Detection of Foodborne Biohazards Using Antibody Modified Electrospun Conducting Fibres

by

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ABSTRACT

DETECTION OF FOODBORNE BIOHAZARDS USING ANTIBODY MODIFIED ELECTROSPUN CONDUCTING FIBRES

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The objective of the research was to fabricate an integrated sensor that could detect *Escherichia coli* K-12 from sample matrices. The sensor was based on antibodies immobilized on the surface of conducting polymer coated electrospun nylon fibres. Baseline studies optimized the conditions required for consistent depositions of polyaniline on the surface of nylon fibres. Antibodies with affinity towards *E. coli* K-12 were coupled to the conducting polymer and binding of the bacterium was followed using impedance spectroscopy using a 4-electrode configuration. It was demonstrated that the sensor membrane could recover >80% *E. coli* K-12 when placed in suspensions containing 1 – 5 log CFU *E. coli* K-12. In terms of detection, the increase in charge transfer resistance could be correlated to cell density of *E. coli* within the range of 1-6 log CFU. The study
provides a proof of an integrated capture and label-free detection method for microbial targets.

**Keywords:** Biosensor, label-free immunosensor; electrospining, nylon 6, polyaniline, immobilization, *E. coli.*
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# Table of Contents

**ABSTRACT** ................................................................................................................................. ii

**Acknowledgement** ......................................................................................................................... iv

**Table of Contents** ............................................................................................................................ vi

**List of figures** .................................................................................................................................. viii

**List of tables** .................................................................................................................................. x

**Chapter 1** ....................................................................................................................................... 1

1.1. Introduction .................................................................................................................................. 1

1.2. Literature review ........................................................................................................................... 4
   1.2.1. Foodborne diseases or illness ................................................................................................. 4
   1.2.2. Extraction and concentration of microbial toxins ............................................................... 12
   1.2.3. Nanofibres .......................................................................................................................... 14
   1.2.4. Conducting polymers ......................................................................................................... 21
   1.2.5. Polymers used in electrospinning ......................................................................................... 26
   1.2.6. Biosensors .......................................................................................................................... 34
   1.2.7. Enzyme-linked immunosorbent assays (ELISA) ................................................................. 42

1.3. Research Hypothesis ...................................................................................................................... 44

1.4. Objectives .................................................................................................................................... 44

**Chapter 2** ....................................................................................................................................... 45

2.1. Materials and Methods .................................................................................................................. 45
   2.1.1. Materials ........................................................................................................................... 45
   2.1.2. Preparation of nylon 6 electrospun fibres .......................................................................... 46
   2.1.3. Synthesis of nylon 6 / Polyaniline (PANI) conducting fibres by polymerization .......... 47
   2.1.4. Scanning Electron Microscopy (SEM) ................................................................................ 48
   2.1.1. Fourier Transform Infrared Spectroscopy (FTIR) .............................................................. 49
   2.1.2. Measurement of conductivity using impedance spectroscopy ........................................ 49
2.1.3. ELISA- direct ELISA procedure................................................................. 52
2.1.4. Elution of E. coli from the conducting fibres............................................. 55
2.1.5. Detection of bound E. coli K-12 by antibody modified nylon/PANi sensor membrane ............................................................................................................. 60
2.1.6. Statistical analyses....................................................................................... 62

Chapter 3 .............................................................................................................. 63

3.1. Result and discussion.................................................................................. 63

3.1.1. Optimization of nylon 6 electrospun fibre.............................................. 63
3.1.2. Optimization of the polymerization of aniline ........................................... 65
3.1.3. Capture efficiency of the nylon/PANi biosensor and elution of E. coli K-12 ...... 73
3.1.4. Detection of E. coli K-12 using the antibody modified conducting fibre biosensor 77

Chapter 4 ............................................................................................................. 81

4.1. Conclusion and future work ...................................................................... 81

4.1.1. Conclusions............................................................................................... 81
4.1.2. Future Work............................................................................................... 82

References ........................................................................................................... 84
List of figures

Figure 1.1: Worldwide Food Safety testing & diagnostics market value in 2008 and 2013 (modified from: Strategic Consulting Inc., 2013) ........................................ 9

Figure 1.2: Summary of U.S. food-safety testing market value ($ millions), by contaminant type, in 2011 and 2017 (modified from: http://www.bccresearch.com/pressroom/fod/global-markets-technologies-food-safety-testing; accessed on November 2, 2014) ........................................ 11

