Molybdenum Disulphide/Cellulose Acetate Nanofiber Composite on Screen Printed Electrodes for Determining Cardiac Disease by Electrochemical Impedance Spectroscopy

By

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22716

Dissertation submitted in partial fulfilment of

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CERTIFICATION OF APPROVAL

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WITH HONOURS

Approved by,

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January 2020

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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G. KABHILAN A/L GOBALU

ABSTRACT

Acute Myocardial Infraction (AMI) where currently ranked at top for causes of death worldwide, can be diagnose by detecting the concentration level of Troponin I in human blood. However, current problem reflects onto poor diagnose test such as Enzyme-Linked Immunosorbent Assay (ELISAs) because of expensive utilization cost and time limitation in diagnosing and treatment. Thus, to mitigate the number of deaths caused by cardiovascular disease, a nano-biosensor to detect troponin level in blood, developed by optimizing the active material in the biosensor. Molybdenum Disulphide (MoS_2) has great sensing abilities where it has an optimum band gap property as it has efficient conductivity although being a semiconductor transition metal dichalcogenide. With the aid of Cellulose Acetate (CA), synthesis of nanofiber from MoS_2 mixed with CA can be done with ease by electrospinning method. Characterization under Field Emission Scanning Electron Microscope (FESEM) exhibited the nanofiber with diameter of 200nm and analysed the morphology structure to capture the layered nanofiber. Encapsulation of MoS_2 nanosheets inside the nanofiber were observed through Transmission Electron Microscopy (TEM). The crystalline planes such as T(11-1), T (200), T(31-1) and T(130) which belong to the hybridised MoS_2 nanosheet were observed in X-Ray Diffraction (XRD). Moreover, the functional groups present in the nanofiber were analysed in Fourier-Transform Infrared Spectroscopy (FTIR). Electrical Impedance Spectroscopy (EIS) conducted to study the dielectric properties of the nanofiber to detect Troponin I in target concentration and human serum. The developed nano-biosensor analysed to have detection limit up to 10fM of Troponin I concentration. The analytical range extend from 10fM to 1nM of Troponin I concentration. High levels of stability, selectivity, linearity and repeatability of the sensor were obtained.

ACKNOWLEDGEMENT

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Heart and blood vessels are a group which categorized under cardiovascular disease (CVDs). In light of 2017 World Health Organization (WHO) report, CVDs are the fundamental driver of death universally assessed that 31% of every single worldwide passing (~17.7 million passing) in 2017 was identified with CVDs. Recent cardiovascular disease can be detected in one's blood by doing troponin test. Troponins are the protein fiber segments of the contractile cardiovascular and skeletal muscles, yet which are absent in smooth muscle. When a patient experienced cardiovascular disease such as heart attack or stroke, the troponin level in the blood increase. Thus, by doing troponin test will be an indicator of patient's current condition and future medication can be prepared.

Based on Figure 1.1, Troponin is known as a complex of three authoritative proteins—troponin I, troponin C, and troponin T—that are related to skeletal and cardiovascular muscle compressions. The troponin complex has three subunits; troponin I, which quells actin–myosin interchanges; troponin C, which ties calcium and troponin T, which joins the troponin complex by legitimate to tropomyosin and energizes narrowing.

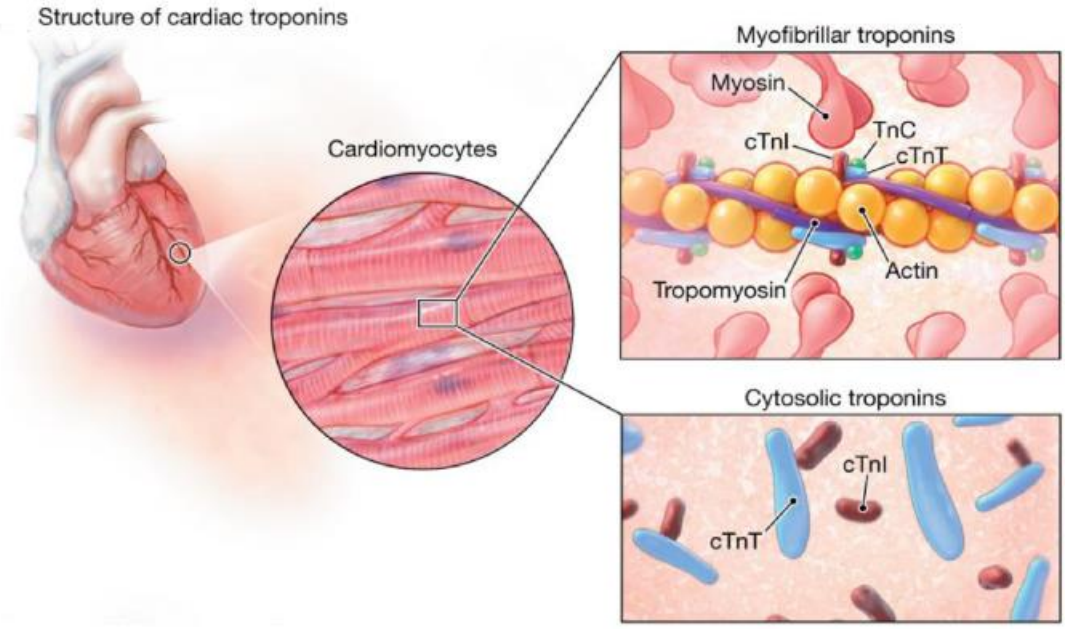


FIGURE 1.1: Structure of the cardiac troponin complex (consists of three proteins: troponin I, T and C). Picture retrieved from [1]

In order to detect the troponin in blood with higher sensitivity and with rapid timing, biosensor plays a vital role in satisfying the requirement mentioned earlier. Biosensor is an expository gadget equipped for creating explicit quantitative or semi-quantitative expository data utilizing a natural acknowledgment component coordinated to a transducer unit [2] . The reason for a biosensor is to give fast, ongoing, precise with accurate, dependable data about the analyte of cross examination. In a perfect world, it is a gadget that is equipped for reacting consistently, reversibly, and does not alter the example [3].A biosensor consist of three main components which are transducer, bioreceptor and signal processing system [3]. A bioreceptor consists of large comprises of an immobilized biocomponent that can recognize the particular analyte.

Developing an efficient and a quality biosensor required a remarkable property of nanoparticles that can sense the specific substrate. As progress metal sulphides, Molybdenum Disulphide, MoS_2, has been the asset of basic research for its functionality including reactant hydride sulfurization of oil, wear restriction and nonaqueous lithium charging batteries. For all of these uses, the huge systems happen on the surface or at the revealed side edges of the MoS_2 layers [4]. MoS_2 is a layered

semiconductor (band hole = 1.2 eV, indirect) that stands up to oxidation even in moist air at temperatures up to 85 C [5]. This characteristic makes MoS_2 an alluring material for nanoscience uses in medical field. Thus, for further upgrade its physical and substance properties, significant attempts have been made to get ready MoS_2 nanomaterials with various structures.

Building up an increasingly powerful and more extensive usefulness of bioreceptor when identifying a particular objective , making a polymer nanofiber is suggested where it show wonderful characteristics, for example, superb porosity and incredible explicit space region [6]. By blending MoS_2 nanosheets with cellulose acetate (CA) will deliver MoS_2/CA nanofiber by electrospinning process. For biomedical applications, cellulose acetate, anacetylated subordinate of cellulose polymer, is a conventional up-and-comer, since this polymer is hydrophilic, non-risky, biodegradable, and boundless with extraordinary processability [7]. Cellulose acetate induction reasoning contains distinctive ether, hydroxyl and carboxyl packages in the essential spine chains, making it significantly ionic in nature and, as such, it will all in all be used as an awe inspiring nanoreactor to join differing synergist and accommodating metal nanoparticles on the polymer surface [8].

1.2 PROBLEM STATEMENT

Reflecting the current biosensor to detect troponin in human blood, it comes with several drawbacks by using several methods. As normal tests, Enzyme-Linked Immunosorbent Assay (ELISAs) have been utilized for troponin measurement and quantification. ELISA's disadvantages fall under time limitation in diagnosing and treatment. Moreover, the utilizing cost of ELISA is expensive where certain considerations of economic should be done before proceeding with this method. Thus, the need of low cost and extremely sensitive to increase the detection time and acquire a stable characterization should be achieved by developing a biosensor. Biosensor performance such way in specificity, accuracy, detection time, sensitivity and require volume can be improved by introducing nanostructure in active material which leads to nano-biosensor. The advantage comprises under usage of nanostructure in a biosensor is as sensitivity of the sensor increase thus required a smaller or lesser

volume of sample for detection compare to traditional biosensor. Moreover, signal sensitivity can be enhanced with the application of nanostructure-based electrode substrate. However, no commercial product is available in market for the detection of a troponin using a nano-biosensor. Hence, the need of developing a nano-biosensor is required where in this research, MoS_2/CA nanofibers are used to satisfy all the advantages mentioned earlier and advancing the application of biosensor for detecting troponin in medical industry.

1.3 OBJECTIVE

1. To synthesize MoS_2/CA nanofibers by using electrospinning method and characterize MoS_2/CA nanofiber chemical properties, electrical properties and morphology structure.

2. To develop a biosensor to detect troponin in human blood with the use of MoS_2/CA nanofiber as an active material.

1.4 SCOPE OF STUDY

This research focusses on developing a biosensor with the aid of an active metal in a nanostructure form to increase the feasibility and sensitivity .The active metal chosen to be applied in this research is MoS_2 as it known for high band gap application which leads to high conductivity and suitable to be used in biosensor as signal enhancing from the bioreceptor to the transducer of the biosensor. To produce a nanofiber from MoS_2, it suggested to be mix with CA solution as a novelty attempt as it will give a good elongation of structure where nanofiber synthesis will be in ease. Electrospinning can be practiced as it most common and convenient method under certain circumstance to produce nanofiber. Detection of troponin I level in blood is the main priority of this study as a reason to investigate or detect any early cardiovascular attack disease. Characterization of the nanofiber will be carried out using Electrical Impedance Spectroscopy (EIS) to study and research on the electrical properties of the nanofiber by interpreting the graph acquired from this test. With the good results from EIS, this research can be approaching the main aim which is the detection of troponin level in blood as a reason to investigate or detect any early cardiovascular attack or disease.

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW

Over the prior decade, different basic creative advances have furnished us with the gadgets and materials expected to fabricate biosensor gadgets. Since the primary improvement of the Clark Oxygen Electrode sensor, there have been different updates in the affectability, selectivity, and multiplexing purpose of restriction of the advanced biosensor [3]**.** Various method of bioreceptors been developed such as the enzyme, DNA, antibodies, biomimetic and cell-based receptors. Each of the bioreceptors have different method of detection with the specific target as their properties different among themselves. Transducer is the most aspect in a biosensor as it convert the signal from the receptor into electrical signal to be interpreted by any data software to understand the characterization of target .Moreover , developing an active metal is essential to make a connection bridge between the transducer and bioreceptor to enhance the detection limit and sensitivity of biosensor . As to optimize and enhance the detection limit, usage of nanoparticles in active metals is recommended because of the enhanced chemical and electrical properties of nanoparticles as it capable to detect any target at highest sensitivity and to acquire more accurate data and functionality of a biosensor in medical industry especially.

This chapter will cover about the purpose of selecting Molybdenum Disulphide as the active material and electrical impedance spectroscopy (EIS) as transducer. As nanofiber structure is required to serve the function in the biosensor, the Molybdenum Disulphide mixed/stirred with Cellulose Acetate solution. This chapter will review the reason behind choosing cellulose acetate solution to be mixed with Molybdenum Disulphide nano-powder where the nanofiber can be produced by electrospinning method. This method procedure and advantages are reviewed in this chapter as well.

Finally, literature review would be focus on application of biosensor which is to detect troponin level with the aid materials used to build the biosensor as mentioned above.

2.2 BIOSENSOR

Three main components exist in a biosensor which are a bioreceptor, a transducer and an amplifying process where it called as signal processing system. Specific or targeted analyte can be detected by the static biocomponent in the bioreceptor. Antibodies, enzyme, cell and nucleic acid are what leads to building called as biocomponents based on Figure 2.1. Transducer play a vital role where it acts as a converter by converting biochemical signal into electrical signal.

