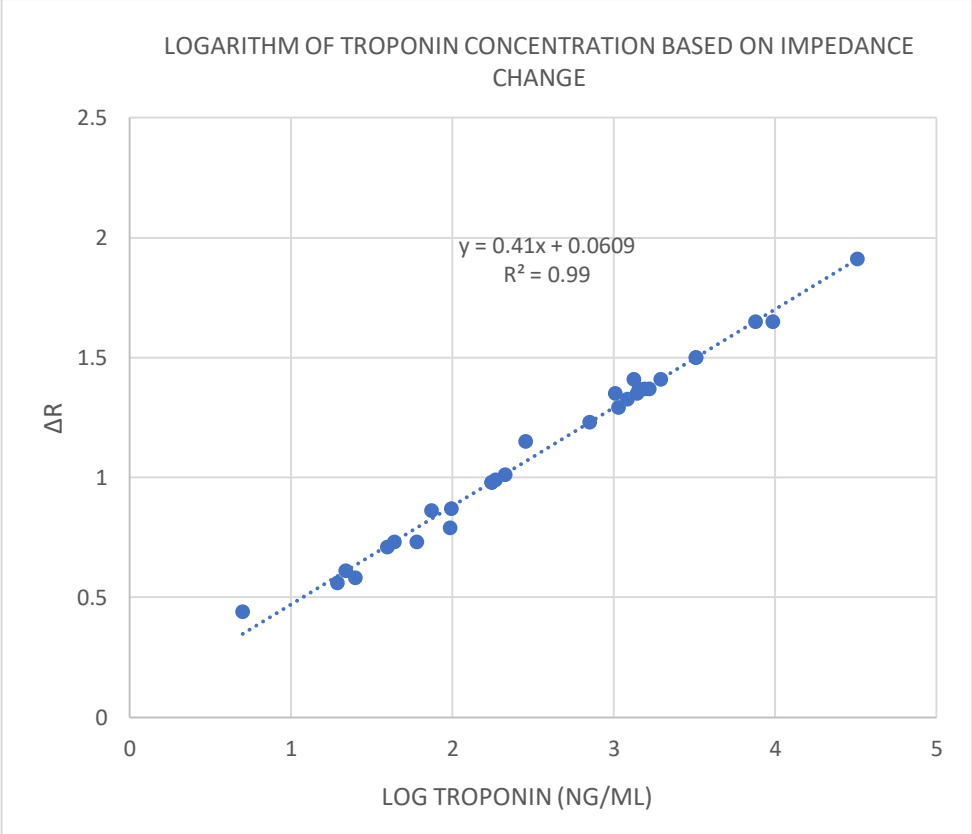

Figure 6. Delta Impedance Diagram in Terms of Troponin Concentration Logarithm.

Table 1. Calculation of Troponin Concentration Logarithm

ΔR	LOG Troponin (ng/ml)
1.01	2.32792
1.35	3.0125
0.56	1.286719512
0.87	1.995756
1.37	3.193512
0.71	1.597853001
0.73	1.643414
1.37	3.219512
0.86	1.87211123
0.58	1.40023
1.35	3.146341463
0.99	2.268292683
0.44	0.7007
1.29	3.0321439
1.15	2.455697
1.91	4.512195122
0.61	1.341463415
1.5	3.512195122
1.65	3.9858
1.41	3.125487
0.98	2.243902439
0.79	1.987867
1.23	2.853658537
1.325	3.085365854
0.73	1.7823342
1.37	3.157987
1.5	3.512195122
1.65	3.87804878
1.41	3.292682927
0.98	2.243902439

Table 2. Comparison of Actual Troponene Concentration Entering the System and the Measured Concentration

Troponin Concentration (ng /ml) According to Calculations	Actual Troponin Concentration (ng /ml)
212.77	234
1029.20	1043
19.35	31
99.03	132
1561.39	1535
39.61	62
44.00	72
1657.72	1660
74.49	81
25.13	36
1400.69	1410
185.48	195
5.02	18
1076.82	1090
285.56	296
32523.34	32540
21.95	32
3252.33	3270
9678.32	9660
1335.02	1365
175.35	150
97.24	120
713.93	790
1217.21	1240
60.58	92
1438.76	1489
3252.33	3264
7551.77	7594
1961.93	1998
175.35	202

Figure 5 shows the result of running a simulated program in Proteus, which shows that each concentration of troponin enzyme can be measured according to the program written for the Arduino impedance board. Also, the measured impedance behaves according to the Nyquist diagram.