Figure 1.3: A schematic diagram of the electrospinning process. A polymer solution held in a syringe (A) is fed to a metal needle (B). A high voltage supply (C) is connected to the needle, producing a fine jet of polymer solution (D). This dries out in transit, resulting in fine fibres which are collected on an earthed target (E). .................................................................................. 18

Figure 1.4: The structure of various of conducting polymers (collected from Park and Lee, 2005) .......................................................................................................................... 24

Figure 1.5: Chemical structure of nylon 6,6 (a) and nylon 6 (b) ..................... 29

Figure 1.6: The three oxidation states of PANI: (a) a fully reduced leucoemeraldine base (LEB); (b) a fully oxidized pernigraniline base (PNB); and (c) a half oxidized/half reduced emeraldine base (EB) state (Bhadra et al., 2009). .......................................................................................................................... 31

Figure 1.7: Schematic of a simple biosensor ...................................................... 35

Figure 2.1: Electrospinning apparatus set-up (packaging Lab, University of Guelph). Arrow indicates the collector and the spinneret ......................... 46

Figure 2.2: Mechanism of oxidative polymerization of Aniline (recreated from Nicolau and Beadle, 2001) ................................................................. 48

Figure 2.3: Nyquist plot for nylon 6/PANi electrospun fiber. The fibre sample was mounted onto a 4-electrode cell and 0.1 N NaCl was used as the supportive electrolyte to ease the electrotransfer. Impedance was run when initial frequency was 100 KHz, final frequency was 50 Hz, AC amplitude was 50 mV, and DC potential was 0 V. .................................................................................. 51

Figure 2.4: Illustration of direct ELISA ................................................................ 53

Figure 2.5: Illustration of antibody immobilization on the electrospun conducting fibre (a) and capture of E. coli K-12 by antibody modified electrospun fibre. 58
Figure 2.6: Illustration of elution of *E. coli* K-12 with 10% NaCl solution. 59

Figure 3.1: Scanning Electron micrographs of nylon 6 electrospun fibres of different concentrations of w/v nylon 6/formic acid solution: (a) 18% showing presence of beads and fibres (b) 20% and (c) 22% not showing any beads. 64

Figure 3.2: Scanning Electron micrographs of nylon 6/PANi electrospun fibres following the oxidative polymerization of aniline with different concentrations: (a) 0.3 M (b) 0.45 M (c) 0.5 M (d) 0.6 M for 60 min. 67

Figure 3.3: Impedance spectroscopy measurement of the change of charge transfer resistance in 20% w/v and 22% w/v conducting nylon/PANi composite fibres with polymerization time 60 min and aniline concentration (0.45 M and 0.5 M). Values of bars with the different letter above them are significantly different for the given attribute at p ≤ 0.05. 71

Figure 3.4: FTIR spectra of 22% w/v nylon 6 electrospun fibre (control) and 22% w/v nylon 6/PANi composite fibre. 72

Figure 3.5: Elution volumes measured using different buffers to elute Bovine Serum Albumin (BSA) from antibody modified nylon 6/PANi sensor membrane. As elution buffer 10% NaCl solution, tris buffer solution and phosphate buffer solution were used. Control fibres weren’t treated with anti-BSA. 74

Figure 3.6: Scanning electron microscopy images of electrospun fibres after bacteria detection (a) *E. coli* K-12 was captured onto antibody modified nylon 6/PANi electrospun fibre and (b) no bacteria was observed on the electrospun fibre without antibody treatment. 75

Figure 3.7: % Recovery of *E. coli* K-12 after elution with 10% NaCl solution. % recovery was calculated from equation 2-2. 76

Figure 3.8: Biosensor response verses test time for different target concentration of *E. coli* K-12 bacteria sample. Detection time was established at around 20 minutes and the detection limit was from 1-5 log CFU (1.6×10^4 CFU/mL - 3×10^8 CFU/mL). 77

Figure 3.9: Charge transfer resistance of antibody modified electrospun fibre after treating with *E. coli* K-12 (10^1-10^5 CFU) for 20 min. 79
List of tables

**Table 1.1**: List of pathogens reported by CDC causing illness, hospitalization, and death of American population (CDC, 2011) .......................................................... 6

