Electrochemical Techniques-Based approaches for Mycobacterium Tuberculosis Detection: Last Decade Review

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Abstract. Mycobacterium Tuberculosis (MTB) is a common airborne infectious disease that leads to millions of deaths every year worldwide. It is still one of the top ten causes of death and the victims of TB are more than HIV/AIDS in 2017. Traditional approaches for MTB detection are either take a long time, unreliable or high cost. The electrochemical techniques (ECTs) as improved and inexpensive approaches to detect the MTB. Many of ECTs were used in MTB detection such as differential pulse voltammetric (DPV), cyclic voltammetric (CV), square wave voltammetric (SWV), amperometric and impedimetric. Principle of the MTB detection using ECTs depends on DNA hybridization of the MTB on the working electrode of ECTs. The researchers developed biosensors or aptasensors and used them for ECTs analyzing to detect the MTB. They developed various biosensors from various composite and DNA probes but all the developed composite of the biosensors were used to coat the electrodes that used in ECTs. Many types of electrodes and electrolytes were used in MTB detection. The most used ECTs in MTB detection is DPV and CV while the least used is amperometric. The ECTs for MTB detection achieved high sensitivity, reliability, low detection time and very low detection limits.

1. Introduction

Mycobacterium Tuberculosis (MTB) is a common airborne infectious disease that leads to millions of deaths every year worldwide [1]. In 2014, about one million children got TB out of which 14% died [2]. TB is still one of the top ten causes of death and the victims of TB are more than HIV/AIDS in 2017 [3]. Usually, MTB attacks and affects lungs but some times it affects bones, abdomen, lymph nodes and genitourinary tract [3]. Traditional approaches for MTB detection are either take a long time or high cost. Approaches such as the chest X-ray [4], sputum smear microscopy and skin test is lack specificity [5]. Also, there are other approaches for MTB detection but these approaches are considered as expensive such as antibody-based Enzyme-Linked Immunosorbent Assay (Antibody ELISA) [6] and polymerase chain reactions (PCR) [7].

Recently, many studies directed to use electrochemical techniques (ECTs) as improved and inexpensive approaches to detect the MTB. A bunch of ECTs were used in MTB detection such as differential pulse voltammetric (DPV), cyclic voltammetric (CV), square wave voltammetric (SWV), amperometric and impedimetric. The ECTs are electrical measurements through chemical solutions. These measurements are classified into three types:
Potential difference (voltammetric) like DPV, CV, SWV and so on.
- Changes in current (amperometric).
- Changes in impedance (impedimetric).

Generally, the basic principle of ECTs measurements as shown in Figure 1 is based on measuring the current passed through the chemical solution (electrolyte). This current measured between two electrodes; namely, the working electrode (WE) and the counter electrode (CE). There is a third electrode also is dipped in the same chemical solution and it is called the reference electrode (RE) except the amperometric does not have this electrode or in sometimes it is shortened with WE, RE and WE are used to provide the excitation electrical signal to the chemical solution while the current is measured between WE and CE. In the amperometric, the measured current between WE and CE is used directly for the analyzing. Voltammetric is the study of the current as a function of applied potential that means the measured current through WE and CE electrodes is converted to a voltage. Impedimetric is the ratio of an incremental change in voltage to the resulting change in current that means the measured current through the WE and CE is used to find the impedance in the chemical solution [8, 9].

![Figure 1. The basic principle and setup of ECTs (1- WE, 2- CE and 3- RE) [10]](image)

In this paper, the ECTs that were used for MTB detection using DNA will be reviewed. We will focus on only three ECTs types were used in MTB detection; namely, voltammetric, amperometric and impedimetric. Some techniques of voltammetric will be reviewed in details and these techniques are DPV, CV and SWV. Also, the types and materials of the electrodes will be reviewed.