In this research, the delta impedance diagram was drawn according to the logarithm of troponin concentration according to Figure 6 and its linear equation was calculated. Its linear equation is: $y = 0.41x + 0.060$ ($R^2 = 0.99$).

In the following, the logarithm of troponin concentration is calculated using electrochemical impedance and line equation, which is mentioned in Table 1.

This device was tested by samples with a certain concentration of troponin enzyme. The amount of impedance is in the line formula and the logarithm of the concentration of troponin is calculated. Finally, the concentration of troponin is calculated and compared with its real value, which compares the amount of real troponin. The troponin calculated by the device is listed in Table 2.

Discussion

In the model presented in this research, the operation of separating the troponin enzyme from the analyte is done by carbon nanotubes and specific aptamers, and then with the help of the cyclic voltameter method, using an electronic circuit containing a microcontroller and by applying voltage, the resulting current is measured. The microcontroller manages the final current and calculates the troponin concentration. This circuit is a constant voltage electronic circuit that is supposed to generate an alternating voltage to run the cyclic voltammeter method. Previous studies have used qualitative diagnostic kits that measure regardless of the basal level of troponin in the body. In laboratories, methods such as cyclic voltammeter and chromatography have been used. However, this method combines the cyclic voltammeter method to measure the enzyme troponin with the combination of chromatography and carbon nanotubes to separate the enzyme in the analyte and calculate its value on a microcontroller. In this proposed method, by combining the mentioned methods, we presented a model that can calculate the enzyme concentration in a short time, and this device can be used on a mobile basis and is easy to use and does not require specialized training.

As rapid diagnosis is extremely important in the treatment of myocardial infarction, diagnosing it in the golden time after a myocardial infarction and taking the necessary measures to treat it, can prevent serious damage to the heart muscle. This is due to the fact that the symptoms of a heart attack are different and are felt differently in each person and people may actually confuse it with other illnesses such as stomach pain. It is possible to perform a troponin test nowadays either in a laboratory or qualitatively, with only a yes/no answer. However, it only indicates whether the enzyme in the blood is more or less than the amount specified in the kit, which is not an acceptable reference method for confirming heart damage. Due to the fact that the amount of enzyme base in each person's body is different, and the test

can only be done in a laboratory, so it is not practical to use this method in ambulances and homes. After going to the hospital and taking a blood sample in a well-equipped laboratory, tests are performed, which is a waste of time. As a result, a device is needed that can be portable to be used in homes and ambulances or even emergencies, and the test result must be announce at high speed so that it can be effective in early detection of heart damage. On the other hand, troponin enzyme is a type of enzyme that is found exclusively in the blood following heart damage.

The electrochemical biosensors designed to diagnose acute myocardial infarction, Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) techniques have been used to measure cardiac troponin I (cTnI).[13]

Conclusion

The need for a prognostic tool is felt for the risk of heart attack due to the high prevalence of deaths caused by myocardial infarction. So far, different sensors and tools have been used to measure the amount of troponin in the blood, and different methods have been used to measure the enzyme troponin. In the present study, lab-on-chips, troponin enzyme, troponin assay methods, and research of predecessors in this field have been introduced. In the following, using electrochemical methods, a model for making a new lab-on-chip has been presented, and the important points that should be considered for presenting the model of this laboratory device are as follows: A device that has the ability to receive samples, separate samples into necessary and unnecessary components, extract the required information from the sample, analysis the obtained information, and provide the final report to the user.

Table 3 shows the comparison between the use of different identifiers in the electrochemical method.

Table 4 compares the test methods by different diagnostics and different measurement methods, which indicate the measurement range of each method and their detection limit.