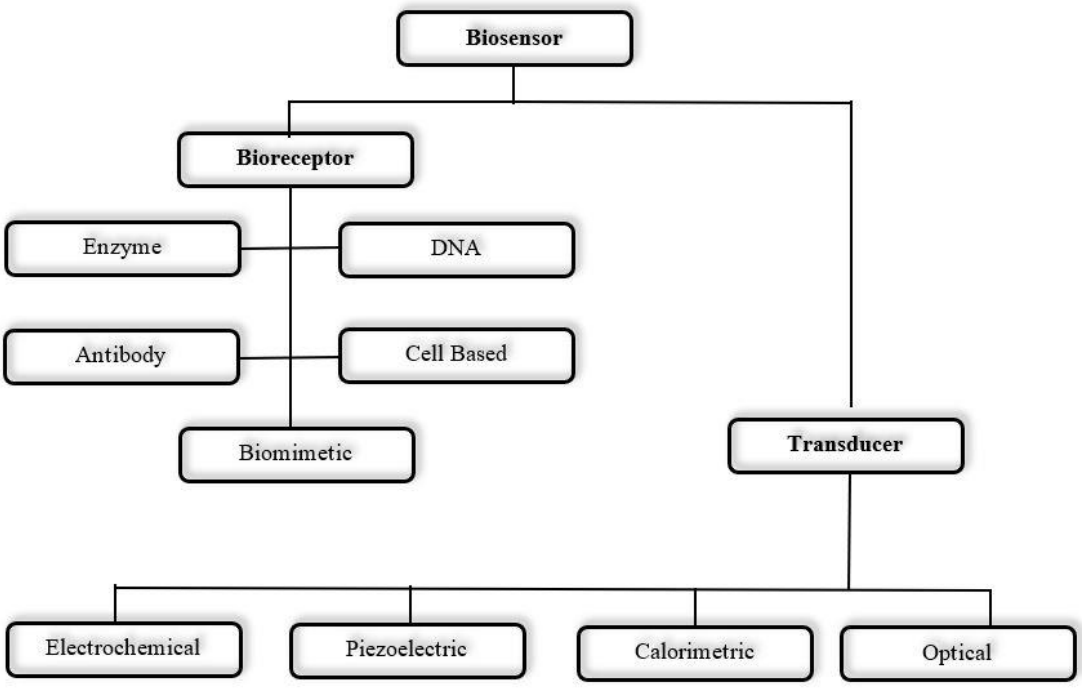


FIGURE 2.1: Different categories of biosensor [3]

2.2.1 Bioreceptor

Since the first biosensor, enzyme-based biosensors have faced up to the present a gigantic development in utilization for various applications. Enzymes are exceptionally effective biocatalysts, capable of explicitly perceiving their substrates and catalysing their transformation. There have been vigorous endeavours made to build up an immunosensor which is made out of an antigen/counter/ antibody acting

agent as bioreceptor as an instrument for clinical diagnostics. A neutralizer is "Y" moulded immunoglobin (Ig) that is comprised of two substantial chains (H) and two light chains (L). Some late examinations demonstrate that the immunosensor is generally investigated for its utilization in the recognition of malignancy/tumours.

Suitably, such devices rely upon the immobilization of a solitary stranded (ss) DNA atom for hybridizing with the correlative ("target") strand in a given model .DNA hybridization biosensors offer amazing assurance for obtaining gathering express information in a speedier, simpler and more affordable path appeared differently in relation to customary hybridization looks at.

A synthetic or manufactured sensor that copies the usefulness of a certified sensor is the thing that a biomimetic biosensor. Aptasensors where it uses aptamers as the biocomponent. Hence, to perceive amino acids, peptides, proteins and oligosaccharides, aptamers with engineered strands of nucleic acids are planned [7]. For instance, aptamer properties, their high expresses, little size, adaptability in alteration and immobilization, regenerability or conformational change started by target confinement have been adequately misused to redesign a scope of bio-detection structures. All methods of biosensing illustrated in Figure 2.2.

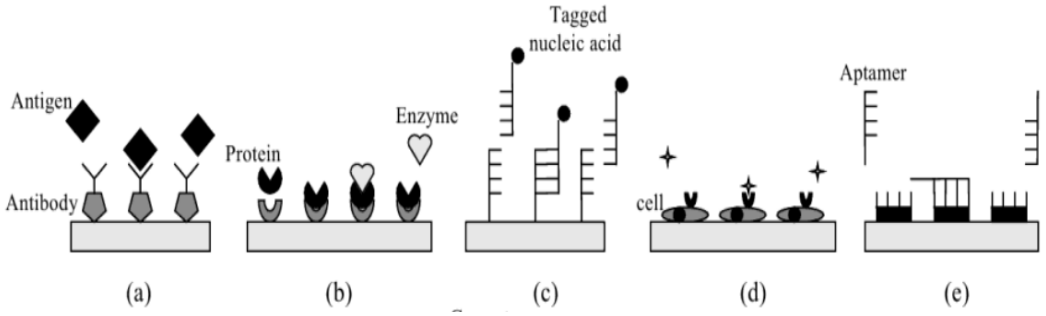


FIGURE 2.2: Methods of biosensing: (a) antibody/antigen; (b) enzyme catalysed; (c) nucleic acid; (d) cell-based; (e) biomimetic [7].

2.2.2 Transducer

The electrochemical sensor wherein a terminal is utilized as the transduction part, addresses a basic subclass of sensors. An electrochemical biosensor is a self-governing joined gadget, which is set up for giving unequivocal quantitative or semi-quantitative sensible data utilizing a characteristic assertion fragment. There are four

perceive classes fall under electrochemical biosensors, for instance, amperometric, conductometric, potentionmeter and Electrical Impedance Spectroscopy (EIS)

The electrical impedance spectroscopy (EIS) based transduction methodology is genuinely not a generally used procedure for electrochemical distinguishing proof; in any case, this system has starting late transformed into a standard mechanical assembly for bioreceptor transduction. This procedure is known to resemble other electrochemical acknowledgment contraptions yet with a conductivity recognizable proof that yields the revelation volume with an electrical repeat clear in the extent of 10 kHz and 10 MHz [3]. EIS estimations can be made with an alternate number of terminals in various designs, among which the most regular ones (for the most part called two-, three-and four-electrode usage) are introduced in Figure 2.3.

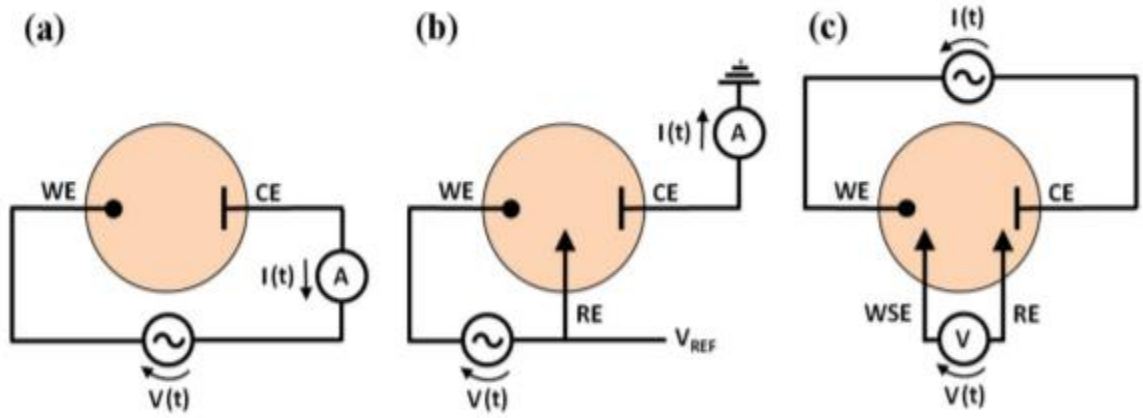


FIGURE 2.3:Measurement setup configurations used in EIS featuring (a) two electrodes, (b) three electrodes and (c) four electrodes [9]

Most of the time, three electrodes system are widely used as transducer for biosensor. The EIS also compromise of a frequency response analyser (FRA) and a potentiostat. The three main electrodes are the counter electrode that supply current to the cell, the working electrode that take the measurement of current and the reference electrode for measurement of the voltage. High input impedance provider is the function of potentiostat in order to maintain voltage across the electrode. Essentially, the FRA is fused to supply an excitation waveform and to give a helpful, high accuracy, wide band strategy for estimating the impedance.

2.3 ACTIVE MATERIAL – MOLYBDEUNUM DISULPHIDE (MoS_2)

2.3.1 Molybdenum Disulphide (MoS_2) Properties & Applications

Molybdenum disulphide (MoS_2) is a normal layered compound as shown in Figure 2.4 with feeble interlaminar van der Walls control, and has been comprehensively thought about because of its potential applications as solid oils, impetuses, hydrogen accumulating material, and so on. Starting late, with the necessity for progressively dumbfounding execution, the mix of diminished size MoS_2 with high surface area has started to be inspected. A critical instance of these is that nanoMoS_2 present different properties from their mass accomplices because of their particular measure and structure[10]. A wide scope of approach, for instance, solvothermal ,aqueous and electrochemical/substance have been represented to prepare nanosized MoS_2 [11].

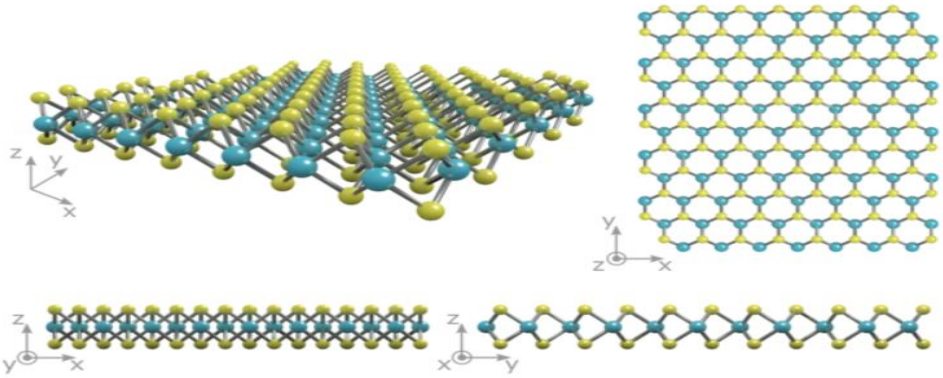
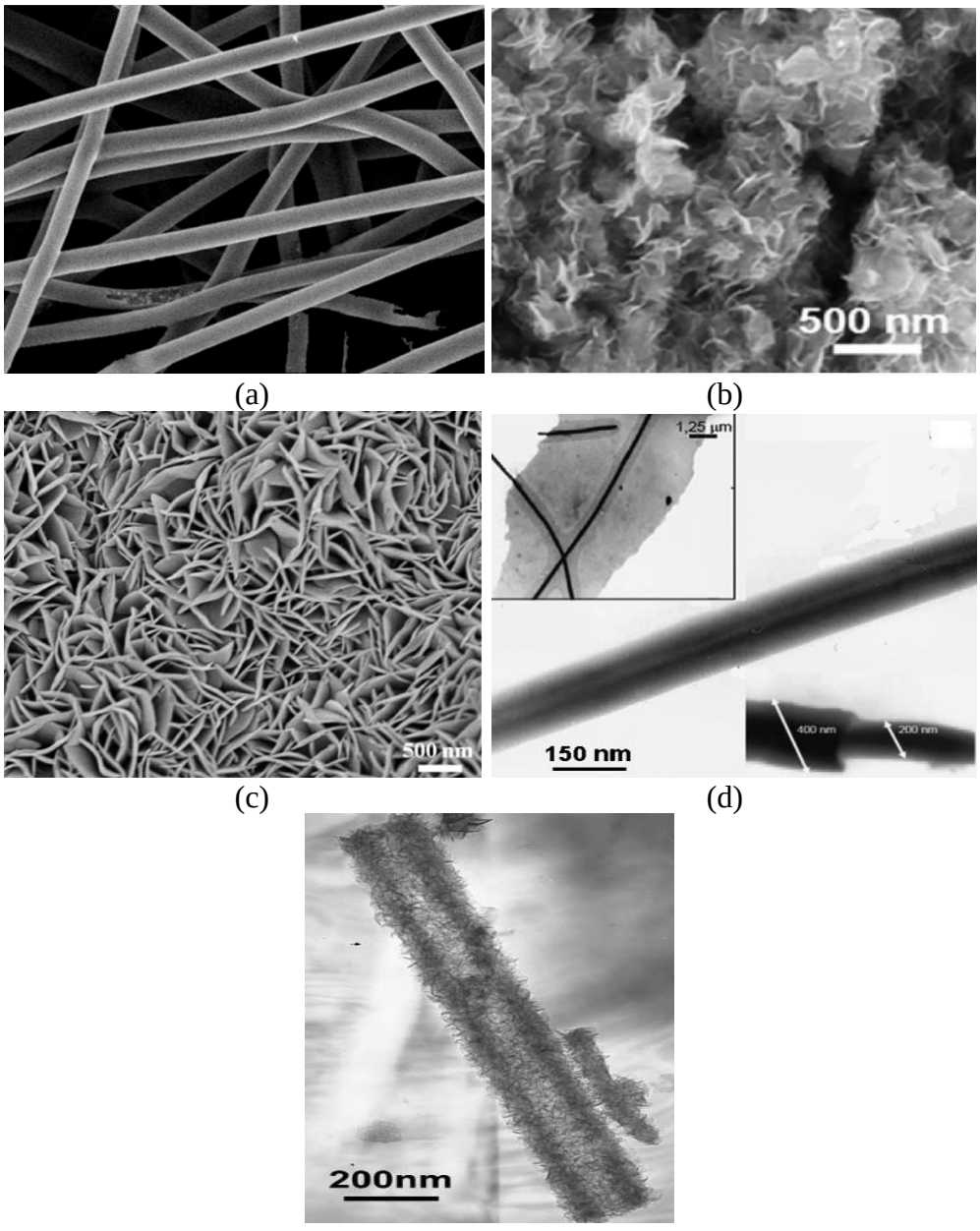


FIGURE 2.4: Precious structure of monolayer MoS_2 demonstrating a layer of molybdenum molecules (blue) fortified between two layers of sulphur iotas (yellow).

As named as progress metal dichalcogenides, molybdenum disulphide (MoS_2) exhibits the captivating properties, for instance, a tremendous direct bandgap an amazing on/off extent of 108,4 and high transporter versatility up to 200 $cm^2/(V.s)$ at room temperature [4]. Because of the high band hole of MoS_2 and mass MoS_2 being where the band hole of the single layered MoS_2 modifies from aberrant to coordinate (~1.9 eV) and roundabout band hole of ~1.2eV. Additionally, the band hole of MoS_2 is tunable because expanding with decrement of number of stacked layers quantum limited. These exceptional properties displays MoS_2 more remarkable than its analogue for sensor, energy capacity and light-emanating gadget applications [4].

