ENGINEERING RESEARCH JOURNAL (ERJ)

Vol. 1, No. 49 Jul. 2021, pp 163-175

Journal Homepage: http://erj.bu.edu.eg

A Novel Electronic System for Detecting Foodborne Bacteria in Vegetables and Fruits

Mourine F. Azmy[a], Michael N. Mikhael[b], Mohamed T. Elewa[c] and Gihan M.El Moghazy[d]

[a]Faculty of Engineering, Benha University, Cairo, Egypt,
morn_fayez@yahoo.com

[b]Faculty of Engineering, Benha University, Cairo, Egypt,
Michael.gerges@feng.bu.edu.eg

[c]Faculty of Engineering, Benha University, Cairo, Egypt,
tarek_elewa@yahoo.com

[d]Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt, gihanelmoghazy@gmail.com

Abstract Foodborne diseases are critical and become a greater problem over a while. World Health Organization shows that every year 420 000 die from eating contaminated food. Foodborne diseases are any illness resulting from pathogenic bacteria, viruses, and contaminated food. It is important to analyze the food and detect foodborne Bacteria to prevent or minimize diseases and ensure food safety. The designed system aims to detect the foodborne bacteria present in fruits using rapid detection technique instead of traditional methods. Many areas will be impacted by this early detection, for example, the economy, health, and agriculture societies. The system is divided into 3 main parts. First, Extracting DNA from food sample. Then sensing and signal manipulation part and finally signal analysis and bacteria detection. Electrochemical biosensors used for bacteria examination. 27 samples including tomato and apple samples were tested. The results show that each test takes from 12 to 500 seconds not including DNA extraction period with accurate results and cost-friendly.

Keywords: pathogenic bacteria, rapid detection methods, DNA, Biosensor, Cyclic Voltammetry.

1. Introduction

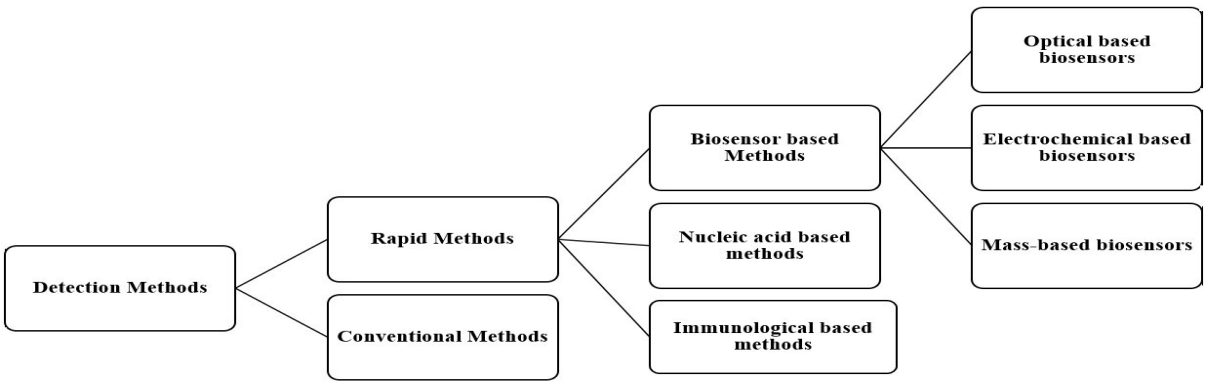
Food that includes harmful bacteria or viruses cause infections and Foodborne illnesses. It kills 420 000 every year from eating contaminated food worldwide [1]. Pathogenic Bacteria are any harmful bacteria that give rises to infections or poisoning. The general diseases/ symptoms result from Pathogenic bacteria are a miscarriage, premature delivery of a newborn baby, septicemia (blood poisoning), kidney failure, toxic shock syndrome, fever, malaise, headache, rash, whooping cough, bubonic plague, tuberculosis Salmonella and E.Coli are the most types of bacteria that the studies carried on in pathogens detection field [2].

As the detection is done in vegetables and fruits, Its good for the pathogenic bacteria detection to be rapid before food samples are expired. This will protect consumers from potential infections in time. Pathogens monitoring designs desired to be portable and highly robust systems. This required issue aims to enhance new studies and designs in the field of pathogenic bacteria detection. The obtained results from those design expected to be real-time, fast, on-site and reliable.

2. Literature Review

This section reviews a variety of methods that have been developed for the detection of foodborne pathogens as its required in many food analysis fields, as shown in figure(1).

Figure (1): Detection Methods.



2.1. The conventional methods

They are based on culturing the microorganisms on plates followed by standard biochemical identifications. The conventional method requires several days to give results [3]. The conventional Methods is inexpensive and simple method but it is time-consuming as they depend on the ability of the microorganisms to grow in different culture media with low sensitivity.

2.2. The rapid methods

They can be done in a few minutes to a few hours [4]. Rapid methods have high sensitivity. It can detect the presence of pathogens in raw and processed foods immediately, time-efficient, labor-saving and able to reduce human errors. But, it can detect only one specific pathogen. Rapid methods include Nucleic acid, biosensors, Immunological based methods [5]. Nucleic acid-based method is a higher sensitive method. It can be used for all categories of microbes not only bacteria but also many types such as viruses or fungi. But, they require trained personnel and specialized expensive instruments. Also, they require effective sample preparation to prevent contamination for successful detection [6]. Biosensors based method is a rapid, cost-effective, and easy to operate method and they do not require trained personnel but it has defects such as ambient disturbance which occurs to biological material that causes a drift in the output signal under measurements. The Immunological based method has high sensitivity. It is simple but sometimes false-positive results can be obtained and unable to indicate the viability of organisms [4].

2.2.1. Biosensor-based Methods

These Methods require a bio-receptor element responsible for recognizing the target substance whose chemical constituent being identified (analyte) such as nucleic acids, enzymes, or antibodies and a transducer that converts the biological interactions into an optical, electrochemical, mass-based, thermometric, micromechanical or magnetic measurable electrical signal. Unlike nucleic-acid based methods and immunological methods, those methods are easy to operate and do not require a pre-enrichment step which is used to promote the growth of a particular organism by enriching the sample of interest with the essential nutrients. Optical, electrochemical and mass-based biosensors are the most commonly used biosensors nowadays [4]. A Biosensor is short for "Biological Sensor". Generally, it is known as an analytical device that converts a biological reaction into an electronic processable signal. Biosensor systems consist of two main divisions: transducer and electronic sub-system.