Table 3. Comparison of Limits of Different Detectors Detection in the Electrochemical Method

The Study Method	Research Fellow	Used Identifier	Linear Range	Recognition Limit
Electrochemical sensor	11	BSA/anti-cTnI/APTES/ZrO_2/ITO	0.1–100 ng/ml	0.036 ng/ml
Electrochemical sensor	12	AnticTnI/didodecyldimethylammoniumbromide/AuNPel	32 0.1 ng/ml	0.1ng/ml
Electrochemical sensor	13	Anti-cTnI/PrGO/GCE	0.1–10 ng/ml	0.07 ng/ml
Electrochemical sensor	10	Aptamer/Rgo/MWCNT/IL/AuNPs	12-12×10^5 ng/ml	0.043 ng/ml

Table 4. Comparing the Performance of Different Methods

The Measurement Method	Identifier	Used Materials	Recognition Limit	The Measurement Range	Reference
Electrochemistry	Aptamer	Au-Ag Alloy Nus	0.045 pg/ml	0.1-200	14
Electrochemical	Aptamer	Array of Au nanodumbbells	8.0 pg/ml	0.05-500 ng/ml	15
Flourescence	Antibody	HRP-Au NCs	0.03	0.1-100	16
ELISA	Antibody	Horseradish peroxidase	-	0.0312–1.0	17
Fluorescence immunoassay	Antibody	(FMGC)-based sandwich immunoassay	0.1 µg/L	0.1-100	18
Electrochemical	Aptamer	Array of Au nanodumbbells	0.8 pg/ml	0.05–500 ng/ml	19
Electrical detection	Antibody	Monolithic graphene sheets	0.0001ng/ml	0.0001-1 ng/ml	20
Electrochemical	Antibody	Gold Nanoparticle- ITO Electrode	0.5 ng/ml	1-100 ng/ml	21
Electrogenerated chemiluminescence	Aptamer	Gold electrode	0.4 pg/ml	1-300 pg/ml	22
Surface plasmon resonance	Antibody	Gold film	0.68 ng/L	0-160 µg/l	23
Fluorescence	Antibody	Fluorescent labels with 532 nm and 650 nm lasers	-	-	24
Electrochemical	Aptamer	Rgo/MWCNT/IL/AuNPs	0.043 ng/ml	12-12×10^5 ng/ml	10
Optic biosensor	Antibody	Ellipsometry, atomic force microscopy	0.00012 ng/ml	0.001–8.0 ng/ml	25
Spectroscopy	Aptamer	Rapid gold Nanoparticle-quantum dot plexcitonic	0.3 fM	0.4-2500 fM	26
Electrochemical	Aptamer	pRNA-3WJ/Au (AuNS)	-	-	27
Fluorescence based on surface plasmon	Antibody	SPCE fluorescence chips from thin gold (2 nm) to silver (50 nm)	21.2 ag/mL	21.2 ag/ml (atto-g/ml)	28
Electrochemical	Antibody	Pulse voltammetry	0.01 ng/ml	0.01-100 ng/ml	29
Electrochemical	Antibody	ion-sensitive field-effect transistor	0.3 pg/ml	1-1000 pg/ml	30
Electrochemical	Antibody	Dry chemistry-based sample-to-answer, Ultrasensitive closed bipolar electrode-ECL (CBP-ECL)	0.4416 pg/ml	0.001-100 pg/ml	31
Magnetic nanotags	Antibody	Functionalized Magnetic Nanotags	0.08 ng/ml	-	32

Finally, the device is made by electrochemical method with the method presented in this research. The initial measurement by the device showed a 55% error, which was decreased to 13.5% error by calibrating the device and changing the measuring factors of the device. The device must reach 95% reliability by changing the electronic structural factors of the device, such as resistance, etc. The behavior of the simulated model was the same in the presence of disturbing factors and the absence of disturbing factors, which concludes that this method has the necessary

accuracy in the isolation of troponin enzyme.

Authors' Contributions

Study concept and design by MR and GH; Analysis and interpretation of data by MR and SJ; Drafting of the manuscript by SJ; Critical revision of the manuscript for important intellectual content by GH and MR; Statistical analysis by SJ and MR.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