2.3.2 Molybdenum Disulphide (MoS_2) Nanoparticles

In order to enhance the chemical, physical and electrical properties MoS_2, making or preparing MoS_2 nanoparticles would be recommended. Different shape of MoS_2 's nanostructure shown in Figure 2.5 contribute different properties such as nanofiber exhibit excellent mechanical properties as well [12],nanosheets have higher surface ratio [13] , nanotubes may also make it useful in hydrogen storage [14] , nanorods have remarkable properties in optical and electrical [15] and nano-petals can be used as adsorbent for the removal of dye [16].



(a) (b) (c) (d) (e)

FIGURE 2.5: (a) MoS_2 nanofiber [17] (b) MoS_2 nanosheet[18] (c) MoS_2 nano-petals[19] (d) MoS_2 nanotube [20] (e) MoS_2 nanorod [17]

2.4 ELECTROSPINNING METHOD

Electrospinning is the most appropriate strategy for creation of nanofibers. The favourable circumstances incorporate its relative low cost, ease, fast, tremendous materials determination, and adaptability. Besides, the procedure permits power over fiber arrangement, microstructure, and diameter [21]. Fiber in the nano-scale range are framed as the charged polymer arrangement is extended persistently because of electrostatic repulsions of the surface charges and the solvent. This strategy for utilizing electrical potential to draw strands was licensed in 1934 by Formhals [22].It has been utilized as a fruitful apparatus to turn a heap of manufactured and regular polymers into 30–2000 nm strands expanding up to numerous kilometres long.

As shown in Figure 2.6, this system depends upon three basic parts: a great voltage producer, a hair-like chamber containing polymer course of action joined to a little estimation of a needle, and a metal properties power. To cause an electrically charged fly of polymer blueprint/to gather out of the needle, a high current times resistance is applied between two anodes related with the turning game-plan/conciliate and to the collector (normally grounded). The electrical current at the tip of the needle pulverizes the outside of the speck of the polymer strategy engineered on it. Repulsiveness between charges exits on the surface, comparatively as their appreciation for the contrary terminal, initiate a power that thrashings the surface weight. Thusly, a charged stream is kicked off out from the top of the needle. Because of the ordinarily undesirable powers of the current in the planes, the polymer game-plan stream experiences a bowing insecurity, consequently a development and decreasing framework. Meanwhile, scattering of the dissolvable prompts the game plan of a charged polymer nanofiber, assembled as an interconnected web on the authority [23].

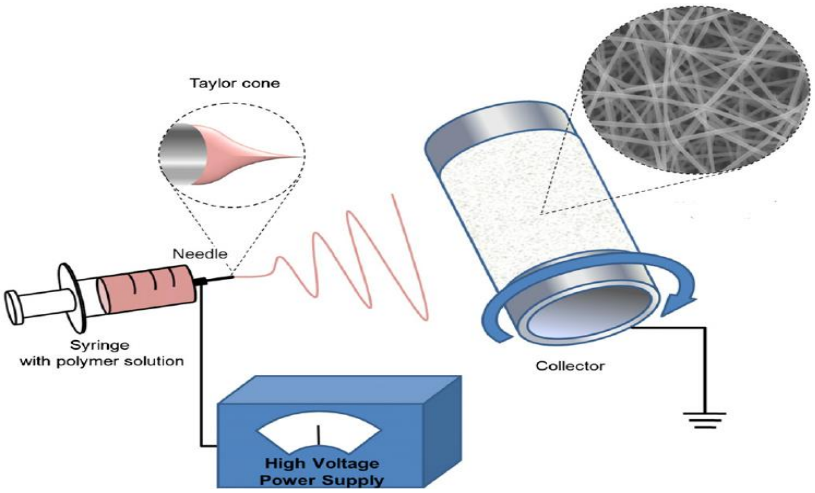


FIGURE 2.6: Electrospinning process and setup

2.5 CELLULOSE ACETATE

2.5.1 Cellulose acetate (CA) ‘s Properties & Applications

Cellulose acetate (CA) is the most resourceful and widely utilized sustainable polymer asset due to its great warm solidness, biodegradability, and chemical resistance [24].Acetate ester of cellulose which also a cellulose acetate and it plays a vital role in green plant as it is the primary structural component of the cell wall. These brilliant properties make an incredible possibility for different applications, for example, biosensors, chemosensors, sacffolds against microbial specialists,membranes ,and bundling films fortified with nanocomposites [25]. By reacting cellulose with acetic acid and acetic anhydride using sulfuric acid can produce CA.

2.5.2 Cellulose acetate (CA) ‘s Nanofiber

Nanofiber-type cellulose acetate as illustrated in Figure 2.7, has pulled in particularly huge technological and scientific consideration because of its high surface region to-mass proportion, its high porosity with magnificent pore interconnectivity, also, its adaptability with sensible quality [26]. Besides, CA nanofiber exhibits an excellent filtration property due to their narrow shape fiber, fine diameter, high surface area and narrow fiber distribution range. As needs be, it might be attractive to break down the microfibrillar idea of cellulose acetate derivation into a caught nanofibril

structures for being utilized as a biocompatible nanoreactor in biosensor application. CA nanofibers have stayed point of convergence of research in numerous businesses including the material and even more as of late in the biomedical area exhibited in Figure 2.8.

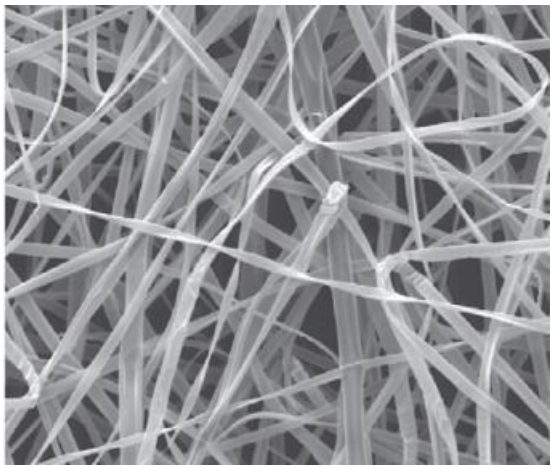


FIGURE 2.7: SEM structure of the CA nanofibers [27]

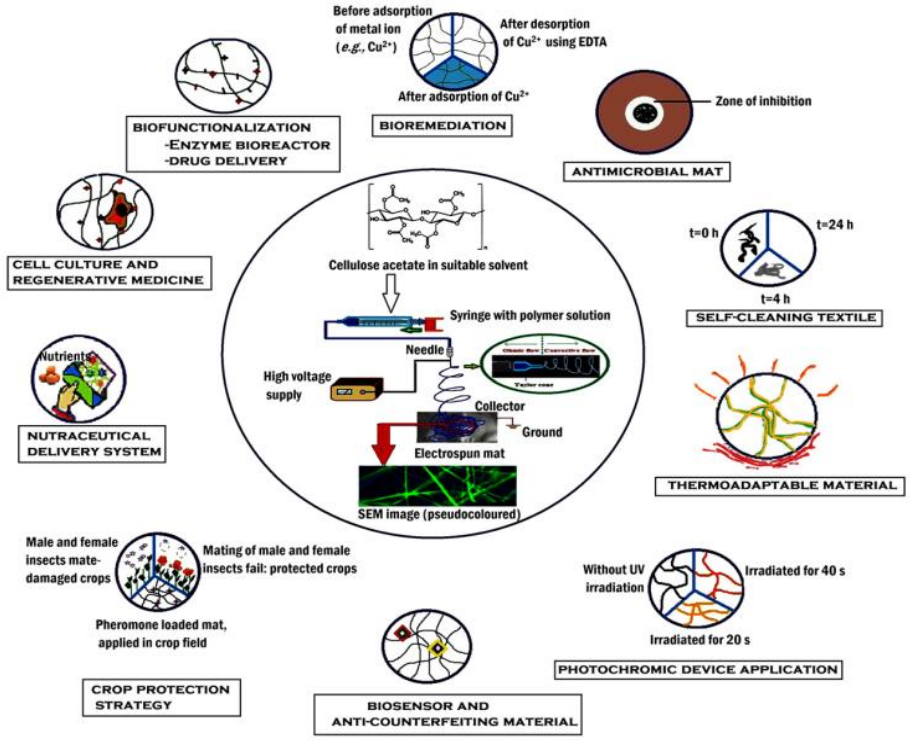


FIGURE 2.8: Application of cellulose acetate nanofibers [28]

2.6 APPLICATION OF BIOSENSOR

2.6.1 Detection of Troponin Level in Human Blood

Acute myocardial infraction (AMI) is the most notable disease classed as cardiovascular disease. After a barrier of a coronary store course and nonappearance of blood supply (ischemia), the heart muscle getting injured and inciting the AMI. The rottenness of heart muscle is could not be reversed and 85% of heart hurt advances inside the fundamental two hours after the beginning of a coronary scene, and yielded restorative operation expands the likelihood of fatality. As such, early and definite completion of AMI and brief treatment are basic to develop the continuance change over time [29].

Cardiovascular troponins are the favoured biomarkers for assessment of myocardial dead tissue considering their high affectability and affectability for myocardial mischief. Cardiovascular Troponin I and heart Troponin T are sensible for confirmation and these biomarkers considered as "greatest levels" to AMI end [33]. The troponin examining or level augmentation inside 4 hours after the start of AMI while its half-life is around 2 hours and close to the end in blood for 4 - 10 days as shown in Figure 2.9. Heart Troponin I holds an estimation of 0.4 ng mL^{-1} at standard level and if higher than 2.0 ng mL^{-1} shows that risk for future certifiable cardiovascular contamination [30].

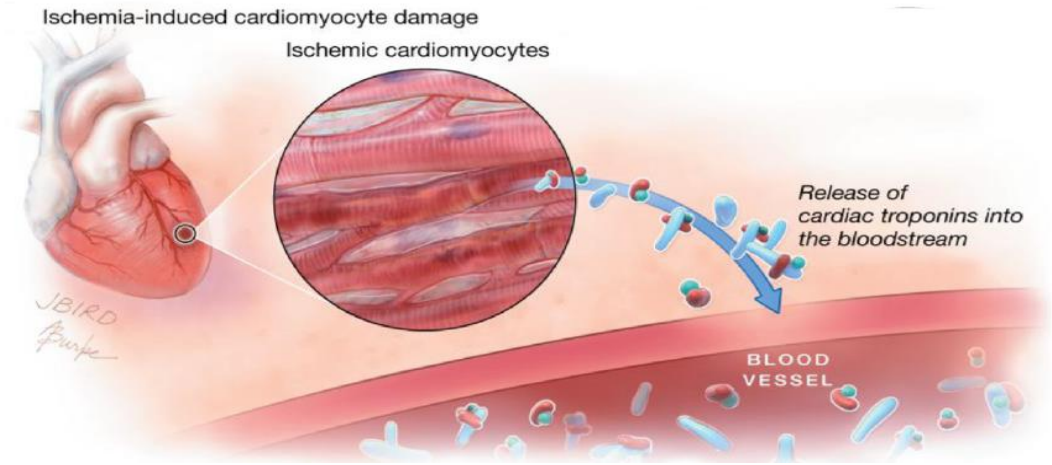


FIGURE 2.9: Arrival of cardiovascular troponins following ischemic heart damage (troponin C; cardiovascular troponin I; heart troponin T)[1].

TABLE 2.1: Common application of MoS_2 nanostructure in biosensors

Material	Structure	Surface Modification	Aptamer	Detection Limit	Reference
MoS_2	Nanoflake	Hydrophobic interaction	anti-Bovine Serum Albumin (BSA) antibodies	0.006 ng/mL	[31]
MoS_2	Nanosheet	-	Plasmodium lactate dehydrogenase	0.6nM	[32]
MoS_2	Nanosheet	AFP aptamer functionalized green concealed Au NCs (510 nm) and CEA aptamer functionalized red shaded Au NCs (650 nm)	Tumour marker- AFP aptamer & CEA aptamer	CEA: 0.16 ng/mL AFP: 0.21 ng/mL	[33]
MoS_2	Nanosheet	Adjust the HfO2 layer covering the MoS_2 surface with silane particles to display surface aldehyde gatherings, which further respond with amino bunches in the counter PSA antibodies	Prostate-specific antigens (PSA)	15nM	[34]
MoS_2-rGO	Nanosheet	Modification of a glassy carbon electrode (GCE) with MoS_2-rGO (MG)	Folic acid protein	10nM	[35]

TABLE 2.2: Biosensors application in detecting troponin

Material	Structure	Surface Modification	Aptamer	Detection Limit	Reference
Silicon	Nanowire	Aldehyde was first utilized to modify the SiNx layer	cTnI monoclonal antibodies.	0.092 ng/mL	[36]
Graphene oxide (GO) Carbon Nanotube (CNT)	Nanohybrid	Direct electron transfer (DET) between redox protein and electrode surface	Anti-myoglobin (Mb)	0.34 ng/mL	[37]
Carbon	Nanofiber	Individual VACNFs were electrically separated from one another by saving SiO2 as a dielectric utilizing synthetic fume affidavit with tetraethoxysilane a	anti-cTnI Antibodies	0.2 ng/mL	[38]
Carbon, Colloidal gold	Nano-gold	Silver upgrade: anode surface was submerged in a silver intensifying arrangement containing silver nitrate and hydroquinone	Antibody Colloidal Gold Conjugated IgG2	0.8 ng/ml	[39]
Single Walled Carbon	Nanotubes	Chitosan-wrapped carbon nanotubes are cross-linked to form a thin gel that is further functionalized with nitrilotriacetic acid (NTA)	Anti-cTnT antibodies with NTA groups as anchor point	100 ng/mL	[40]

TABLE 2.3: Types of nanofiber biosensors in detecting protein

Material	Target	Surface Modification	Aptamer	Detection Limit	Reference
gold nanoparticles bacterial cellulose nanofibers (Au–BC) nanocomposite	Heme Proteins	-	H2O2	0.1µm	[41]
Carbon Nanofibers	dopamine and serotonin	CNF electrode with presence of ascorbic acid	-	Dopamine :50 nM Serotonin: 250 nM	[42]
Carbon Nanofiber	C-reactive protein (CRP)	Freshly created carboxylic acid groups on the CNFs tips were used for probe immobilization using carbodiimide crosslinking	anti-CRP	90 pM or 11 ng/ml	[43]
Copper Carbon	Catechol	Copper/ Carbon Composite Nanofibers	CNFs/Lac/ Nafion/GCE and Cu/CNFs /Lac /Nafion/ GCE	1.18 µM	[44]
Polystyrene Poly(styrene-co-maleic anhydride)	Thrombin	Polystyrene–poly(styrene-co-maleic anhydride) nanofiber	Fluorescein-labeled TBA15 (F-TBA15)	10pM	[45]

2.7 SUMMARY

The most important advantages of this project in developing biosensor to detect troponin level is the no commercial product/biosensor in nano level to detect troponin. Hence, this research will create a remarkable achievement after the objectives of this project been achieved. Taking detection of troponin as consideration to develop this biosensor because number of cardiovascular diseases have been increasing day by day where a solution to cope this number is outlined by developing this biosensor. Choosing the MoS_2 as the active metal enhance the properties of biosensor as it has higher band gap making it as good conductor and the band gap is tunable. Moreover, cellulose acetate solution mix with Molybdenum Disulphide nano-powder to produce the Molybdenum Disulphide/Cellulose Acetate nanofiber as it would produce nanoparticles that has higher surface area which make more compatible to be use as nanoparticle active metal in the biosensor. In order to produce the nanofiber, electrospinning method is chosen as it is low cost, easy to operate and more efficient when comes to produce nanofiber.