(a)The transducer: It is used to produce electrical measurable signals from non-electrical energy resulting from a bio-recognition event according to interactions while analyte binding to the bio-receptor [4].

(b) An electronic sub-system: it is used for signal processing, amplification and results visualization [7].

The Electrochemical DNA Biosensor detection principles can be summarized as follows: an electrode acts as a conductor which directly contacts with the inspected solution. In electrochemical biosensors, both input and output are realized through the electrode.

The Detection principle is based on the variation in the electrical properties of the electrode after chemical reactions which causes measurable changes in current, impedance and voltage.

The three-electrode system is the most commonly used technique which consists of three electrodes; a reference electrode, a working electrode and a counter electrode fabricated in an insulating substrate. The working or counter electrodes can be from carbon or ceramic (noble metal such as gold or platinum) substrate [8]. The Carbon electrode is compatible only with aqueous solutions in which the solvent is water and can be used for single analysis. Despite ceramic electrode can be reused but it has a high cost. The reference electrode option is silver/silver chloride material for aqueous solutions because of its good stability in an aqueous solution containing chloride ions. Figure (2) shows the equivalent circuit of the 3-electrode system. The current generator responds to analyte chemical reacting at the working electrode. R and C are the resistance and capacitance respectively of a simplified model of the electric double-layer that forms at the electrode-electrolyte interface. The capacitor represents the double layer capacitance due to the interface between a conductive electrode and a substance which produces electrically conducting solution when dissolved in a solvent such as water (electrolyte) [9].

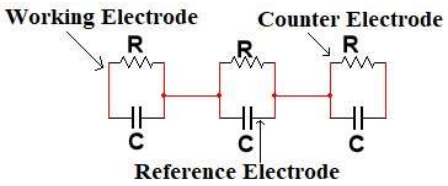


Figure (2): The Equivalent Circuit of 3-electrode System.

The working electrode surface is the place where the analyte makes contact with and the chemical reaction occurs. The electronic circuit path will be completed by the reference electrode and counter, the counter electrode which allows current to pass through it [10] [11].

The advantage of 3-electrode system over the 2-electrode system is that the 2-electrode cell just gives the current flowing between them and none of the electrode potential is fixed so the potential at which the reaction occurs is still unmeasurable due to counter electrode potential changing during measurements.

Electrochemical detection methods includes several types such as amperometry, potentiometry, impedimetry, and voltammetry [12]. Voltammetric methods include so many types but linear sweep voltammetry and cyclic voltammetry are the most commonly used techniques. In cyclic voltammetry, a set of potential ranges are applied to the working electrode with respect to the reference electrode. The applied voltages has a triangular shape characteristic as shown in figure (3). The waveform consists of a forward scanning process (increasing potential with respect to time) and a reverse scanning process (decreasing potential from switching point with respect to time).

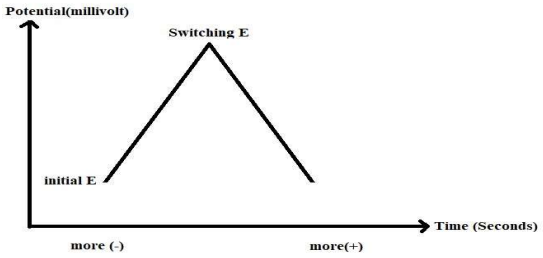


Figure (3): Cyclic Voltammetry Potential Waveform.

Then, a voltammogram curve is obtained from measuring the current passing through counter electrode as shown in figure (4). The current is proportional to the concentration of an analyte when varying the applied potential on the working electrode with respect to the reference electrode at some scanning rate (millivolt/second) in the forward and reverse processes [7]. The obtained voltammogram has two peaks if the process is reversible, one if the process is irreversible. I_{pa},E_{pa} for anodic (Oxidation) positive current and potential respectively. I_{pc},E_{pc} for cathodic (reduction) negative current and potential respectively [13].

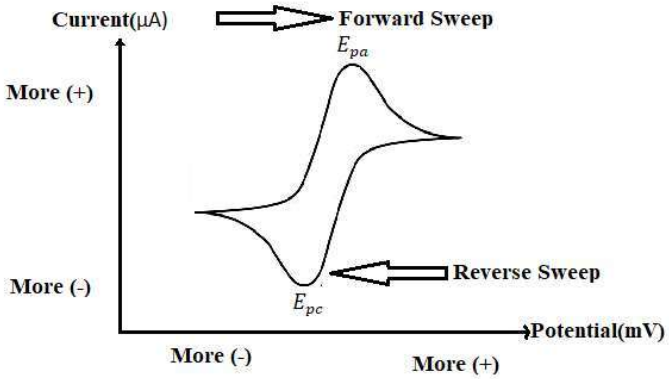


Figure (4): Cyclic voltammogram [14].

Electrochemical biosensors have advantages such as simple, cost-effective, and real-time sensors without sacrificing their sensitivity and selectivity. But, they are not suitable for analyzing the low amount of microorganism samples.

3. Experimental Methodology

Figure (5) shows the design of the electronic system for detecting foodborne bacteria.