2. Detection of MTB Using Electrochemical Techniques

ECTs are rapid, easy and inexpensive approaches of MTB detection. For these reasons, many researchers worked on developing new and advanced approaches for MTB detection using ECTs. All studies in our literature focused on developing a DNA hybridization and immobilization (using DNA probes) for MTB detection using ECTs. The principle behind MTB detection using ECTs depends on DNA hybridization of the MTB on the working electrode of ECTs [11]. Hybridization of the targeted DNA on the electrode changes the impedance value of the electrode so that leads to change in the measured electrical current [11]. In MTB detection using ECTs, the researchers developed biosensors or aptasensors and used them for ECTs analyzing to detect the MTB. The researchers developed various biosensors from various composites and DNA probes but all the developed composites of the biosensors were used to coat the electrodes that used in ECTs [12]. There are four common type of working electrodes used in MTB detection screen-printed carbon electrode (SPCE) [12, 13, 14, 15, 16, 17], graphite [18] and a mechanical pencil as a graphite electrode (PGE), graphene or graphene oxide electrode (GOE) [19, 16] and bare gold.
disk electrode (BGE) [1]. To increase the surface area of the electrodes, the gold in nano-forms was used like gold nanoparticles (AuNPs) [13, 14, 19, 15, 11] and gold nanotubes (AuNTs) [20]. As mentioned before, the ECTs used for chemical analyzing by measure the electrical current through the electrolyte, in MTB detection also the electrolyte or indicator is an important part of ECTs and play a vital role in MTB detection. Methylene blue (MB) widely used as an indicator/electrolyte in MTB detection [12, 4, 18, 20, 21]. By monitoring the changes in MB oxidation current can indicate the MTB, because MB has a strong affinity for the free guanine bases [22]. Also, MB has high stability and extraordinary ability to transfer electrons[4]. There are other studies used other types of electrolytes like phosphate buffered saline (PBS) [23, 24] and potassium chloride (KCl) [17]. ECTs like DPV, CV, SWV, Amperometric and impedimetric will be reviewed and explained in brief below:

2.1. Differential Pulse Voltammetric (DPV)

DPV is a method of voltammetric with a small influence of capacitive current. It has better signal-to-noise ratio by attenuating the background currents. In DPV, the excitation electrical signal has constant magnitude and linear ramped electrical pulses [25]. The current sample is measured twice in each pulse period (once at the beginning of the pulse, and at the pulse’s end), and the difference between these two current values is used in the measurements [25, 26]. The electrical wave of the DPV is shown in Figure 2. DPV is widely used in MTB detection [11, 7, 19, 27] with various setups like voltage range and scan rate.

2.2. Cyclic Voltammetric (CV)

The CV is a potential-controlled reverse electrochemical process [28] also, it is widely used ECT in MTB detection [29, 11, 19]. Normally, the experiment of CV starts by scanning the potential from a user-defined initial potential value to final user-defined potential value then reverse the scanning to the initial value [28], as shown in Figure 3. There are many researchers used the CV in MTB detection with various setups like initial and final potential values, scanning rate and so on [11, 7, 19, 30].

2.3. Square Wave Voltammetric (SWV)

The SWV is a linear potential sweep voltammetric technique that uses a constant amplitude square wave formed in a staircase manner. In the SWV the current is measured at the end of each peak, and the current measured on the button peak (I_b) is subtracted from the current measured on the top peak (I_t). This difference current (I_t - I_b) is displayed as a function of the applied potential [32, 26]. The waveform of the SWV is shown in Figure 4. Since SWV and DPV have similar voltage sequences and exhibit similar peak shapes [33] it is a commonly used method in MTB detection [34, 35, 36].
2.4. Impedimetric

Impedimetric or electrochemical impedance spectroscopy (EIS) is an ECT used to measure the electrical impedance in the electrolyte. The impedimetric uses a range of frequencies to scan the electrolyte and this range of frequencies can be set by the user [37]. In our review, there are many studies that used the impedimetric in MTB detection [38, 1].

2.5. Amperometric

It is simple and basic ECT that used in chemical analyzing and it is depending on the changing in the direct electrical current between two electrodes dipped in the electrolyte (WE and CE) [39]. Amperometric is used in MTB detection for its simplicity, and it gives good results for MTB detection by using various configuration [7, 40, 30].

3. Summary

In this section, we will summarize (in Table 1) all studies that we reviewed about MTB detection using ECTs and some details about these studies like types of ECTs, potential range in volt (V), scan rate in millivolt.second\(^{-1}\) (mV .sec\(^{-1}\)), electrodes’ materials, electrolytes, DNA detection limits and other parameters like the range of frequencies of EIS. Only, working electrodes will be mentioned in the table because most studies use Ag/AgCl as a reference electrode and platinum as a counter (auxiliary) electrode.

Table 1. The summary of the reviewed studies about MTB detection using ECTs

| Ref. | ECTs | Potential range (V) | Scan rate (mV .sec\(^{-1}\)) | WE material | Electrolyte and/or indicator | Detection limit | Other parameters |
|------|------|---------------------|-----------------------------|-------------|----------------------------|----------------|-----------------|
| [1]  | EIS  | 0.01                | 50                          | Bare gold disk electrode (BGE) | Ferricyanide and KCl | 6 ng.μL\(^{-1}\) | EIS frequency =0.1 to 100 Hz |
| [12] | DPV  | 0.01                | 20                          | SPCE        | MB                          | 15 μg.mL\(^{-1}\) | -               |
| [18] | DPV  | 0.01                | 20                          | PGE         | MB                          | 15 μg.mL\(^{-1}\) | -               |
| [38] | DPV  | -0.5 to 0.5         | 50                          | BGE         | Ferricyanide and KCl        | 20 fg.mL\(^{-1}\) | -               |
| [11] | DPV  | -0.4 to 0.3         | 50                          | BGE         | buffered saline (PBS)       | 50 fM           | -               |