**Table 1.2**: Examples of some expensive foodborne disease outbreaks/recalls in the world (Hussain and Dawson, 2013) ............................................................................ 7

**Table 1.3**: Parameters that affect the properties of electrospun fibres (Bhardwaj and Kundu, 2010; Teo and Ramakrishna, 2006; Ramakrishna *et al.*, 2005; Yang *et al.*, 2005) .................................................................................................................. 20

**Table 1.4**: Properties and possible applications of Polyaniline and its composites on biosensors .......................................................................................................................... 32

**Table 1.5**: Different modes of electrochemical based foodborne pathogen detection ................................................................................................................................. 40

**Table 1.6**: Conducting polymers systems used for foodborne pathogen detection 42

**Table 3.1**: Comparison of charge transfer resistance of 20% w/v nylon/PANi electrospun fibres with different monomer concentration (M) and polymerization time (min) ........................................................................................................ 69

**Table 3.2**: Comparison of charge transfer resistance of 22% w/v nylon/PANi electrospun fibres with different monomer concentration and polymerization time ........................................................................................................ 69
Chapter 1

1.1. Introduction

Food safety is a global health goal. Foodborne disease represents a major health crisis. There is a continuing demand for rapid diagnostic techniques for detection of pathogenic microbes in foods and environmental samples (Hoorfar, 2011; Velusamy et al., 2010). Rapid detection of pathogenic microorganisms or their toxins can prevent contaminated foods from being consumed and ensure food safety by eliminating or minimizing the risk of foodborne illness outbreak (Binet et al., 2014). A new innovation in the diagnostics market is to capture and/or to detect microbes with a single assay (Sharma and Mutharasan, 2013). Methods that have been used for years to detect biohazards are mainly laboratory based; they are time consuming and laborious. To ease these difficulties, methods have been developed by combining the recognition properties of macromolecular biological molecules with the sensitivity of transducers or detector elements, such as: optical, thermal, gravimetric, or electrochemical detectors (Cosnier, 2005). The resulting biosensor can be used to detect microorganisms on site in food processing plants, on both food contact surfaces (surfaces that come into direct contact with food) and non-food contact surfaces (surfaces that are not always obvious during the processing operation), in small volume samples, as well as, in multi-step protocols (Cosnier, 2005; Sapsford et al., 2004; Clark and Lyons et al., 1962). In the development a
biosensor electrospun fibres play a significant role because of their high porosity, interconnectivity, microscale interstitial space and their large surface area to volume ratio.

Because of their versatility and promising advantages electrospinning technology and nanotechnology have been used in different aspects of society including the development of different types of sensors (Liang et al., 2007). Recently, the nanotechnology, including electrospinning has been introduced into the agriculture and food sector, and especially in the food safety area (Nazzaro et al., 2012). One of the areas that has undergone considerable growth is the use of electrospun fibres to develop sensors (Bhardwaj and Kundu, 2010; Liang et al., 2007). The advantages of using electrospun fibres are:

- High surface area to volume ratio (this ratio for nanofibres or submicron fibres can be $10^3$ times larger than that of microfibres) (Burger et al., 2006);

- Tuneable porosity and the ability to manipulate fibre composition in order to get desired properties;

- Flexibility in surface functionalities.

The scope of using nanotechnology in the food and agricultural sectors has become even broader; the use of conductive polymers adds a new dimension to it. The use of conducting polymers, that is polymer material with metallic and
semiconductor characteristics, in biosensors has been introduced because of their many advantages (Gerard et al., 2002). For example, conducting polymers improve the biocompatibility and sensitivity in-vivo (Balint et al., 2004); Also, conducting polymers can be designed to regulate parameters, such as, polymer layer thickness, electrical properties (conductivity, ionization potential) and bio-reagent loading (Malhotra et al., 2006). Nonetheless, conductive, flexible and biocompatible fibres produced through electrospinning are very attractive substrates for detection applications i.e., biosensor application.