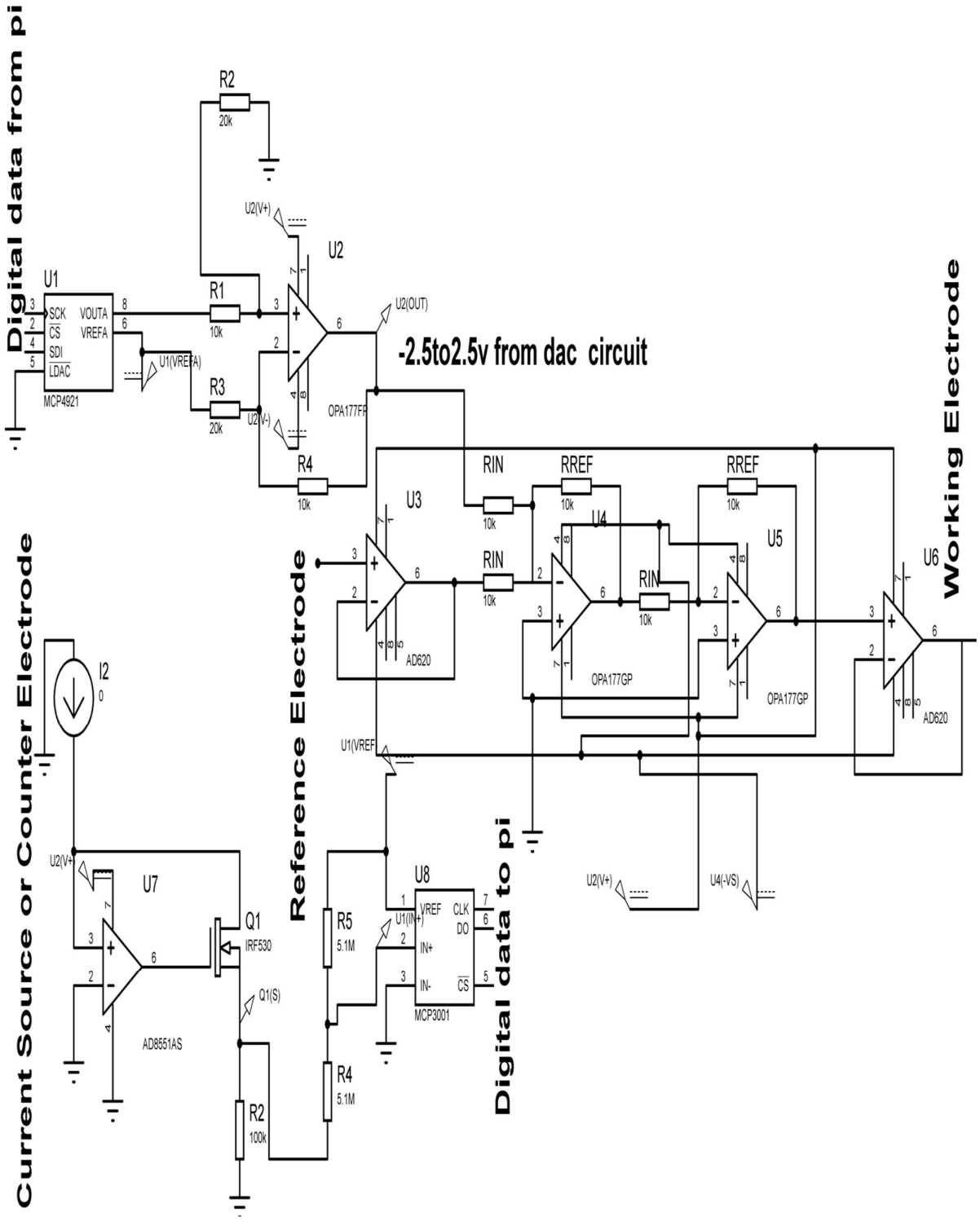


Figure (5): The electronic System.

System methodology describes the overall steps for bacteria detection. Besides, it includes the protocols used for both extracting DNA from the sample and amplifying its concentration as shown in figure (6).

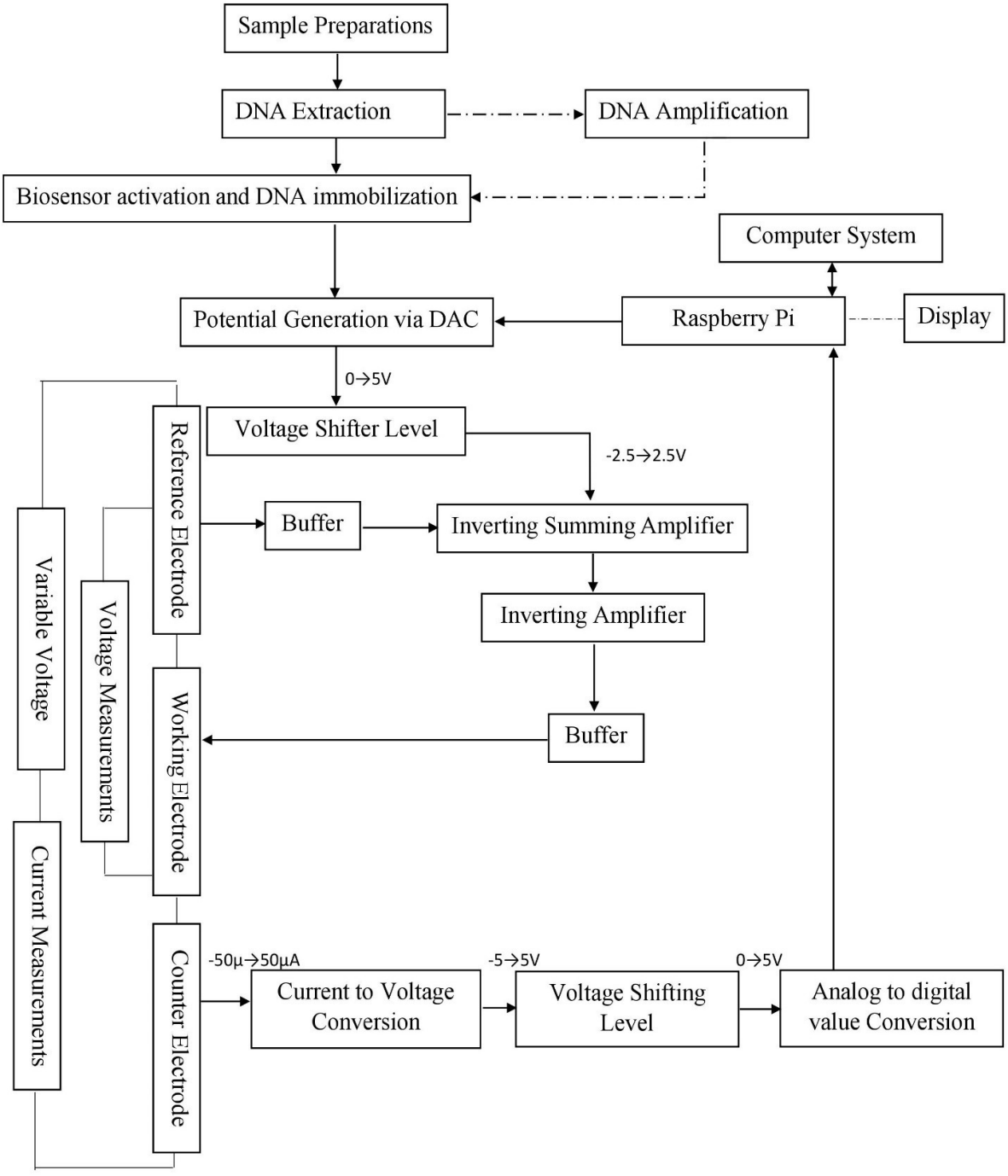


Figure (6): Bacteria Detection Process.