CHAPTER 3

METHODOLOGY

3.1 OVERVIEW

The planned methodology compromise of 3 main experiment as shown in Figure 3.1 starting from preparation of Cellulose Acetate solution by stirring the solvents and CA powder on hot plate. The preparation continued with preparing MoS_2/CA solution by mixing the MoS_2 into the CA solution on hot plate. After acquiring the MoS_2/CA solution, synthesizing of the MoS_2/CA carried out with electrospinning method by using the electrospinning machine with specific setup specification for optimum production of nanofiber.

3.2 PROJECT ACTIVITY

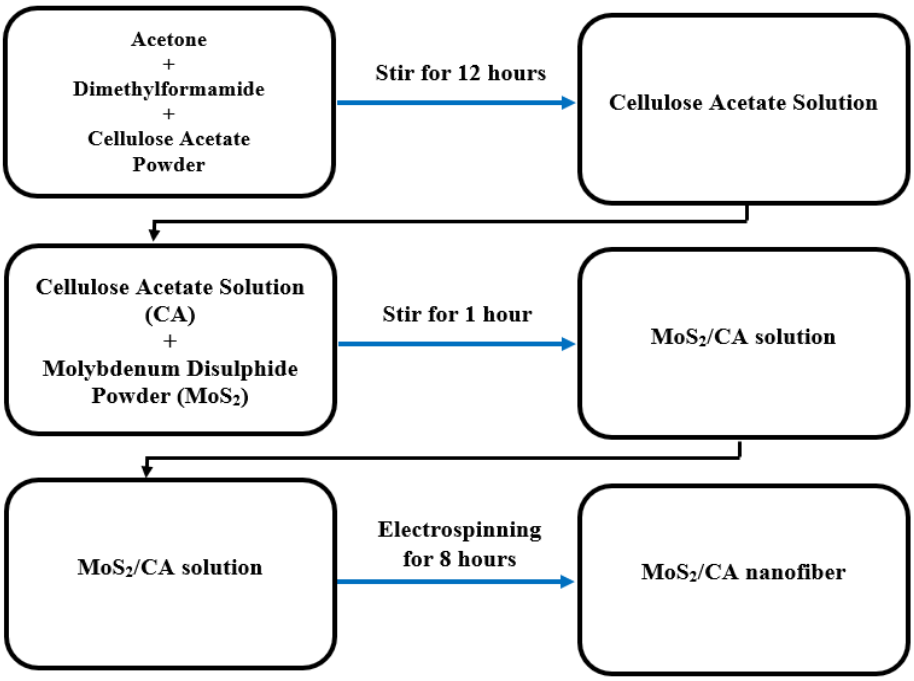


FIGURE 3.1: Flowchart to achieve MoS_2/CA nanofiber

The experiment carried out with 6 different mass of MoS_2 nanosheet powder where explained in Table 3.1 below.

TABLE 3.1: List of experiments need to be done

Experiment	Cellulose Acetate Solution	Mass of MoS_2 Nanosheet(g)
1st	30 ml of Acetone + 20 ml of Dimethylformamide + 10.1g of Cellulose Acetate powder	0.025
2nd		0.05
3rd		0.075
4th		0.1
5th		0.2
6th		0.3

3.3 EXPERIMENTAL PROCEDURE

3.3.1 Cellulose Acetate (CA) Solution Preparation

Based on Figure 3.2, preparation began with pouring 30ml of Acetone into the beaker. Next, with the aid of pipette, 20 ml of dimethylformamide (DMF) added into the same beaker and the mixture stirred using magnetic stirrer with hot plate. Meanwhile stirring, 10.1 gram of cellulose acetate powder weighed using precision weigh machine to get most accurate amount before the next procedure. After weighed, cellulose acetate powder added into the solution in the beaker. In order to ensure the cellulose acetate powder dissolve completely with the Acetone/DMF solution, the solution stirred for 12 hours, under room temperature and at 400 rpm.

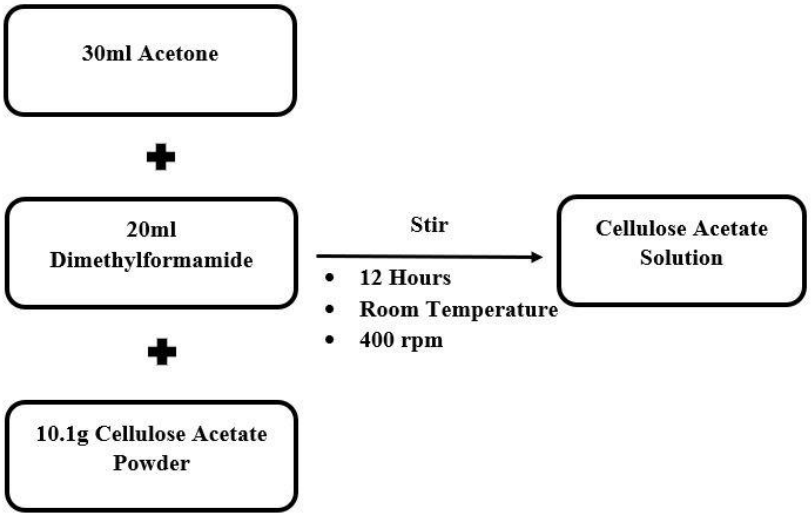


FIGURE 3.2: Synthesis of cellulose acetate solution

3.3.2 Molybdenum Disulphide/Cellulose Acetate Solution Preparation

The first experiment carried out with 0.025 gram of MoS_2. After stirring the CA solution for 12 hours, with the aid of precision weigh machine, the MoS_2 powder weighed to 0.025 gram. Next, mixed the MoS_2 nanosheet powder into the CA solution in the same beaker and stirred for 1 hour and under room temperature to ensure the MoS_2 disperse completely with the CA solution as existence of powder particles can cause blockage in tube during electrospinning process. Depends on the amount of MoS_2 being mixed with CA solution, the solution to be in the range of slight black to dark black as MoS_2 is in black in colour while CA solution normally colourless. Figure 3.3 shows summarization of the solution preparation process.

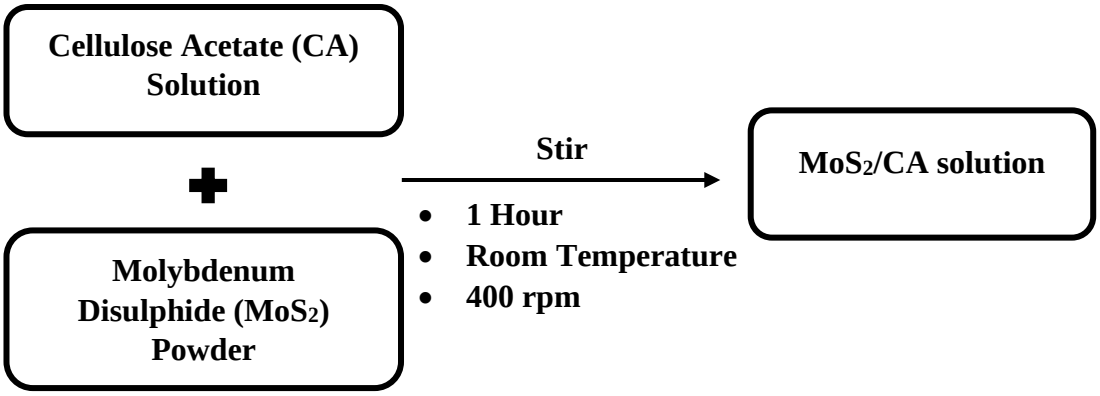


FIGURE 3.3: Process flow of preparing MoS_2/CA solution

3.3.3 Producing MoS_2/CA Nanofiber Using Electrospinning Method

As discussed in literature review, using electrospinning method to produce MoS_2/CA is the most convenient and cheapest method. The electrospinning was setup before proceeding utilizing it such as cleaning the interior of machine with acetone solvent , preparing aluminium foil to cover the drum as medium surface to collect the nanofiber, calibrating the drum speed at 1200 rpm and supply voltage at 10V.After the 1 hour stirring process, MoS_2/CA solution injected into the syringe at optimum volume of 20 ml . Next, the syringe connected with the needle with tube and ensure no air bubbles exist in the syringe and the tube as well to prevent air trapped in the medium as it will disrupt the uniform distribution of nanofiber on aluminium foil/drum. ran at least for 8 hours as observed in Figure 3.4 to get the right or uniform thickness of nanofiber distribution onto the drum. Table 3.2 shows the setup specification of the electrospinning machine that need to be calibrated before starting the process. All steps

mentioned from the beginning were repeated with different mass of MoS_2 nanosheet as implies in Table 3.1.

TABLE 3.2: Electrospinning setup specification for nanofiber production experiment

Specifications of Electrospinning Machine	**Input Value**
Supply Voltage	10 V
Drum Speed	1200 rpm
Feeding Rate	0.4 ml/hr
Distance of Needle from Drum	15 cm

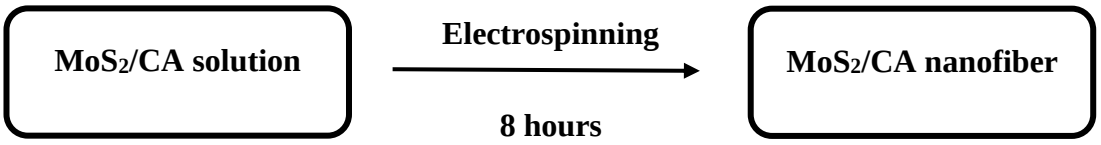


FIGURE 3.4: Process flow of synthesizing MoS_2/CA nanofiber

3.3.4 Surface Functionalization to Capture Troponin I

Initially the Screen-Printed Electrodes (SPE) were washed with 10 µL of 10mM PBS of pH7.4 and subsequently reading for bare SPE chips were taken to detect any manufacturing error of the electrodes. The chips later incubated with 2% of APTES for 1 hour in room temperature. A constant interval time of check on the chip must be done during the incubation process to ensure the APTES is present on the chip. After 1 hour of incubation process, the unattached APTES on the surface was removed with the aid of washing process using 10mM PBS of pH7.4. APTES bonded on electrode surface were proceeded with readings to analyse the effectiveness of the modification. The pre-modified SPE chips with APTES were further enhanced by incubating with 10 µL of Complex Mixture for 15 minutes. further enhanced by incubating with 10 µL of Complex Mixture for 15 minutes .16-mercaptohexadecanoic acid mixed with NHS and EDC are what contribute to the formation of Complex Mixture. With 250 µL of ethanol and 250 µL of water, the 16-mercaptohexadecanoic acid powder were diluted. The diluted 16-mercaptohexadecanoic acid were further mixed with 25 µL of NHS and 25 µL of EDC. In order to obtain white pellet where it

will be used as Complex Mixture modified on the electrode surface, the current mixture undergone centrifugation process lasted for 2 minutes.

Next, a liquid form of MoS_2/CA nanofiber was prepared by having a mass of 0.005g of MoS_2/CA nanofiber placed into 5 mL of Toluene where the solution was let to sonicate for 80 minutes and in 60°C. The obtained solution from the process earlier was added onto the modified SPE electrode where modified into MoS_2_CA/SPE electrode by letting the solution to incubate for 30 minutes. The MoS_2_CA/SPE electrodes were modified by adding 10 µL of 1 Molar Streptavidin while incubating for 30 minutes. To block the deficient structure of Streptavidin/MoS_2_CA/SPE, the electrodes were further modified by dropping 1 Molar of ethanolamine as a blocking agent and incubated for 30 minutes as well [46].After each immobilisation steps , the SPE electrodes were washed with 10 µL of 10mM PBS and impedance readings had taken [47].