In the current study, an immunosensor was developed based on electrospun nylon 6 fibres coated with polyaniline to capture and detect Escherichia coli K-12 (E. coli K-12). Although electrospinning has been applied in various areas such as biomedical, sensor and filtration applications, the use of electrospun fibres in conjunction with a conducting polymer for food safety applications has not yet been reported in the literature. The idea of this study was to develop a rapid detection method for microorganism responsible for foodborne illness and food poisoning. In the current study, the high surface area to volume ratio of electrospun fibres was combined with the conductivity of polyaniline to develop a biosensor membrane. Nylon 6 was selected because of its mechanical strength (Ding et al., 2009), its hydrophilic properties and excellent affinity toward aniline and polyaniline (Nasybulin et al., 2009).
Chapter one will introduce the background and present justification for the current research in terms of its relevance and value. The aims and objectives emanate from the research agenda which will be outlined along with the structure of the thesis.

1.2. Literature review

1.2.1. Foodborne diseases or illness

Foodborne diseases are mainly caused by consuming foods or beverages contaminated by biohazards (an organism or a by-product from an organism). In other word, foodborne illness is an infection or irritation of the gastrointestinal (GI) tract caused by foods or beverages that contain harmful bacteria, parasites, viruses, or chemicals (www.stopfoodborneillness.org, 2014; Cliver and Riemann, 2002). It was reported that between 1987 and 1992, around 79% of foodborne illness outbreaks were caused by bacteria (Collins, 1997). The Canada Communicable Disease Report (CCDR) mentioned that harmful bacteria (especially, Norovirus, Clostridium perfringens, Campylobacter and Nontyphoidal) are mostly responsible for 90% of 1.6 million foodborne diseases caused by foodborne pathogens (CCDR, 2014). The chances of foodborne illness outbreak are increasing.

There are many controversies about the pathogens responsible for the majority of foodborne illnesses. In 2011, Scallan and team reported that each year in the
United States 31 major pathogens caused 9.4 million episodes of foodborne illness (Scallan et al., 2011). According to the Center for Disease Control and Prevention’s (CDC) report of 2011, there are eight known pathogens which are responsible for the vast majority of illnesses, hospitalizations, and deaths (Table 1.1) (http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html; accessed on June 6, 2015). Whilst, University of Nebraska–Lincoln reported 12 major bacteria which are responsible for foodborne illness: *Aeromonas hydrophilia, Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Escherichia coli* O157:H7 (E. coli), *Listeria monocytogenes, Salmonella spp., Shigella, Staphylococcus aureus, Vibrio, Yersinia enterocolitica* (http://food.unl.edu/safety/pathogenic-org; accessed on November 11, 2014).
**Table 1.1**: List of pathogens reported by CDC causing illness, hospitalization, and death of American population (CDC, 2011)

<table>
<thead>
<tr>
<th>Pathogen responsible</th>
<th>Estimated number of people reported</th>
<th>% population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top five pathogens that contribute to foodborne illnesses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norovirus</td>
<td>5,461,731</td>
<td>58</td>
</tr>
<tr>
<td><em>Salmonella</em> (non-typhoidal)</td>
<td>1,027,561</td>
<td>11</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>965,958</td>
<td>10</td>
</tr>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>845,024</td>
<td>9</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>241,148</td>
<td>3</td>
</tr>
<tr>
<td>Top five pathogens contribute to foodborne illnesses resulting in hospitalization</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> (non-typhoidal)</td>
<td>19,336</td>
<td>35</td>
</tr>
<tr>
<td>Norovirus</td>
<td>14,663</td>
<td>26</td>
</tr>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>8,463</td>
<td>15</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>4,428</td>
<td>8</td>
</tr>
<tr>
<td><em>E.coli</em> (STEC) O157</td>
<td>2,138</td>
<td>4</td>
</tr>
<tr>
<td>Top five pathogens contribute to foodborne illnesses resulting in death</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> (non-typhoidal)</td>
<td>378</td>
<td>28</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>327</td>
<td>24</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>255</td>
<td>19</td>
</tr>
<tr>
<td>Norovirus</td>
<td>149</td>
<td>11</td>
</tr>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>76</td>
<td>6</td>
</tr>
</tbody>
</table>
1.2.1.1. Impact of foodborne diseases or illness on the economy of a country

The outbreak of foodborne diseases has a remarkable effect on both the human health and economic condition of the country. Eighty-four studies evaluated the cost of foodborne illness in humans during the last two decades (1992–2012) in North America and Europe (Mclinden et al., 2014). The most common estimated costs were due to foodborne illnesses caused by bacteria (McLinden et al., 2014). In the United States, the estimated cost of food safety incidents (e.g., recall-notifying consumers, removing food from shelves, and paying damages as a result of lawsuits) was around $7 billion per year (Hussain and Dawson, 2013).