The following steps indicating the process for the bacteria detection starting from food sample preparation to the pathogenic bacteria detection:

1. The first step of the developed electronic system for bacteria detection is sample preparations then DNA extraction from inspected sample. Also, some results are carried by amplifying DNA samples to produce multiple copies of a sequence of DNA in order to enhance the system sensitivity. Figure (7) shows the protocols used for both extracting and amplifying DNA samples.

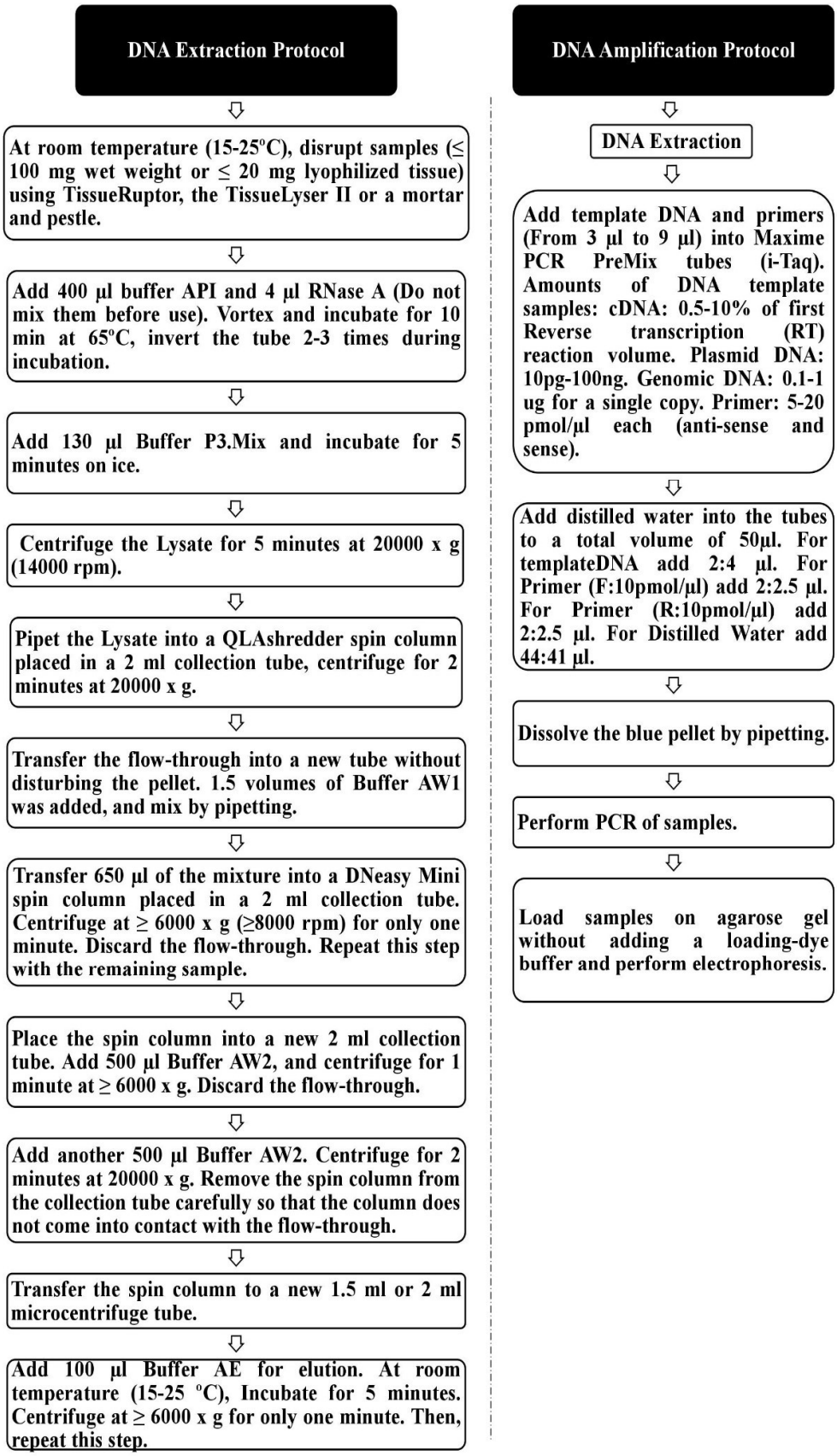

DNA Extraction Protocol
At room temperature (15-25°C), disrupt samples (≤ 100 mg wet weight or ≤ 20 mg lyophilized tissue) using TissueRuptor, the TissueLyser II or a mortar and pestle.
Add 400 µl buffer API and 4 µl RNase A (Do not mix them before use). Vortex and incubate for 10 min at 65°C, invert the tube 2-3 times during incubation.
Add 130 µl Buffer P3.Mix and incubate for 5 minutes on ice.
Centrifuge the Lysate for 5 minutes at 20000 x g (14000 rpm).
Pipet the Lysate into a QLAshredder spin column placed in a 2 ml collection tube, centrifuge for 2 minutes at 20000 x g.
Transfer the flow-through into a new tube without disturbing the pellet. 1.5 volumes of Buffer AW1 was added, and mix by pipetting.
Transfer 650 µl of the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at ≥ 6000 x g (≥8000 rpm) for only one minute. Discard the flow-through. Repeat this step with the remaining sample.
Place the spin column into a new 2 ml collection tube. Add 500 µl Buffer AW2, and centrifuge for 1 minute at ≥ 6000 x g. Discard the flow-through.
Add another 500 µl Buffer AW2. Centrifuge for 2 minutes at 20000 x g. Remove the spin column from the collection tube carefully so that the column does not come into contact with the flow-through.
Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.
Add 100 µl Buffer AE for elution. At room temperature (15-25 °C), Incubate for 5 minutes. Centrifuge at ≥ 6000 x g for only one minute. Then, repeat this step.
DNA Amplification Protocol
DNA Extraction
Add template DNA and primers (From 3 µl to 9 µl) into Maxime PCR PreMix tubes (i-Taq). Amounts of DNA template samples: cDNA: 0.5-10% of first Reverse transcription (RT) reaction volume. Plasmid DNA: 10pg-100ng. Genomic DNA: 0.1-1 ug for a single copy. Primer: 5-20 pmol/µl each (anti-sense and sense).
Add distilled water into the tubes to a total volume of 50µl. For templateDNA add 2:4 µl. For Primer (F:10pmol/µl) add 2:2.5 µl. For Primer (R:10pmol/µl) add 2:2.5 µl. For Distilled Water add 44:41 µl.
Dissolve the blue pellet by pipetting.
Perform PCR of samples.
Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.

Figure (7): Protocols for DNA Extraction and Amplification.

Figure (8) shows a compact voltammetry cell which is used in electrochemical measurements and to hold the sample inspected.

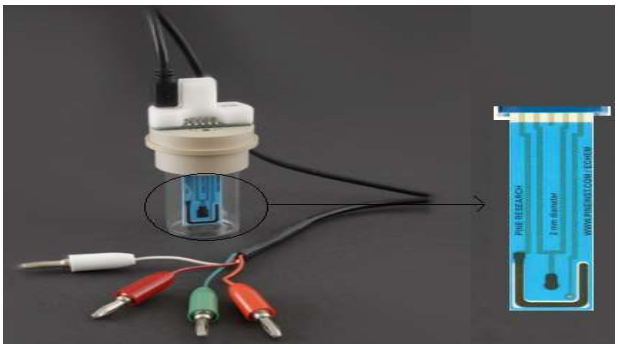


Figure (8): Compact voltammetry cell.

Equation (1) shows the relation between the peak current, the analyte bulk concentration and the scan rate.

$$I_P = 2.99\text{x}10^5 \text{ nAC}\sqrt{\acute{\alpha} n_a D v} \quad \textbf{eq.1}$$

Where

- I_P is the peak current in amperes.
- n is equal to the number of electrons gained in the reduction.
- A is the surface area of the working electrode in (cm^2).
- C is the bulk concentration of the analyte (mol/cm^3).
- D is the diffusion coefficient of the analyte (cm^2 /seconds).
- v is the scan rate (V/s).
- ά is the transfer coefficient.
- n_a is the number of electrons involved in the charge-transfer step.

The peak current is increased with the square root of the scan rate or at faster voltage sweep. Also, the current measured is directly proportional to the concentration of DNA or analyte [15].

2. Before testing any sample, the biosensor was activated via cyclic voltammetry in 0.1 NH_2SO_4sulfuric acid at extreme anodic and cathodic potentials (1mM, sweep rate = 200 mV/sec).