References

1. Shamsi J, Yaghmaei P, Seyed MM, Haeri RS. Investigation of the level of serum troponin i and comparing it with serum ck-mb in diagnosis of myocardial infarction. J Anim Biol. 2015;7(4):19-25.
2. Voleti S, Olivieri L, Hamann K, Gordish-Dressman H, Spurney C. Troponin I levels correlate with cardiac MR LGE and native T1 values in Duchenne muscular dystrophy cardiomyopathy and identify early disease progression. Pediatr Cardiol. 2020;41:1173-9. doi:10.1007/s00246-020-02372-5
3. Januzzi JL, Lewandrowski K, MacGillivray TE, Newell JB, Kathiresan S, Servoss SJ, et al. A comparison of cardiac troponin T and creatine kinase-MB for patient evaluation after cardiac surgery. J Am Coll Cardiol. 2002;39(9):1518-23. doi:10.1016/j.cca.2018.10.040
4. Shahsavari G, Anbari K, Dehkourdi N, Jalalvand M. A comparative study of rapid test and routine methods for the measurement of cardiac markers in patients with suspected acute myocardial infarction. Yafte. 2014;16(1):5-13.
5. Mehrabi A, Salesi M, Pasavand P. Comparison of the effect of the exercise time (morning or evening) and the amount of Troponin T in men with cardiovascular diseases. RJMS. 2015; 22(134):107-14.
6. Ahammad AS, Choi YH, Koh K, Kim JH, Lee JJ, Lee M. Electrochemical detection of cardiac biomarker troponin I at gold nanoparticle-modified ITO electrode by using open circuit potential. Int J Electrochem Sci. 2011;6(6):1906-16.
7. Haddad R. Introducing microchip technology with application in crime detection. karagah. 2019;12(48):142-66.
8. Whitesides GM. The origins and the future of microfluidics. Nature. 2006;442(7101):368-73. doi:10.1038/nature05058
9. Zhan T, Su Y, Lai W, Chen Z, Zhang C. A dry chemistry-based ultrasensitive electrochemiluminescence immunosensor for sample-to-answer detection of Cardiac Troponin I. Biosens Bioelectron. 2022;214:114494. doi:10.1016/j.bios.2022.114494
10. Khosravi Mashizi F. Electrochemical troponin sensor for detecting acute myocardial infarction. Master's Thesis, University of Valiasr Rafsanjan. 2021. pp. 54.
11. Anoop AE, Mohan NM, Guruvayurappan K. Simulation of a multi-strip blood glucometer. TENCON 2014-2014 IEEE Region 10 Conference, IEEE. 2014. pp. 1-4. doi:10.1109/TENCON.2014.7022473
12. Wang Y, Liu T, Yang M, Wu C, Zhang W, Chu Z, et al. A handheld testing device for the fast and ultrasensitive recognition of cardiac troponin I via an ion-sensitive field-effect transistor. Biosens Bioelectron. 2021;193:113554. doi:10.1016/j.bios.2021.113554
13. Shumkov AA, Suprun EV, Shatinina SZ, Lisitsa AV, Shumyantseva VV, Archakov AI. Gold and silver nanoparticles for electrochemical detection of cardiac troponin I based on stripping voltammetry. BioNanoScience. 2013;3:216-22. doi:10.1007/s12668-013-0090-9
14. Wang S, Esfahani M, Gurkan UA, Inci F, Kuritzkes DR, Demirci U. Efficient on-chip isolation of HIV subtypes. Lab on a Chip. 2012;12(8):1508-15. doi:10.1039/C2LC20706K
15. Lee T, Lee Y, Park SY, Hong K, Kim Y, Park C, et al. Fabrication of electrochemical biosensor composed of multi-functional DNA structure/Au nanospike on micro-gap/PCB system for detecting troponin I in human serum. Colloids Surf B. 2019;175:343-50. doi:10.1016/j.colsurfb.2018.11.078
16. Wen F, Dong Y, Feng L, Wang S, Zhang S, Zhang X. Horseradish peroxidase functionalized fluorescent gold nanoclusters for hydrogen peroxide sensing. Anal Chem. 2011;83(4):1193-6. doi:10.1021/ac1031447
17. Sun D, Luo Z, Lu J, Zhang S, Che T, Chen Z, et al. Electrochemical dual-aptamer-based biosensor for nonenzymatic detection of cardiac troponin I by nanohybrid electrocatalysts labeling combined with DNA nanotetrahedron structure. Biosens Bioelectron. 2019;134:49-56. doi:10.1016/j.bios.2019.03.049
18. Song SY, Han YD, Kim K, Yang SS, Yoon HC. A fluoro-microbead guiding chip for simple and quantifiable immunoassay of cardiac troponin I (cTnI). Biosens Bioelectron. 2011;26(9):3818-24. doi:10.1016/j.bios.2011.02.036
19. Fathil MF, Arshad MM, Gopinath SC, Hashim U, Adzhri R, Ayub RM, et al. Diagnostics on acute myocardial infarction: Cardiac troponin biomarkers. Biosens Bioelectron. 2015;70:209-20. doi:10.1016/j.bios.2015.03.037
20. Tuteja SK, Bhalla V, Deep A, Paul AK, Suri CR. Graphene-gated biochip for the detection of cardiac marker Troponin I. Anal Chim Acta. 2014;809:148-54. doi:10.1016/j.aca.2013.11.047
21. Ahammad AS, Choi YH, Koh K, Kim JH, Lee JJ, Lee M. Electrochemical detection of cardiac biomarker troponin I at gold nanoparticle-modified ITO electrode by using open circuit potential. Int J Electrochem Sci. 2011;6(6):1906-16.
22. Shan M, Li M, Qiu X, Qi H, Gao Q, Zhang C. Sensitive electrogenerated chemiluminescence peptide-based biosensor for the determination of troponin I with gold nanoparticles amplification. Gold Bull. 2014;47:57-64. doi:10.1007/s13404-013-0113-x
23. Kwon YC, Kim MG, Kim EM, Shin YB, Lee SK, Lee SD, et al. Development of a surface plasmon resonance-based immunosensor for the rapid detection of cardiac troponin I. Biotechnol Lett. 2011;33:921-7. doi:10.1007/s10529-010-0509-0
24. Huttunen A, Aikio S, Kurkinen M, Mäkinen JT, Mitikka R, Kivimäki L, et al. Portable low-cost fluorescence reader for LFA measurements. IEEE Sens J. 2020;20(17):10275-82. doi:10.1109/JSEN.2020.2992894
25. Çimen D, Bereli N, Günaydın S, Denizli A. Detection of cardiac troponin-I by optic biosensors with immobilized anti-cardiac troponin-I monoclonal antibody. Talanta. 2020;219:121259. doi:10.1016/j.talanta.2020.121259
26. Rezaei Z, Ranjbar B. Ultra-Sensitive, Rapid Gold Nano Rod-Quantum Dot (RQ) Plexcitonic Self-Assembled Aptamer-based Nanobiosensor for the Detection of Human Cardiac Troponin I. Nanoscale. 2015;2(3):131-67.
27. Lee T, Yagati AK, Pi F, Sharma A, Choi JW, Guo P. Construction of RNA–quantum dot chimera for nanoscale resistive biomemory application. ACS Nano.