Table 1.2 lists some of the costly food outbreaks in the world.

**Table 1.2: Examples of some expensive foodborne disease outbreaks/recalls in the world (Hussain and Dawson, 2013)**

<table>
<thead>
<tr>
<th>Year</th>
<th>contamination/Food Product</th>
<th>Estimated Economic Loss</th>
<th>Region/Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Clostridium botulinum/Whey concentrate</td>
<td>Unknown</td>
<td>New Zealand</td>
</tr>
<tr>
<td>2009</td>
<td>Salmonella/Peanut products</td>
<td>$70 million</td>
<td>USA</td>
</tr>
<tr>
<td>2008</td>
<td>Salmonella/Tomatoes</td>
<td>$250 million</td>
<td>USA</td>
</tr>
<tr>
<td>2008</td>
<td>Mad cow disease/Meat</td>
<td>$117 million</td>
<td>USA</td>
</tr>
<tr>
<td>2007</td>
<td>Salmonella/Peanut butter</td>
<td>$133 million</td>
<td>USA</td>
</tr>
<tr>
<td>Year</td>
<td>Contaminant</td>
<td>Cost</td>
<td>Location</td>
</tr>
<tr>
<td>------</td>
<td>------------------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>2006</td>
<td>E. coli/Spinach</td>
<td>$350 million</td>
<td>USA</td>
</tr>
<tr>
<td>1992</td>
<td>E. coli/Hamburgers</td>
<td>$160 million</td>
<td>USA</td>
</tr>
</tbody>
</table>

According to Food Sentry’s data, between July 2012 and July 2013, there were 610 food recalls in North America. Among them, 37.6% were caused by salmonella contamination, 21.6% by allergens and 20.2% were related to *L. monocytogenes* (http://www.foodsentry.org/understanding-food-recalls-the-recall-process-explained/; accessed on September 6, 2014).

### 1.2.1.2. Global food market and food safety diagnosis

The World Health Organization (WHO) defines foodborne illnesses as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food (Velusamy *et al.*, 2010). It has been reported that, in the industrialized countries, more than 30% of the population suffers from foodborne diseases each year (Velusamy *et al.*, 2010).

To avoid foodborne illness and poisoning, food manufacturers are implementing Food Safety Management systems and various diagnostic testing procedures. Food Safety diagnostics can be used for the detection of hazards or verification of the performance of food safety management systems. Food Safety diagnostics is a process of testing foods to detect the contaminations that occur as a result of exposure to contaminated equipment or raw materials, contaminated water, improper handling, and inadequate treatment.
Diagnostic testing by food producers varies based on: geographic area, the predominant organisms tested (e.g., *Salmonella*, *Listeria*, and *Campylobacter*), the type of food product produced (meat, dairy, fruits and vegetables, or processed food), sampling method (including the point in the food production chain at which samples were collected), and the test methods used for analysis (http://www.strategic-consult.com/2013/09/food_microbiology_testing/; accessed on October 13, 2013). According to Strategic Consulting Inc. (July, 2013), the market value of food microbiology testing has increased by 40%, or $832 million, in the past five years (Figure 1.1).

![Market Value Worldwide](image)

**Figure 1.1:** Worldwide Food Safety testing & diagnostics market value in 2008 and 2013 (modified from: Strategic Consulting Inc., 2013)

On the other hand, in a report published in 2012, based on market research conducted on worldwide food markets (http://www.marketsandmarkets.com/Market-Reports/food-safety-365.html), it was stated that the North American food safety testing market was segmented on the basis of contaminants, technologies, food types, and geography. The U.S. was recorded as the largest food testing market in the North America. In 2012, Shiga toxin-producing *E. coli* (STEC) was the highest threat for the North American food market and salmonella for the European food market. Contamination from different pathogens in food was one of the driving forces to develop various types of rapid testing methods.
BCC research reported that the global food safety testing market is projected to grow from $3.3 billion in 2011 to $4.3 billion by 2017 (http://www.bccresearch.com/pressroom/fod/global-markets-technologies-food-safety-testing; accessed on November 2, 2014). The food safety testing market was divided into five segments based on contaminant type: pathogens, toxins, GMOs (genetically modified organisms), residues, and others. Figure 1.2 represents an overview of food safety testing market in 2012 and 2017 (an estimation). As shown the greatest focus for food safety testing is for pathogen detection.
1.2.2. Extraction and concentration of microbial toxins