3. After DNA extraction from the sample, it is required to maintain the sensed reference electrode voltage at its value irrespective to cell activity so it is connected to the buffer amplifier (U3 AD620 in figure 5).
4. Voltage range (0→5volts) is generated from the controller via digital to analog converter. One channel with 10-bits resolution digital-to-analog converter is used to interface the analog signal conditioning circuit with raspberry pi controller board via SPI interface.

Equation (2) shows the relation between the analog output voltage and the digital input value of DAC (U1 MCP4921 in figure 5).

$$\text{Analog output voltage} = \frac{V_{ref} \times G \times D}{2^{10}} \quad \textbf{eq.2}$$

Where

- V_{ref} is the voltage reference value.
- G is the gain selection option which can be 1 or 2.
- D is the digital input value of DAC from 0 to 2^{10}.

5. The generated voltage range (0→5volts) is shifted by the differential amplifier (U2 OPA177FP in figure 5) to the range (-2.5→2.5volts) in order to get the maximum voltage applied to the biosensor according to its technical specifications [16].

Equation (3) shows the relation between the shifted voltage value and the analog voltage from DAC.

$$V_{shifted} = V_1 . \frac{R_2}{R_1 + R_2} \times \left(1 + \frac{R_4}{R_3}\right) - V_2 \times \frac{R_4}{R_3} \quad \textbf{eq.3}$$

Where

- $V_{shifted}$ is the output shifted voltage.
- V_1 is the Analog voltage from DAC.
- V_2 is a constant voltage to meet the system requirements.

In order to shift V_{out} according to equation 4:

$$V_{out} = V_1 - 2.5 \quad \textbf{eq.4}$$

Put V_2 =5Volts, R_3=2R_4 and R_2=2R_1.

6. The shifted voltage values are added to reference electrode voltage value (which results from buffer amplifier) via the inverting summing amplifier (U4 OPA177 in figure 5).
7. The summed value is inverted via the inverting buffer (U5 OPA177 in figure 5).
   In order to improve the system performance a precision operational amplifier OPA177 is used with low offset voltage (i.e. 25µV) and a high common-Mode rejection ratio of 140db [17].
8. It order to maintain the summed voltage constant and independent of the cell activity so it is important to buffer the working electrode. A low power instrumentation amplifier (U6 AD620 in figure 5) is used with low offset voltage (i.e. 50µV) and a high common-Mode rejection ratio of 100db [18].
9. When the working electrode is excited by one voltage value, the current is sensed immediately at the counter electrode.
10. The measured current range (-50→50µA) is then converted to a corresponding voltage value range (-5→5volts) via current to voltage converter circuit. It is preferable to use zero drift, low offset voltage, rail-to-rail Input/Output operational amplifier so AD8551 (U7 in figure 5) is selected for this purpose with the following specifications [19]:
    - Low offset voltage: 1 µV.
    - Input offset drift: 0.005 µV/°C.
    - Power Supply Rejection Ratio (PSRR): 130dB.
    - Ultralow input bias current: 20 pA.
11. The converted voltage value is then shifted to a range (0→5 volts) using a voltage divider circuit with a very high resistance values (i.e. 5.1MΩ).
12. A controller is used to acquire the shifted voltage values through a 10-bits resolution analog to digital converter (U8 MCP3001 in figure 5). Then, the controller is responsible for transferring the acquired data to a PC for further processing.
    For a better performance, the Raspberry pi 3 model B board was selected with the following specifications [20]:
    - CPU: 1.2 GHZ quad-core ARM Cortex A53.
    - Memory: 1 GB LPDDR2-900 SDRAM.
    - Support Micro-SD for external storage.
    - HDMI port for external monitor's connection.
13. These steps are repeated for each generated value that excites the working electrode. As a result, the current is sensed at the counter electrode producing a vector pair of current and voltage values to obtain a cyclic voltammetry curve. Hence, bacteria can be detected.