Figure 4. The waveform of the SWV [26]
| Method | Range | Sample Volume (μL) | Electrode Type | PBS Concentration | Ring Electrode | Ring Electrolyte | Frequency Range (Hz) | Accuracy |
|--------|-------|-------------------|----------------|------------------|----------------|----------------|---------------------|----------|
| DPV    | -0.4 to 0.3 | 50 | Glass carbon electrode (GCE) | KCl 0.33 mM | - | - | - | - |
| CV     | -1.5 to +0.7 | 20 | BGE MB and PBS | - | - | - | - | - |
| DPV    | -0.2 to +0.8 | 100 | SPCE MB | 7.853×10⁻⁷ M | - | - | - | - |
| DPV    | -1 to +1 | 50 | BGE PBS | 2.7046 μg.mL⁻¹ | accuracy=99.22% | - | - | - |
| DPV    | 0.1 to -0.3 | 50 | PBS | - | - | - | - | - |
| EIS    | 0.01 | - | BGE Ferricyanide and KCl | 10 fg.mL⁻¹ | EIS freq.=0.01 to 10⁵ Hz | - | - | - |
| DPV    | -0.3 to +0.4 | 100 | GCE PBS | - | - | - | - | - |
| DPV    | -0.6 to -0.1 | 50 | SPCE MB and PBS | 8.9×10⁻¹³ M EIS freq.=10⁻⁴ to 0.1 Hz | - | - | - | - |
| DPV    | 0.2 to 0.8 | 60 | Carbon nanotubes (CNT) PBS | 0.5 ± 0.2 fg.mL⁻¹ EIS freq.=0.1 to 10⁵ Hz | - | - | - | - |
| EIS    | 0.24 | - | GOE PBS | 0.9 fg.mL⁻¹ | - | - | - | - |
| DPV    | 0 to 0.4 | 50 | Fabricated PBS | 1.25 ng.mL⁻¹ EIS freq.=0.01 to 10⁵ Hz | - | - | - | - |
| DPV    | 0.2 to 0.6 | 50 | Silk screen printing ITO | 0.01 ng.mL⁻¹ | - | - | - | - |
| DPV    | -0.6 to 0.1 | 50 | Indium-tin-oxide (ITO) PBS | 1×10⁻¹² M EIS freq.=0.01 to 10⁵ Hz | - | - | - | - |
| DPV    | 0.7 to -1.1 | 50 | BGE Sodium chloride | - | - | - | - | - |
| DPV    | -0.6 to 0.8 | - | indium-tin-oxide (ITO) PBS | 1×10⁻¹² M EIS freq.=0.01 to 10⁵ Hz | - | - | - | - |
| EIS    | 0.01 | - | - | - | - | - | - | - |
| DPV    | 0 to 0.4 | 50 | SPCE MB and PBS | 10 pg Sensitivity=95% | - | - | - | - |
| DPV    | -0.4 to 0.1 | 50 | ITO PBS | 0.00078 μM Sensitivity=6.38×10⁻⁶ AmM⁻¹ | - | - | - | - |
| DPV    | -0.4 to 0.8 | - | SPCE MB and PBS | 10 pg.mL⁻¹ | - | - | - | - |
| CV     | -2.5 to 2.5 | - | BGE PBS | 7.853×10⁻⁷ M | - | - | - | - |
| DPV    | -0.2 to 0.8 | 100 | SPCE MB and PBS | 10⁴ cfu/mL EIS freq.=0.1 to 10⁵ Hz | - | - | - | - |
| CV     | -0.4 to 0.6 | - | BGE PBS | 3.3×10⁻⁵ ng.mL⁻¹ | - | - | - | - |
| EIS    | -0.70 to 0.30 | 100 | GCE PBS | 3.3×10⁻⁵ ng.mL⁻¹ | - | - | - | - |
| EIS    | -0.20 to 0.60 | 100 | - | - | - | - | - | - |
| SWV    | -0.5 to 0 | - | ITO MB | - | - | - | - | - |
4. Conclusion

In this paper, we reviewed the studies about MTB detection using ECTs. Five types of ECTs were reviewed; namely, DPV, CV, SWV, EIS and amperometric. Also, parameters of the ECTs, electrodes composites, electrolytes and DNA detection limits were reviewed. The most used ECTs in MTB detection are DPV and CV then EIS followed by SWV and finally amperometric. The ECTs for MTB detection achieved high sensitivity, reliability, low detection time and very low detection limits. Also, ECTs for MTB detection become widely used in point-of-care as a commercial MTB detection approaches.

5. Acknowledgment

This work financially supported under grant (UPM/800-4/11/MRUN/2018/5539230).

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