Extraction is the process of separating or isolating a substance from a matrix or solution. In other words, extraction facilitates sample preparation or purification in qualitative and quantitative analyses through partitioning of materials between two phases in order to remove interferents. Therefore, it increases the overall sensitivity of the downstream assay. Extraction methods isolate the undesired components from sample matrices that the instruments cannot handle directly (Wang et al., 2011). The extraction techniques together with other analytical methods (chromatography, ELISA, PCR) reduce cost, shorten process time, and improve the efficiency of the analytical methods (Tadeo et al., 2010).

Traditionally, there are various means of extraction and concentration, such as, immuno-magnetic separation (IMS), centrifugation, filtration, and solid phase extraction (SPE).

1.2.2.1. Immuno-Magnetic Separation (IMS)

IMS is a laboratory tool to efficiently separate eukaryotic cells from fluids and prokaryotic organism from heterogeneous samples. It can also be used as a method of quantifying the pathogenicity of contaminated food, blood or feces (Enroth and Engstrand, 1995).

IMS, Which employs uniformly sized polymer paramagnetic beads with an iron oxide core, are known as “Dynabeads™”. These particles are
superparamagnetic and become magnetized in the presence of a magnetic field. They also bind with various bio-reactive molecules or cells with specific affinity to the beads (Uyttendaele et al., 2000).

The Dynabeads™ are magnetically charged polystyrene beads with specific polyclonal and monoclonal antibodies immobilized on their surfaces. When these beads are incubated with suspensions such as enrichment cultures or pathogen cells, these cells bind to the beads via the immobilized antibodies. Thus IMS is used to separate and concentrate microorganism from a vast amount of sample. IMS is also used as a preliminary step during PCR (polymerase chain reaction). For PCR, it is important to remove polymerase inhibitors, so IMS is used to concentrate/extract bacteria from a cultured sample (Enroth and Engstrand, 1995). IMS separates certain organism such as Cryptosporidium, Giardia, Legionella, E. coli O157 H7, E. coli O145, E. coli O111, E. coli O103, E. coli O26, Listeria and Salmonella etc. directly from pre-enriched samples. For example, Uyttendaele and team (2000) used IMS for direct detection of L. monocytogenes (< 10 CFU/g) in cheese (Uyttendaele et al., 2000). At the present time, magnetic separation techniques are commonly used in food diagnostics (Olsvik et al., 1994). Dynabeads™ or IMS techniques have revolutionised the isolation and manipulation process for some biological material, including cells, nucleic acids, proteins and pathogenic microorganisms.
1.2.2.1.1. **Advantages of IMS**

- Effective separation of target organism from competitive microflora to increase concentration (10-100 fold);
- Reduction of the total test time;
- Improvement in the sensitivity of the microbiological test;
- Removal of potential inhibitors.

1.2.2.1.2. **Disadvantages of IMS**

- Non-specific binding or bacterial adherence (at >10^6 CFU/mL);
- Loss of cells during wash steps.

1.2.3. **Nanofibres**

Nanofibres are defined as fibres with diameters less than 100 nanometer (Gaffet, 2011). On the other hand, according to US textile industry and Japanese and Korean strategic research initiative nanofibres are fibres with a diameter of less than 1 µm (Gibson *et al.*, 2007). Nanofibres can be produced by various methods, for example: using air-blast atomization of mesophase pitch; by assembling from individual carbon nanotubes molecules (Tseng and Ellenbogen, 2001); via pulling of non-polymer molecules by an atomic force microscope
(AFM) tip (Ondarcuhu and Joachim, 1998); through depositing materials on linear templates; or using whiskers of the semiconductor which spontaneously grow out of gold particles placed in the reactor chamber (Cobden, 2001). The aforementioned methods, except air-blast atomization of mesophase pitch allowed for good process control. However, these methods are not very flexible with respect to choice of material which being used for nanofibres production. That is why scientists are looking for a more suitable method to obtain nanofibre and nanowires. Electrospinning is a versatile method to produce nanofibres (fibres with diameter < 100nm), nanowires, nanotubes and fibres with diameter >100 nm, e.g., ultrafine fibres, submicron fibres, microfibers (Hassan et al., 2014). Electrospinning allows for manufacturing of long fibres (of the order of 10 cm) with controlled diameter, high surface area and porous structure (Hassan et al., 2014). Instrumentation is relatively easy and the process can be manipulated easily (Yarin et al., 2001).