4. Results analysis

Two types of fruits (i.e. apple and tomato DNA samples) are used, some of the samples are tested as it is and the rest of the DNA samples were amplified in order to increase the DNA concentration to enhance the bacteria detection results. Fruits samples were injected by some types of bacteria like salmonella or E.coli bacteria to ensure that the sample contains bacteria before detection process. Bacteria in DNA samples were detected by Polymerase Chain Reaction (PCR) device in Agriculture Research Centre and compared with the designed system output and both (i.e. the designed system output and the PCR output) results were agreed together.

The following section summarizes some results from DNA samples using the designed system.

4.1. Cyclic voltammetry curves analysis

Figure (9) shows the cyclic voltammetry curves for an amplified DNA from tomato samples (i.e. one sample without bacteria and the other with E.coli).

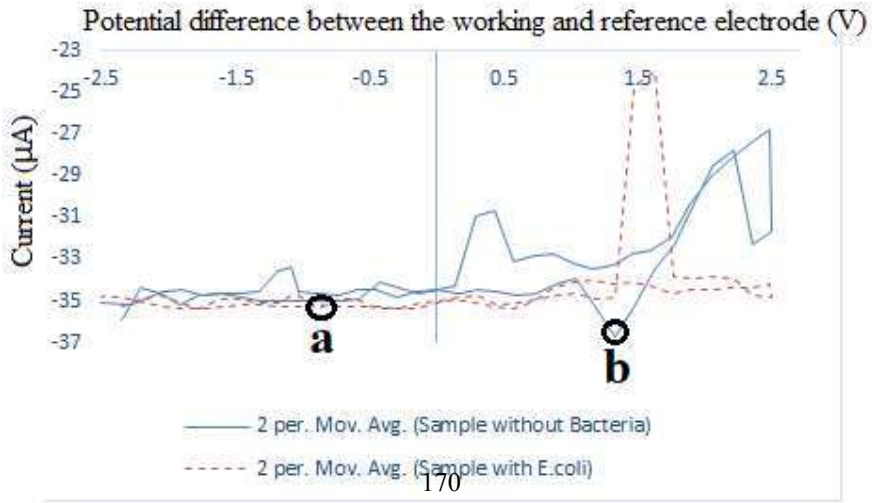


Figure (9): Cyclic Voltammetry Curve for an Amplified DNA from tomato samples contaminated with E.coli.
Figure (10) shows the cyclic voltammetry curves for DNA from apple Samples at Scan rate 0.1V/seconds (i.e. one sample without bacteria and the other with salmonella).

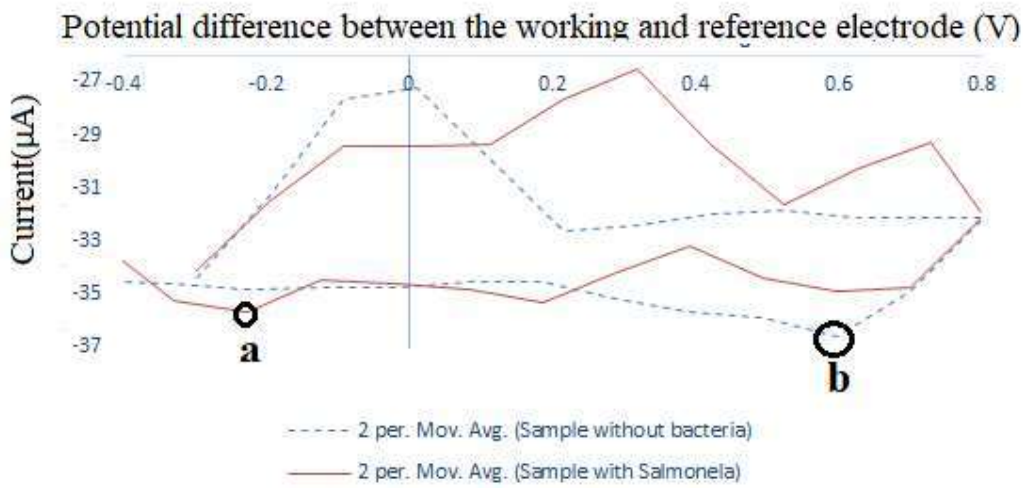


Figure (10): Cyclic Voltammetry Curve for an Amplified DNA from apple samples.

The results show that the current is in negative polarity which indicates that it is a reduction reaction (i.e. a reactant in a reaction gains one or more electrons). The obtained cyclic voltammograms exhibit one cathodic peak (point **a** or **b**) at reverse scan without any anodic peaks in the forward scan. The existence of cathodic peak means that the reaction is an irreversible electron transfer processes [21].

Figure (11) shows the cyclic voltammetry curve for an amplified DNA from tomato sample with salmonella at Scan rate 0.1V/seconds.