3.3.5 Immobilisation and Hybridisation of Aptamer-Biotin Linker and Target

Dilution process of the probe is a very crucial procedure before surface immobilization of the probe onto the electrode surface. In order to form 100µM of biotin end linker so that the biotin end of the linker bind onto the streptavidin modified electrode surface , 973 µL of buffer solution used to dilute the oligo linker sequence of 5'- /5Biosg//IC6Sp/TTT TTT TTT TTT TTT TTT TT-3' which has 97.3 nmoles. Contrarily, to develop 100µM of Aptamer with the sequence of 5'- CGT GCA GTA CGC CAA CCT TTC TCA TGC GCT GCC CCT CTT AAA AAA AAA AAA AAA AAA AAA AAA A -3' which consists 62.3 nmoles were undergone dilution process with 623 µL of buffer solution. First, the biotin linker was immobilised on the Streptavidin/MoS_2_CA/ SPE electrodes before immobilising the aptamer linker on the same modified electrodes and thus completing the probe (Biotin-Aptamer linker) immobilisation process. All washings were done with 10 µL of 100 mM PBS (pH 7.4) and readings taken and performed between each interval of immobilization step.

For target detection, the hybridization took place between the target (Troponin I) and probe by means of by diluting the Troponin I in 10mM PBS of pH 7.4 at room temperature at different concentration of troponin I range from 1 nM to 100 fM with the sequence of 10fm.100fm,1pm,10pm,100pm and 1nm to obtain sequential data

readings. Each target concentration dropped onto the electrode surface was incubated for 30 minutes and later washed with 10mM PBS of pH 7.4 and readings taken.

The steps repeated using control protein (Troponin T) instead of Troponin I with concentration of 100pM and 1nM. Troponin T being the negative control protein as target where used to analyse the modified Aptamer /Streptavidin/MoS_2_CA/ SPE detection sensitivity on different target besides Troponin I. Figure 3.5 demonstrates the steps of surface functionalization from bare electrode to immobilization of Troponin I.

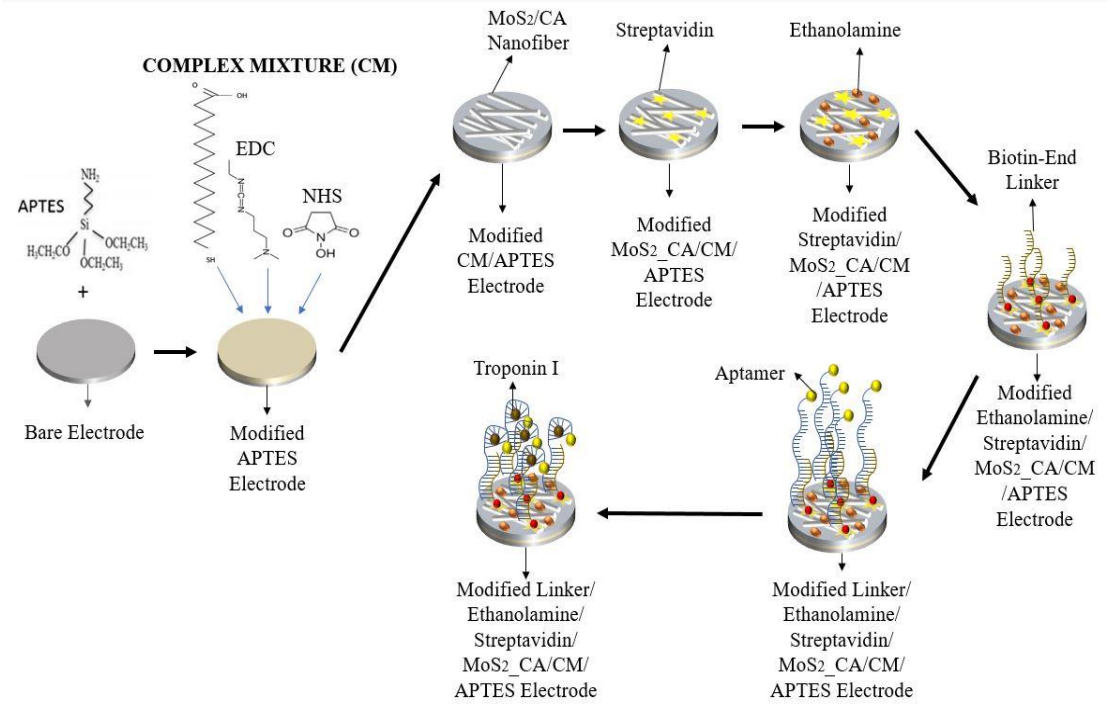


FIGURE 3.5: Surface functionalization of SPE to specifically capture Troponin I

3.4 KEY MILESTONE

TABLE 3.3: Key milestone of project

Week	FYP Markers	FYP 1 Activities
11-14	▲	Prepare MoS_2/CA Solution
11-14	▲	Electrospinning with MoS_2/CA
Week	**FYP Markers**	**FYP 2 Activities**
1-4	❖	MoS_2/CA Nanofiber Characterisation: • FESEM • TEM • FT-IR • XRD
5-7	❖	Data Analysis
8-11	❖	Developing Biosensor using EIS

3.5 PROJECT TIMELINE

Table 3.1 shows the methodological flowchart for the synthesis and characterization of MoS_2/CA Nanofiber process used in this research.

TABLE 3.4: Project Timeline

Progress	FYP															FYP II													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Topic Selection									DEEPAVALI HOLIDAYS						SEMESTER BREAK														
Problem Statement & Objectives																													
Literature Review																													
Develop Methodology																													
Lab Authentication																													
Prepare MoS_2/CA Solution																													
Electrospinning with MoS_2/CA																													
MoS_2/CA Nanofiber Characterisation																													
Data Analysis																													
Developing Biosensor																													
Documentation																													

Planned ❖ Milestone

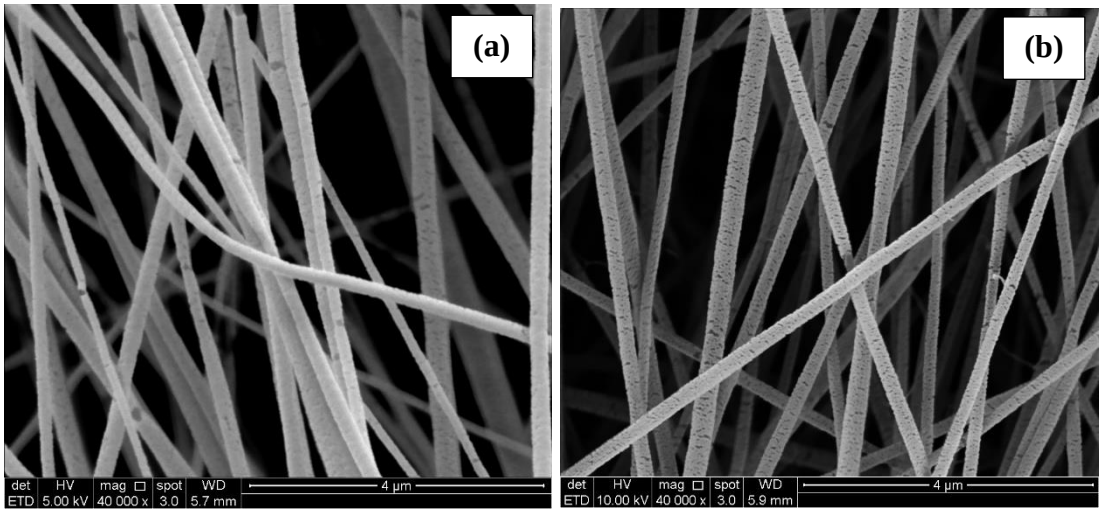
CHAPTER 4

RESULTS AND DISCUSSION

4.1 OVERVIEW

Morphology studies of samples were carried by FESEM and TEM to analyse the nanostructure and layer formed. By depth analysing, sample of 0.05g MoS_2/CA nanofiber chosen for next characterization processes as said to exhibit good nanofiber structure with layered nanofiber stack upon each other which reflects onto good optimization achieved in this sample. FT-IR and XRD were carried out to study the presence of functional group and elements respectively in the produced nanofiber. Dielectric properties of the developed sensor immobilised with MoS_2/CA nanofiber had been analysed through EIS with various Troponin I concentration and different proteins to study the high analytical performance of the sensor.

4.2 FIELD EMISSION SCANNING ELECTRON MICROSCOPE (FESEM)



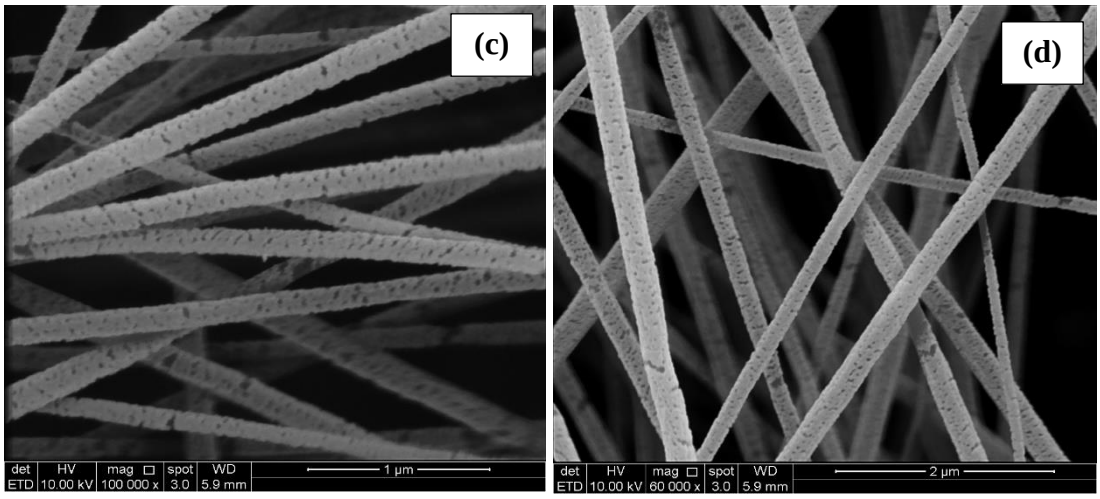


FIGURE 4.1 : (a) Morphology structure for 0.025g MoS_2/CA nanofiber (b) Morphology structure for 0.05g MoS_2/CA nanofiber (c) Morphology structure for 0.075g MoS_2/CA nanofiber (d) Morphology structure for 0.1g MoS_2/CA nanofiber

The morphology structure of all sample studied through FESEM to give a deep and clear analyzation process. For 0.025g of MoS_2 with CA as shown in Figure 4.1 (a), it the surface of the fiber almost smooth as less spores were observed. The diameter of each nanofiber not same in size and in randomly oriented network structure. The structure observed also said to be beads-free structure as there is no undissolved MoS_2 nanoparticles or also known as beads present on the fiber surface. This can conclude that the MoS_2/CA was in form of homogenous solution. The fiber morphology can be further studied by analysing the depth of the nanofiber stacked upon each other. As the figure show different colour concentration of nanofiber where at utmost front bright fiber can be observed and nanofiber stacked below are in dark colour concentration. Hence rather than a one layer, the nanofiber we produced were stacked layer by layer. As in Figure 4.1 (b) and Figure 4.1 (c), the nanofiber formed exhibit similar structure with nanofiber in Figure 4.1 (a) but the difference can be observed by the diameters of nanofiber as the diameters exhibited similar structure and not in different size as oriented in Figure 4.1 (a). Thus, it can be concluded that adding more MoS_2 nanosheet powder into the solution would make the nanofiber to be produced in more stable manner.

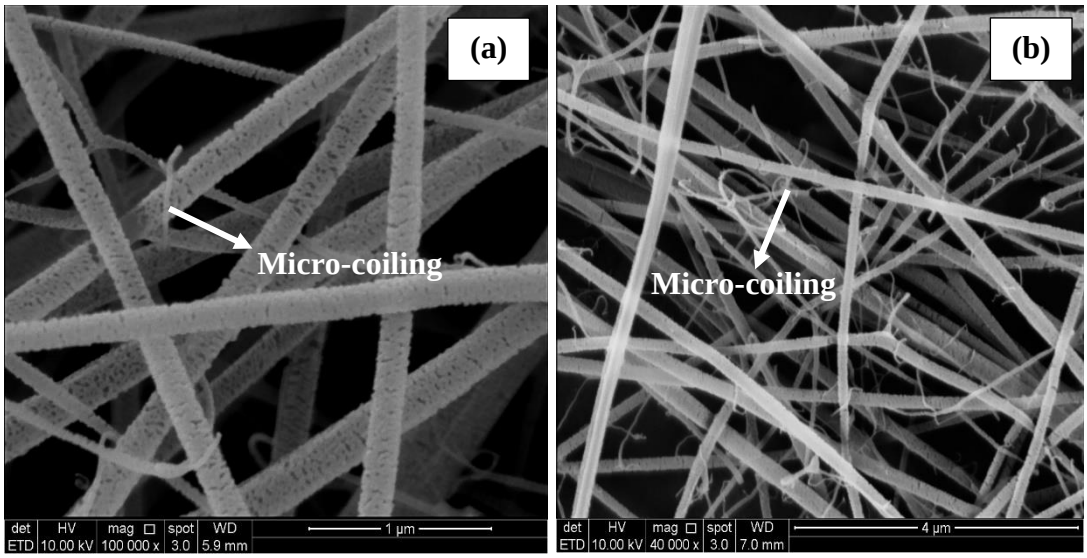


FIGURE 4.2: (a) Morphology structure for 0.2g MoS_2/CA nanofiber (b) Morphology structure for 0.3g MoS_2/CA nanofiber

However, the amount of MoS_2 nanosheet powder added into the solution to produce MoS_2/CA nanofiber has a limit. As can be observed in Fig. 4.2 (a), there were tangling of small diameter nanofiber onto another nanofiber. This phenomenon called as micro-coiling which is a common phenomenon when using electrospinning process to produce nanofiber mostly fall under the cause of bending instability. Micro-coiling can be seen when the amount of MoS_2 increase when mixed with fixed amount CA as previous sample figures do not exhibit any coiling. It can be concluded viscosity plays a vital role in contributing to bending[48].Viscosity of sample increases due to increase amount of MoS_2 added as it making the solution more concentrated. As the amount of MoS_2 increase, more micro-coiling observed in the Figure 4.2 (b). Hence it proved the bending instability is acting much greater due to higher amount of MoS_2 leading to more viscous solution. Thus, making the manipulating variable lower than 0.2g of MoS_2 nanosheet powder is crucial to avoid any consequences especially micro coiling nanofiber as this phenomena disrupt the mechanical and physical properties of MoS_2/CA nanofiber where it makes the surface functionalization process more complex in terms of sensitivity and surface area properties. In conclusion, we devised to carry our further analyse focusing on 1 main, 0.05g MoS_2/CA sample, which exhibited a smooth structure and optimum morphology of a nanofiber which does not possessed any micro-coiling where it can disrupt the sensing capability by causing obstacles for the biomolecules to be bind on the surface of the biosensor.