2015;9(7):6675-82. doi:10.1021/acsnano.5b03269
28. Tran VT, Ju H. Fluorescence Based on Surface Plasmon Coupled Emission for Ultrahigh Sensitivity Immunoassay of Cardiac Troponin I. Biomedicines. 2021;9(5):448. doi:10.3390/biomedicines9050448
29. Gholami MD, O'mullane AP, Sonar P, Ayoko GA, Izake EL. Antibody coated conductive polymer for the electrochemical immunosensing of Human Cardiac Troponin I in blood plasma. Anal Chim Acta. 2021;1185:339082. doi:10.1016/j.aca.2021.339082
30. Wang Y, Liu T, Yang M, Wu C, Zhang W, Chu Z, et al. A handheld testing device for the fast and ultrasensitive recognition of cardiac troponin I via an ion-sensitive field-effect transistor. Biosens Bioelectron. 2021;193:113554. doi:10.1016/j.bios.2021.113554
31. Zhan T, Su Y, Lai W, Chen Z, Zhang C. A dry chemistry-based ultrasensitive electrochemiluminescence immunosensor for sample-to-answer detection of Cardiac Troponin I. Biosens Bioelectron. 2022;214:114494. doi:10.1016/j.bios.2022.114494
32. Orlov AV, Malkerov JA, Novichikhin DO, Znoyko SL, Nikitin PI. Multiplex label-free kinetic characterization of antibodies for Rapid Sensitive Cardiac Troponin I Detection based on Functionalized magnetic nanotags. Int J Mol Sci. 2022;23(9):4474. doi:10.3390/ijms23094474