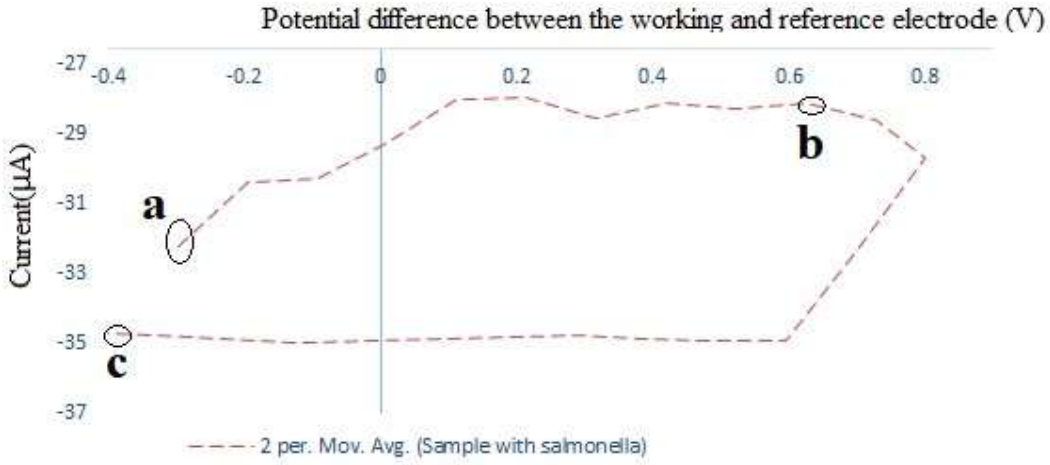


Figure (11): Cyclic Voltammetry Curve for an Amplified DNA from tomato samples contaminated with Salmonella.

For a reduction process, the working electrode at which reduction takes place is a cathode electrode, so DNA molecules gain electrons from the working electrode and diffuses through the surface as electric current. The current at the surface is increased due to the increase in potential difference between the working and reference electrodes until saturation (i.e. point b). Current then begins to decrease due to depletion of the reducing molecules (i.e. point c).

4.2. Cyclic voltammetry curves for amplified DNA samples Vs. not amplified samples

Figure (12) shows the Cyclic Voltammetry curves for DNA and an amplified DNA tomato samples contaminated with Salmonella at scan rate 0.1V/seconds.

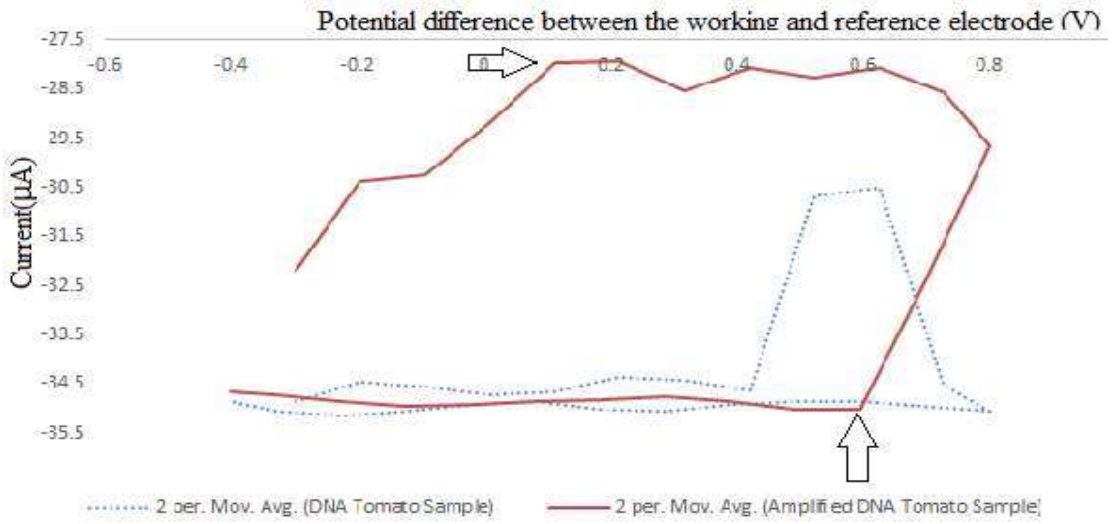
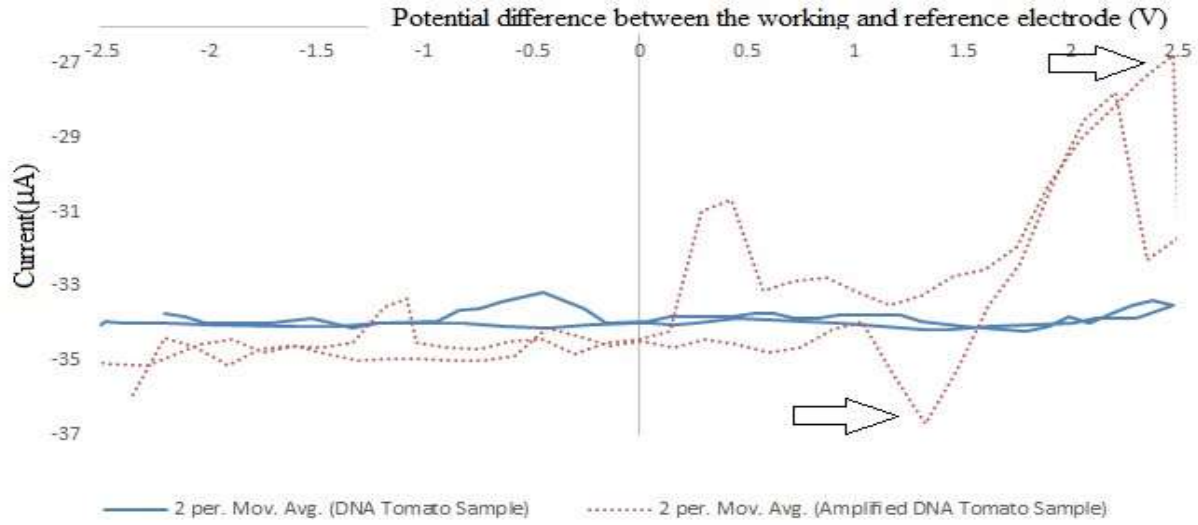


Figure (12): Cyclic Voltammetry Curve for DNA Tomato Samples contaminated with Salmonella.

Figure (13) shows the Cyclic Voltammetry curves for DNA and an amplified DNA tomato samples free from



bacteria.