4.3 TRANSMISSION ELECTRON MICROSCOPY (TEM)

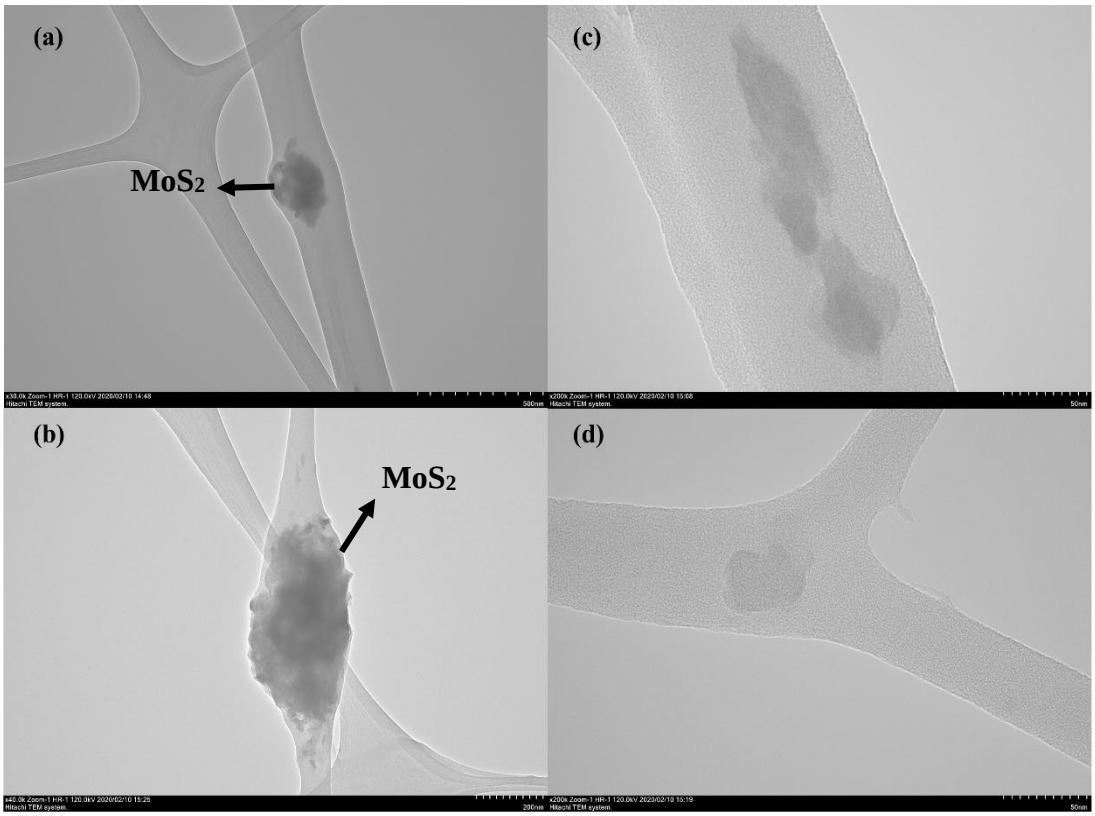


FIGURE 4.3: (a) MoS_2/CA nanofiber under 30 000 magnification. (b) MoS_2/CA nanofiber under 40 000 magnification. (c)(d) MoS_2/CA nanofiber under 200 000 magnification

The morphology structure of MoS_2/CA nanofiber had been further studied with the application of Transmission Electron Microscopy (TEM). As observed in all illustrations in Figure 4.3, the MoS_2 nanosheet embedded or engulfed into the nanofiber. Thus, demonstrated that MoS_2 nanosheets are highly dispersed within cellulose acetate nanofibers. This structural feature is an advantage for increasing the volume expansion [49]. The method of encapsulation had proven by this result as the MoS_2 layered inside CA. Encapsulation structure said to exhibit advantages in terms of good stability and tuning properties [50]. The main purpose of hybridizing MoS_2 nanosheet into CA nanofiber to produce a nanostructure that has good sensing abilities with excellent conductivity properties. Thus, by HRTEM, hybridization process of MoS_2 into CA nanofiber had proven visually successful with the applied method, electrospinning.

4.4 FOURIER TRANSFORM-INFRARED(FTIR) SPECTROSCOPY

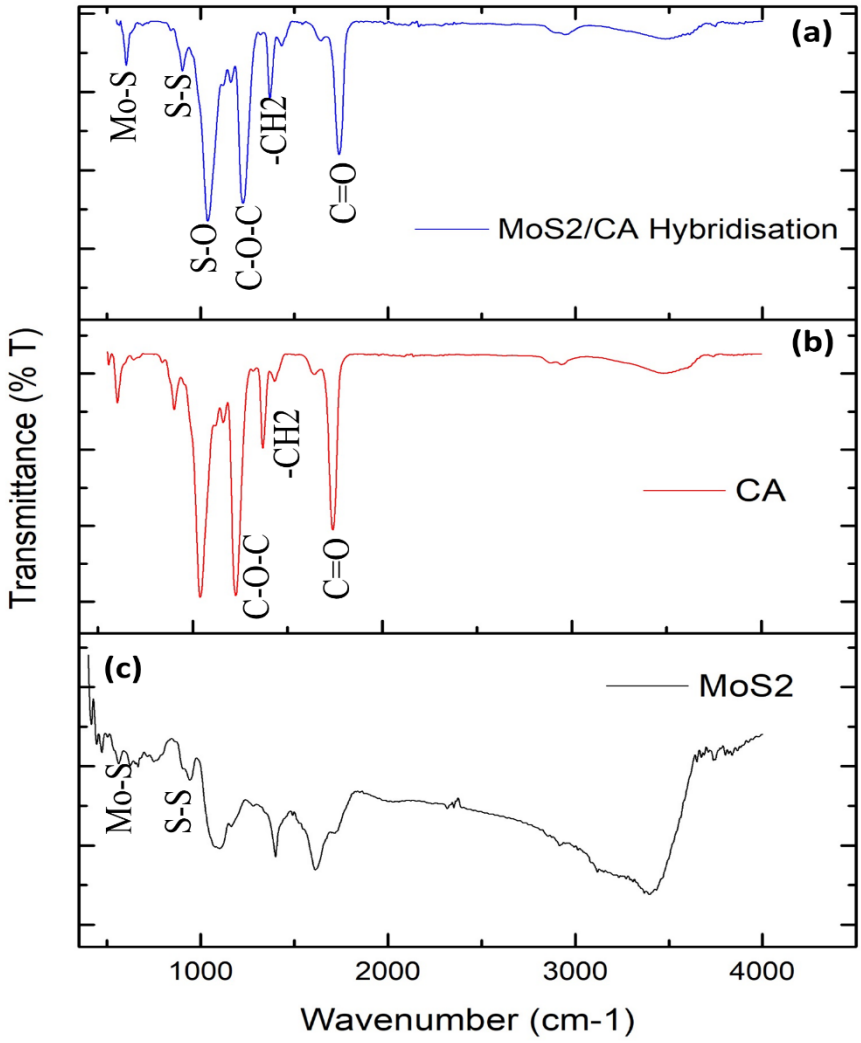


FIGURE 4.4: FTIR spectrum (a) MoS_2/CA nanofiber hybridisation (b) pure cellulose acetate (c) MoS_2 nanosheet

FTIR spectroscopy studies the determination of functional group or element exist in a sample by its vibrational characteristic. FT-IR spectroscopy permits important understanding into the diverse useful gatherings that are available in a framework by estimating the vibrational frequencies of the chemical bonds included. The peak observed at 606cm-1 in Figure 4.4(a) belongs to Mo-S bond as it analysed to be shifted from original spectra value which is 469cm-1 due to mixture reaction of MoS_2 with CA [51]. The peak at 1036 cm-1 contributing to vibration of Sulphur element from MoS_2 together with oxygen element from CA where can be detected by S-O bond [52]. The absorption peak at 906 cm−1 is attributed to the stretching of S-S present in MoS_2. The presence of CA can be proven by main present of bonds which are C-O-C and

C=O that can be observed at 1234 cm-1 and 1758 cm-1 respectively [53]. The -CH2 deformation vibration occurred at 1372 cm-1 for pure cellulose acetate has been shifted to 1369 cm-1 for MoS_2/CA. As shown in Figure 4.4(b) and in Figure 4.4(c), the both FTIR spectra showed peak values for pure cellulose acetate and MoS_2 nanosheet respectively. The analysis of spectra obtained by hybridization of MoS_2/CA further confirmed and compared with the mentioned spectra earlier. The wavenumber obtained for each peak of functional group presents in MoS_2/CA hybridization had exhibited almost similar wavenumber which obtained through only cellulose acetate FT-IR spectra and MoS_2 nanosheet FT-IT spectra hence further proving presence of CA and MoS_2 nanosheet in the MoS_2/CA nanofiber hybridization.

4.5 X-RAY DIFFRACTION SPECTROSCOPY (XRD)

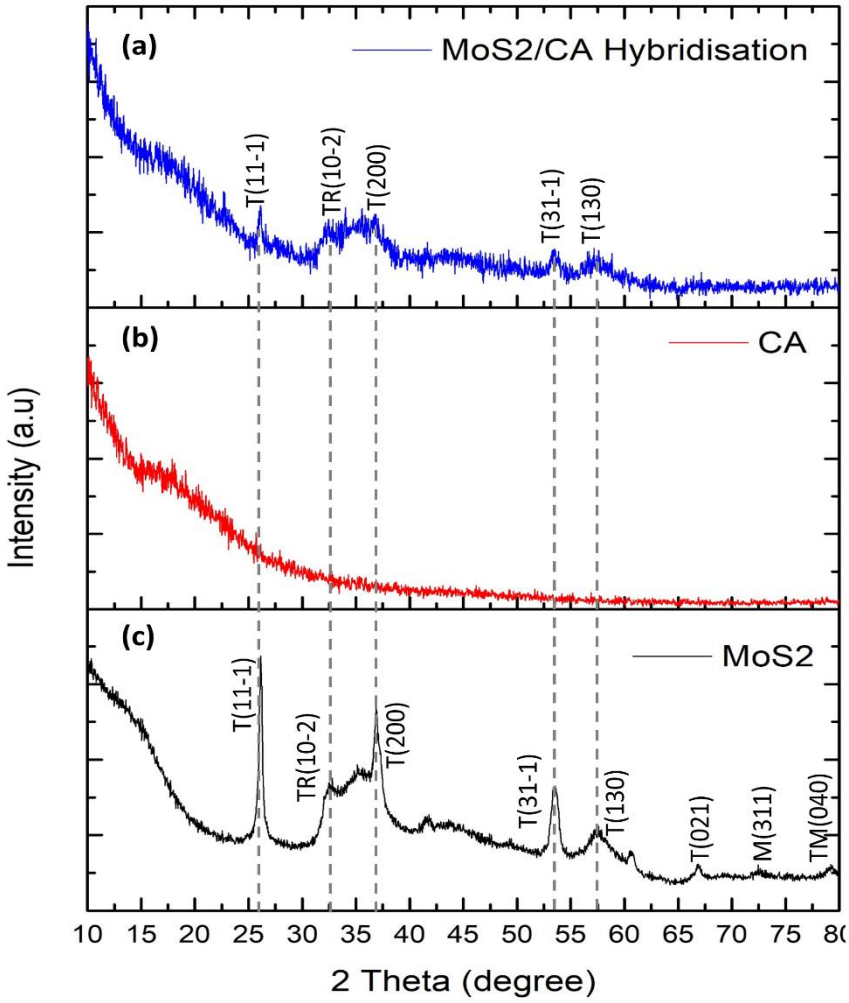


FIGURE 4.5 : XRD spectrum (a) MoS_2/CA nanofiber hybridisation (b) pure cellulose acetate (c) MoS_2 nanosheet.

As shown in XRD Figure 4.5(a), it can be analysed the sample are in amorphous form as there is no clear diffraction peak observed on the XRD spectrum[12]. However, by observing in detailed of XRD spectrum from MoS_2/CA nanofiber hybridisation, there is diffraction at peak of 15° where it resembles same diffraction in cellulose acetate spectrum. Thus, proved the present of cellulose acetate in the hybridisation. Based on Figure 4.5(c) where resembles XRD spectrum of MoS_2 nanosheet only , the diffraction peaks found at 26.1°, 32.8°, 36.9°, 53.5°, 60.6°, 66.9°, 72.9° and 79.5° reflecting onto the T(11-1), TR(10-2) , T(200) , T(31-1), T(130), TM(021), M(311) and TM(040) planes. The diffraction peak (002) at nanosheet result was reduced which recommend that the nanosheet was not firmly stuffed or packed. Observation in Fig. 4a, the spectrum exhibited certain similar diffraction peaks with XRD spectrum obtained in Figure 4.5(b) and Figure 4(c) such as the T(11-1), T (200), T(31-1) and T(130) planes resembles presence of MoS_2 nanosheet in MoS_2/CA nanofiber hybridisation. It had been identified the reason behind minimal shape of peak diffraction in hybridisation compare to MoS_2 nanosheet diffraction although at same degree due to MoS_2 nanosheet were successfully dispersed and layered into the CA nanofiber as can be observed in morphology analysis earlier.