Figure (13): Cyclic Voltammetry Curve for DNA Tomato Samples contaminated Free from Bacteria.

For both figures (12 & 13), the arrows labeled in the figures indicate that the peaks of amplified DNA curve is always higher than the normal DNA curve. It is indicated that the increase in DNA concentration leads to peak current increasing according to the mentioned equation (1).

4.3. Cyclic voltammetry curves for bacteria detection

Figure (14) shows the cyclic voltammetry curve for DNA from apple Samples without amplification at scan rate 0.05V/seconds (i.e. one sample without bacteria and the other with E.coli).

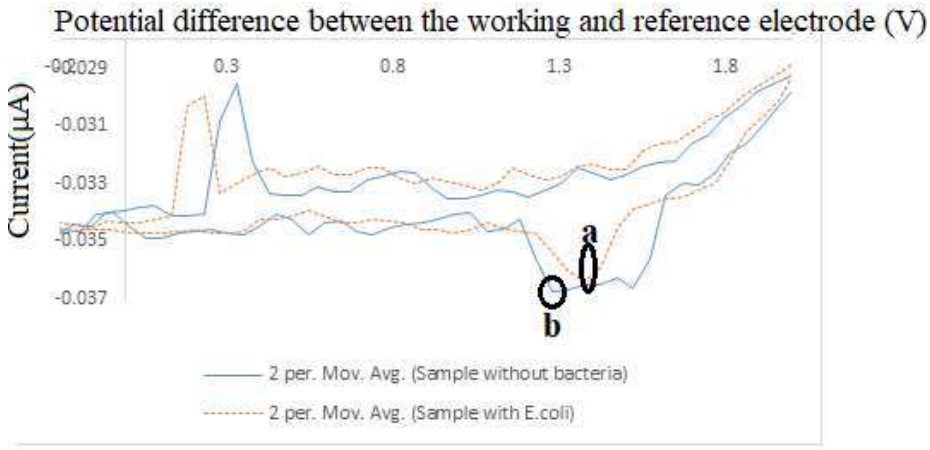


Figure (14): Cyclic Voltammetry Curve for DNA from an apple sample contaminated with E.coli.

Figure (15) shows the cyclic voltammetry curves for tomato samples at scan rate 0.01V/seconds (i.e. one sample without bacteria and the other with E.coli).

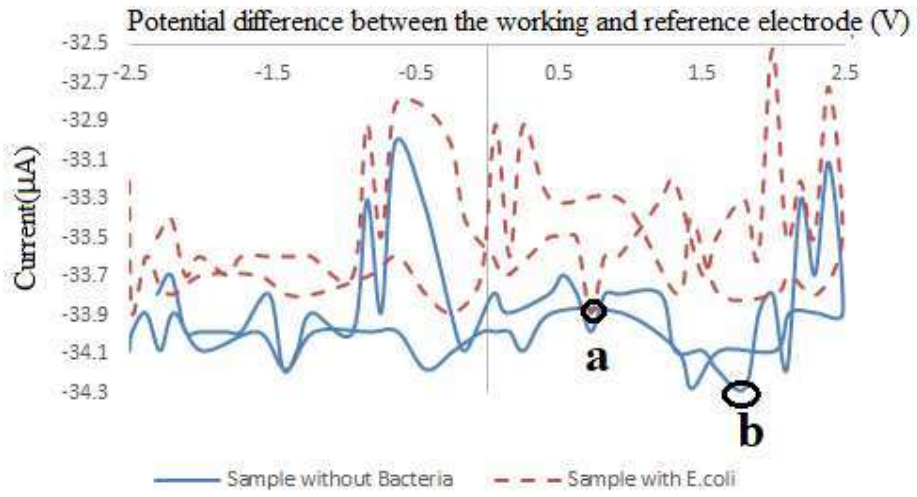


Figure (15): Cyclic Voltammetry Curves for DNA Tomato Samples.

From figures (9, 10, 14&15), it is clear that the cathodic peak of the sample that is free from bacteria (point **b**) always higher in magnitude than the cathodic peak of the samples with bacteria (point **a**) for any scan rate.

The reason for the higher peak for DNA sample is that the ionic strength between the contaminated DNA samples is stronger than the DNA sample free from bacteria. the lower the DNA solution ionic strength ,the faster the DNA diffusion.

The diffusion coefficients decrease with increasing ionic strength and increasing solvent friction [22]. The faster diffusion results in higher current [23]. So, the obtained results indicate that the system is on-site (i.e. low weight) detection system and fast (i.e. it takes for the detection without DNA extraction period 12 seconds minimum and 500 seconds maximum)according to the used scan rate as shown in table (1).

Table (1): Detection Time.

Voltage range (V) / Scan Rate(V/Sec.)	0.01V/Sec.	0.05V/Sec.	0.1V/Sec.
Voltage range (-0.4 to 0.8V)	120 Seconds	24 Seconds	12 Seconds
Voltage Range (-2.5 to 2.5)	500 Seconds	100 Seconds	50 Seconds

Conclusion

Pathogenic bacteria are a threat to the world. Many types of bacteria affect our food and cause foodborne illness. So, bacteria detection is a very important issue. There are many detection ways which take from minutes to several days such as in culturing methods. Rapid methods take many advantages over traditional methods such as it is time-efficient and more sensitive. Electrochemical biosensor based methods are the simplest and cost-effective method. Detection of two types bacteria (Salmonella and E.Coli) were done through the cyclic voltammetry type for tomato and apple samples by applying a continuous potential range on the working electrode of screen printed electrodes biosensor and measure the corresponding current values. After obtaining these coordinates the cyclic voltammetry curve plotted and the bacteria was detected from the cathodic peak of current. The system is characterized by a portable design, easy, rapid (i.e. from 12 to 500 seconds without DNA extraction period) and cost-friendly (i.e. fixed cost is about 11000 EGP and variable cost is about 800 EGP). It is observed that the cathodic peak of the sample that it is free from bacteria always higher than the cathodic peak of the samples with bacteria for any segment with any tested scan rates by noting the magnitude of current values. It is shown that the average value calculated from the obtained curves is approximately from -31 to -35 (μA) the highest value is given for tomato sample and the lowest for apple sample.

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