4.6 IMPEDANCE MEASUREMENT FOR SURFACE FUNCTIONALIZATION

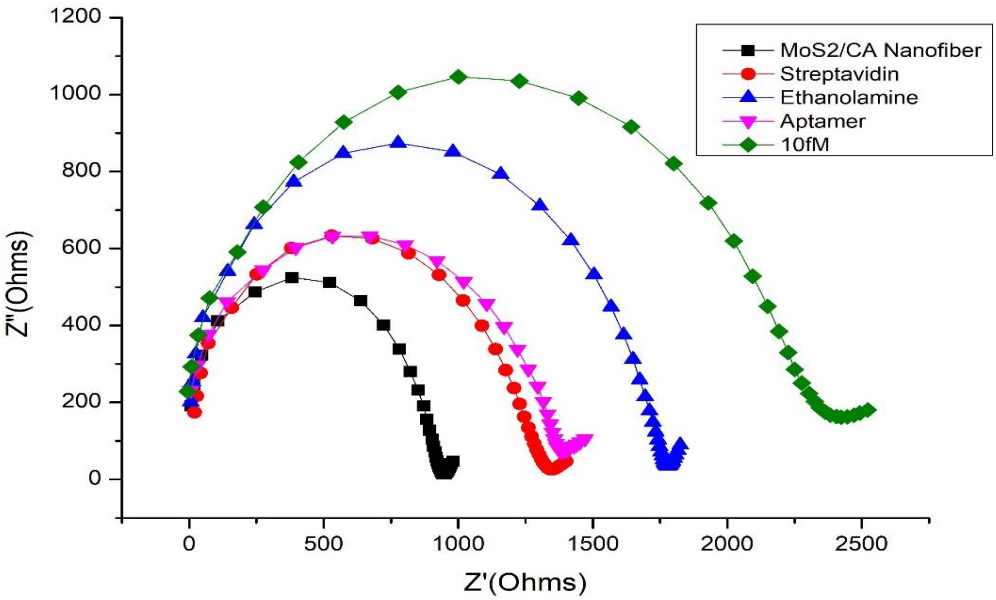


FIGURE 4.6 : Impedance spectra for each surface immobilization process

Before doing detection for serum or target, we had ensured the mentioned surface functionalization were successful procedure by analysing with impedance spectra with the help of Electrical Impedance Spectroscopy (EIS). Randles equivalent circuit exhibit the important and crucial parameters for the analysis which are the Rct, Ra and CPE where Rct resembles the charge transfer resistance, Ra shows the bulk solution resistance and CPE stands for constant phase element. From EIS, Nyquist plot can be obtained where it defines the charge transfer resistance, Rct as the semicircles as observed in Figure 4.6 reflects on the Rct. The progressions in Rct unmistakably show the surface charge resistance move was ended, prompting the expansion in charge transfer resistance. Based on Figure 4.6, the changes of each semicircle diameter indicates the changes in electrical properties of the modified electrode surface upon each immobilization step. By observing the MoS_2/CA nanofiber immobilization impedance reading, it showed the smallest semicircle among others with value of 948Ω.With immobilization of streptavidin , the semicircle diameter increase with value of 1335Ω indicating the streptavidin was successfully immobilized on the MoS_2/CA modified electrode. The charge transfer resistance of the surface said to be increase due to existence of protein molecules in Streptavidin where causing the surface to be less conductive thus increasing the resistance. As for the blocking agent, Ethanolamine, dropped onto the modified surface, a further increment of impedance observed with value of 1774Ω. The impedance said to be increase due to the successful immobilization of ethanolamine to block the target binding onto the surface rather onto the streptavidin. As for the aptamer with biotin-linker added onto the modified electrode, the impedance spectrum exhibit almost similar in size with streptavidin's impedance spectra but with slightly higher value of impedance which was 1382Ω.The reading proved that rather binding onto the surface , the aptamer-biotin linker had bind completely right on the streptavidin binding sites hence exhibiting almost similar electrical characteristics with streptavidin impedance spectrum .Moreover, it also proven that ethanolamine as blocking agent had prevent bio-fouling from binding the aptamer-biotin linker attached onto the non-streptavidin covered electrode surface.

4.7 DETECTION OF TROPONIN I ON IMMOBILISED SURFACE

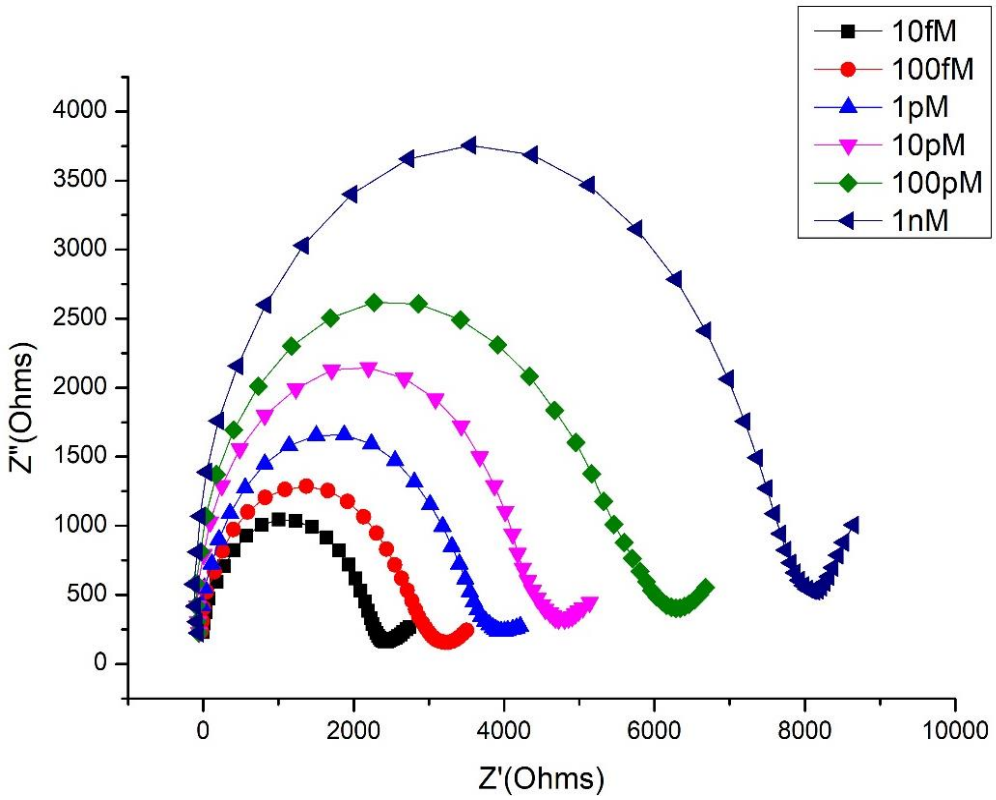


FIGURE 4.7: Impedance spectra of target sequencing

As the surface functionalization proved to be success with immobilization of aptamer, the electrode needed to be analysed further for limit of detection with different range of Troponin I concentrations (10fM - 1nM) applied onto the aptamer modified electrode and measured by EIS. Based on the Figure 4.7, it can be observed that by increasing the concentration of Troponin I hybridized on the modified electrode, the charge transfer resistance increases as well as the diameter of semicircle for each concentration increase from lowest concentration to highest concentration. The lowest concentration of Troponin I, 10fm, recorded value of 2423Ω and the 100fm Troponin I displayed 3163Ω where it showed increment of impedance value numerically as well. Furthermore, the value of impedance for 1pm,10pm,100pm and 1nm are 3805Ω, 4801Ω , 6220Ω and 8131Ω respectively. The Rct value sharply increase as the concentration increase because the number of binding sites increment on the surface of the electrode due to increase of negative charge ions in proteins and increment of the surface coverage of Troponin I [54] .

4.8 ANALYTICAL PERFORMANCE OF TROPONIN BIOSENSOR

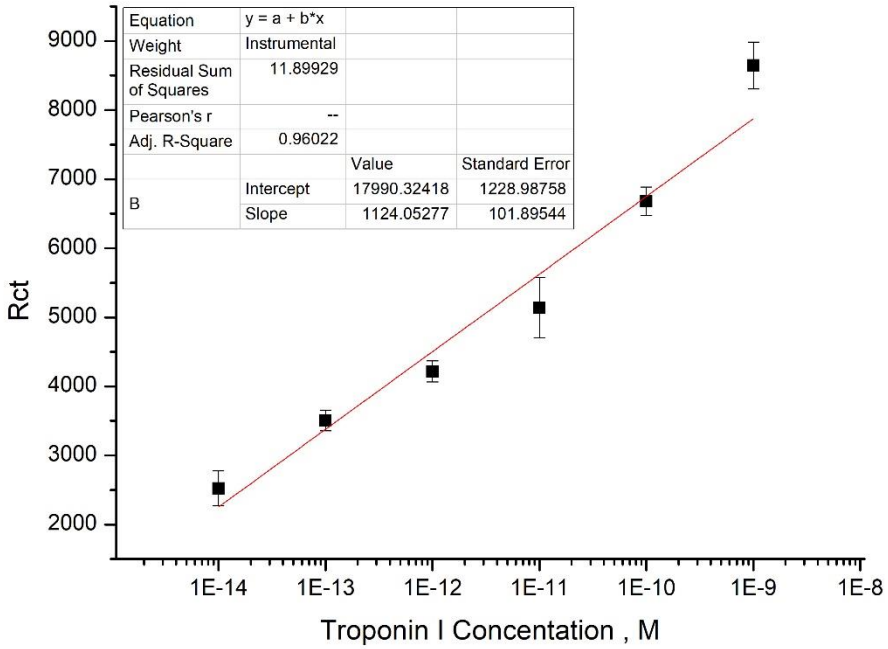


FIGURE 4.8: Linearity of the modified sensor based on different concentrations of Troponin I

The sensitivity of the biosensor can be concluded that it can sense range up to 10fM as the reading previously obtain during hybridization of Troponin I as the impedance spectrum exhibited a solid electrical property by the shape of the curve. Hereby the detection limit of the sensor can be concluded as 10fM (1×10-14) The sensitivity can be further analysed by studying the linear equation illustrated in Figure 4.8. The linear regression, R2, shows a value of 0.96022, where almost equal to 1 and thus contemplating of proving the sensors have good linearity sensing system by showing linear relationship with increment of Troponin I concentration. The sensitivity formula as below [55] :

$$Sensitivity = \frac{Slope\ of\ calibration\ plot, m(\mu A\ \mu M^{-1})}{Active\ surface\ area,\ A(cm^2)}$$

The active surface area of SPE found out to be A=0.1257cm$_2$ and slope of calibration gave a value of the obtained sensitivity or detection limit of this developed sensor has been found out to be better compared to other achievement with other sensors to detect

Troponin I. Sensitivity of the sensor was calculated to be 8942.32 μA μM^{-1} cm^{-2} The current progress shows 90% of sensitivity higher than a recent source that developed sensor with detection limit of 110fm [56].

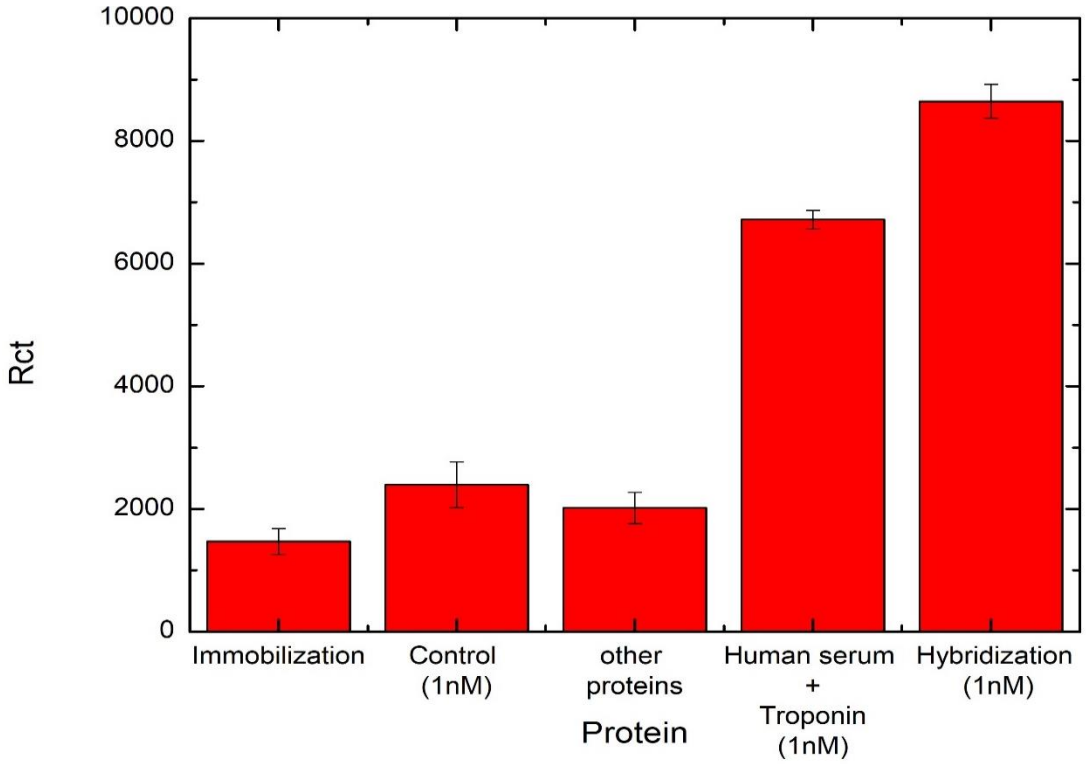


FIGURE 4.9: Selectivity of sensor on specific target

Furthermore, to analyse the selectivity of type of biomolecules detection , different types of concentration had been used such as control (Troponin T) , other proteins and human serum .Figure 4.9 shows the Rct value for each type of concentration or condition immobilized on the electrode. It can be analysed that the value of the charge transfer resistance does not significantly change or differ among the concentration with unrelated molecules such as the control and the concentration with other protein. The values reflect almost same Rct value of the immobilized electrode where it means that the sensor does not able to detect unrelated biomolecules as the aptamer was modified onto the electrode surface to capture specifically Troponin I proteins only. However, for detection of human serum with Troponin I concentration and immobilization of target (Troponin I) only, the values show great Rct readings compare to control and other protein concentration. Hence, it can be proven that the aptamer-biotin linker functionalized on the electrode surface capture and detect only Troponin I proteins.

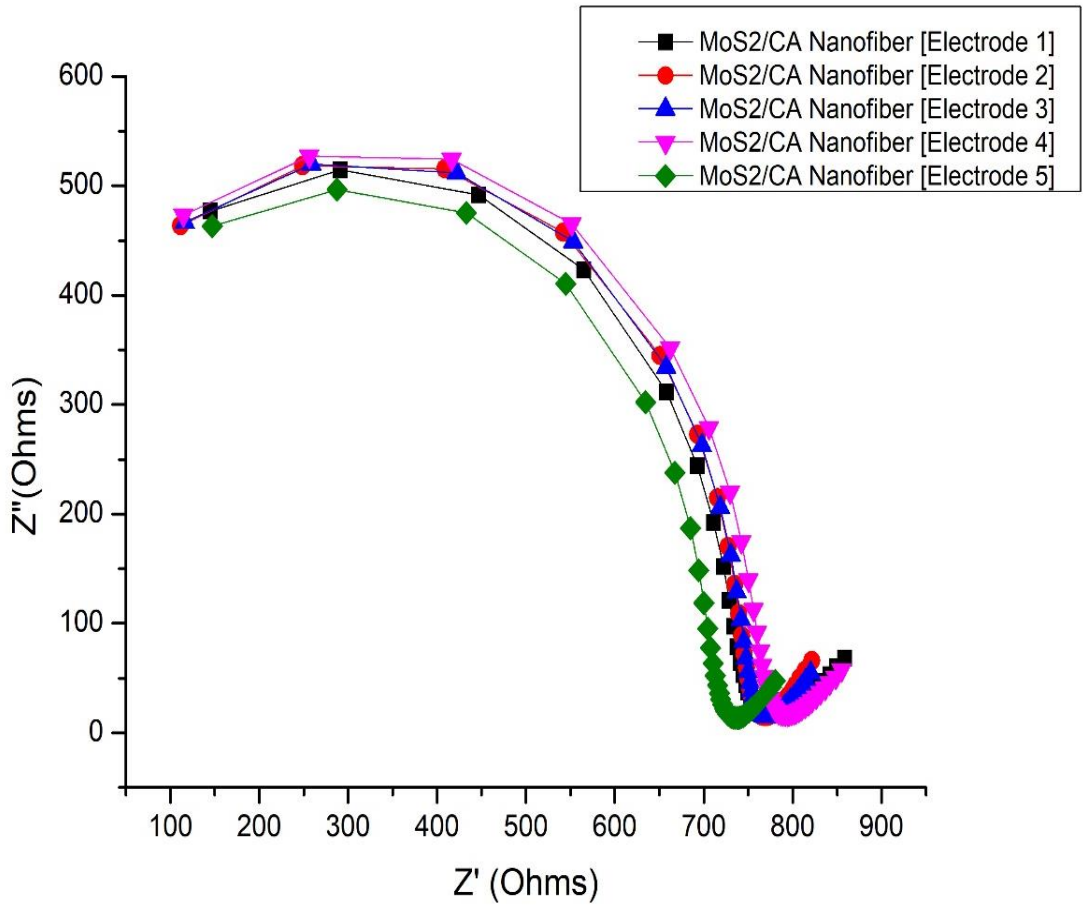


FIGURE 4.10: Impedance spectra on repeatability check of each SPE used in this research

The repeatability check was done by analysing the impedance spectrum for SPE electrodes used for target concentration, human serum and control detection. As shown in Figure 4.10, the impedance spectra of all electrodes used after MoS_2/CA nanofiber immobilization on electrode surfaces showed almost similar impedance spectrum by observing the semicircles diameter of each electrodes. This can be summarized into no significant differences found and can be concluded as high analytical performance of the sensing system of each electrode used in this experiment with immobilisation of MoS_2/CA nanofiber.

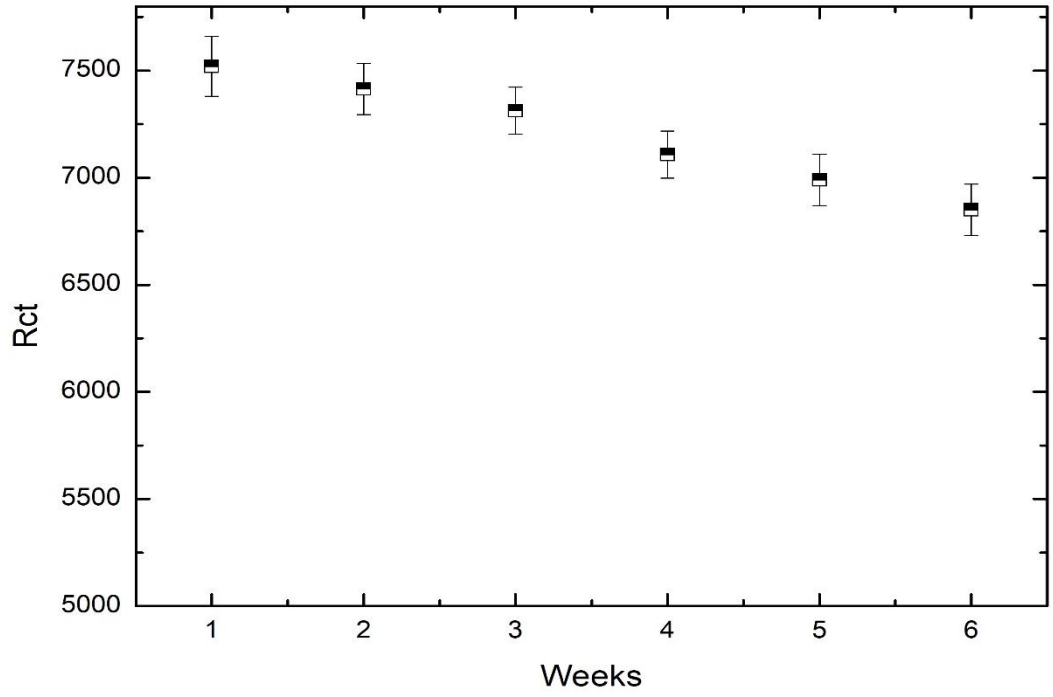


FIGURE 4.11: Stability analysis throughout 6 weeks continuously to determine the performance rate and durability of the developed sensor.

Based on Figure 4.11, rate of functionality declined with small difference between weeks with performance percentage rate of 90 % where it exhibited an excellent durability and functionality over the weeks passed by. The sensor said to be durable as active material build based on nanofiber structure said to be exhibit good mechanical properties in terms of robustness and strength [57]. Although weeks passed by, the electrodes exhibited quality result over troponin detection which reflect the durability of the active material further proving it exceptional mechanical properties to uphold the integrity over long time. The changes of charge resistance transfer between Week 1 (7519.83 Ω) and Week 6 (6850 Ω) calculated to have percentage difference of 8.91% where the margin still fall under high sensitivity and analytical performance of the sensor over the period.

4.9 ASSESSMENT OF TROPONIN I DETECTION IN HUMAN SERUM

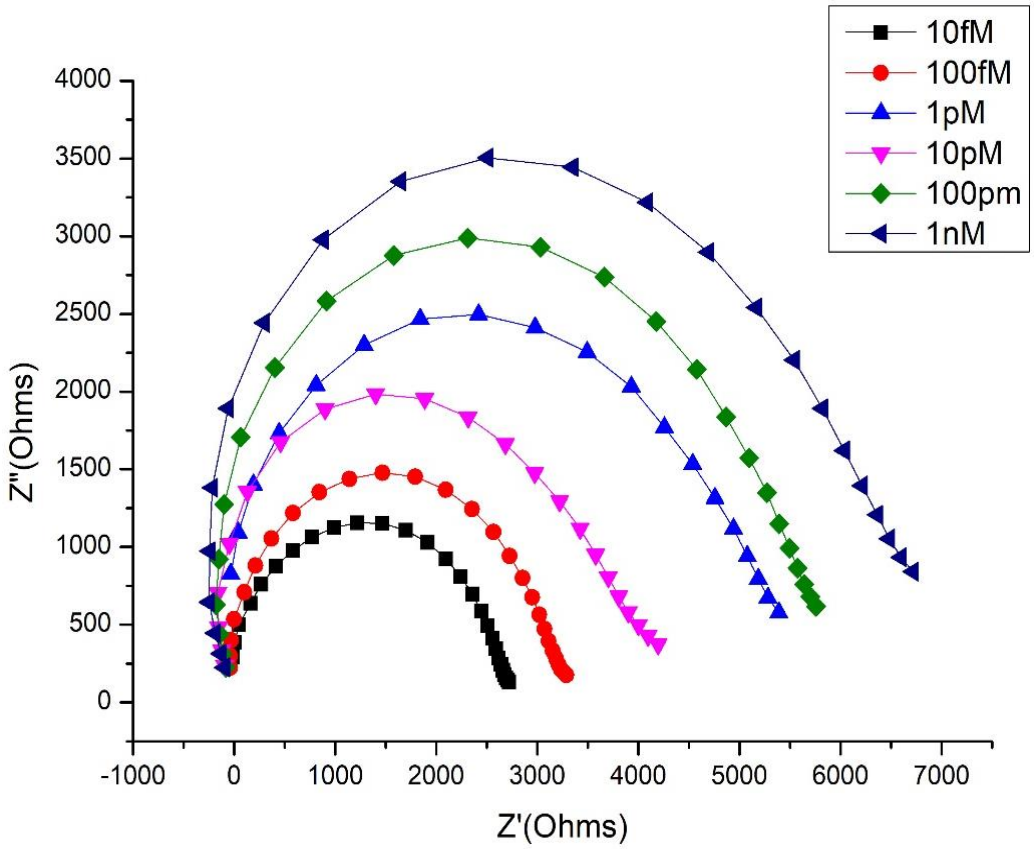


FIGURE 4.12: Impedance spectra of human serum detection with different range concentration

As illustrated in Figure 4.12 , EIS readings for detection of Troponin I in human serum been carried out to study the recognition of modified electrode towards Troponin I mixed among other proteins in human serum and also to explore the selectivity of develop sensor in real life application. Similar to target detection, the charge transfer resistance increases as the human serum concentration increase implies sensor succeed to detect specifically Troponin I in human serum as only the specified protein been captured by the aptamer on electrode. Therefore, the modified aptamer-biotin linker on the MoS_2/CA nanofiber as biological recognition molecule to selectively capture Troponin I during detection can be potentially used for clinical purposes.

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

5.1 CONCLUSION

In this research, using Screen-Printed Electrodes (SPE) and EIS, a nano-level and sensitive sensor developed for detection of Troponin I was demonstrated. SPE used for signification superiorities compared to other conventional electrodes such as great adaptability with surface functionalization, fast response from analyte ,disposability and excellent reproducibility of detection and results [58]. The most crucial and dominant advantages of this sensor based on interpreted results are higher sensitivity by having a detection limit in low concentration of Troponin I, great stable surface, remarkable specificity and durable as active metal nanofiber showcase a great mechanical property with appealing analytical high performances.

One of the objectives which to synthesize MoS_2/CA had been achieved by electrospinning process and the characterization proceeded with FESEM, TEM, FTIR and XRD to analyse the nanofiber properties. With the aid of the aptamer immobilized on the electrodes, the target hybridization done with ease due to higher surface capture ability contributed by the MoS_2/CA nanofiber which immobilized beneath aptamer. Even in the presence of human serum, the results obtained from the detection shows a signification increment of the sensitivity of sensor where the detection limit up to 10fM.Furthermore, repeatability and selectivity had been demonstrated to analyse the performance of used SPEs in this study.

5.2 RECOMMENDATIONS

The main key or the wonder material in this research would be the Molybdenum Disulphide as the application of MoS_2 in current researches are rare and uncommon. Hence, instead of Cellulose Acetate, other polymers such as Polyvinyl Alcohol (PVA),

Polyacrylonitrile (PAN) or Polylactic acid (PLA) can be applied to produce MoS_2 nanofiber. This could be made a separate research to study the effects of different polymers used with MoS_2 on biosensing capabilities of the developed nanofiber material with any specific targets.

This study can increase the horizon of detection for any biomarkers in medical field at high output and multiple diagnoses level. Introducing Internet of Things (IoT) network in biomedical field will further enhance the process of diagnosing and treatment with any related biosensing capable disease. This can be done by the patient to use the biosensor whenever or wherever he or she is, which the data of the sensing will directly send to doctor or medical operators to analyse the person's health condition.

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