1.2.3.1. Electrospinning

Electrospinning is a simple and widely used technique to produce fibres from a polymeric fluid (solution or melt) by utilizing electrostatic forces (Frenot and Chronakis, 2003). The fibre diameter ranges from several microns to lower than 100 nm. The polymer solution or melt is delivered through a needle with diameter in the millimeter range (Bhardwaj and Kundu, 2010; Frenot and Chronakis, 2003).
In 1745, Bose created an aerosol spray by exposing a liquid in a capillary tube to a high electrical potential (Reneker and Chun, 1996). The basis of electrospinning mainly came from his invention (Ko, 2003). In 1952, Vonnegut and Neubauer invented an apparatus to produce filaments, which was based on a glass tube filled with a polymer solution that received high voltage (5-10 kV). In 1955, Drozin studied the dispersion of liquids such as aerosols using high voltage. In 1981, for the first time Larrondo and Manley used a polymer melt method for electrospinning (Frenot and Chronakis, 2003; Larrondo and Manley, 1981). There was little interest in electrospinning or electrospun nanofibres until mid-1990s. Reneker and Chun (1996) revitalized the importance of electrospinning and have drawn attention toward the possibility of using a wide range of polymer solutions for this technique. The development and improvement in electrospinning techniques has been speeding up since the last few decades, and the studies in this area have been increased in the past few years (Bhardwaj and Kundu, 2010; Burger et al., 2006). Since then over a hundred synthetic and natural polymers have been electrospun into fibres (Sawicka and Gouma, 2006).

1.2.3.1.1. Electrospinning Equipment

Basic electrospinning equipment (Figure 1.3) includes three main components: a high power voltage supply, a spinneret, and a collector or target. The spinneret or metal needle is attached to a syringe through which the polymer solution or
melt is forced by a pump (Schiffman and Schauer, 2008). The needle is connected to a high power voltage supply to charge the solution and create an electric field. By inducing an appropriate high voltage (Burger et al., 2006) to a polymer solution, the polymer solution at the tip of the needle becomes highly electrified. Since electrostatic repulsion works against the surface tension the droplet is stretched (Doshi and Reneker, 1995). The drop becomes distorted to form a cone shape known as the Taylor cone (Taylor, 1969). At a critical point, the electrostatic repulsion exceeds the surface tension and a stream of liquid is ejected from the nozzle. Solid fibres are formed when a highly viscous polymer solution within the electrified jet is continuously being stretched due to electrostatic repulsions between the evaporated solvent and surface charge (Li and Xia, 2004; Yarin et al., 2001). If the molecular cohesion of the liquid is not sufficiently high, stream breakup does occur resulting in electrospraying (Li and Xia, 2004).
Figure 1.3: A schematic diagram of the electrospinning process. A polymer solution held in a syringe (A) is fed to a metal needle (B). A high voltage supply (C) is connected to the needle, producing a fine jet of polymer solution (D). This dries out in transit, resulting in fine fibres which are collected on an earthed target (E).

In short, electrospinning is a versatile method which can optimize the morphology of the fibres by adjusting the composition of the solution and the configuration of the electrospinning apparatus, discussed below (Rafiei et al., 2013).
1.2.3.1.2. Parameters for fabricating fibres

The morphology and characteristic of the electrospun fibres are dependent on various parameters (Li and Xia, 2004; Frenot and Chronakis, 2003; Huang et al., 2003; Doshi and Reneker, 1995):

1) Solution and melt properties: the type of polymers, the conformation of the polymer chain, viscosity, elasticity, electrical conductivity, and the polarity and surface tension of the solvent;

2) Process conditions: applied voltage, distance from the spinneret to the collector and the flow rate of the polymer solution;

3) Environmental conditions: atmospheric conditions that include humidity, temperature, and air velocity.

Many studies have been conducted to find the relationship among the different parameters and the properties of electrospun nanofibres as outlined in the Table 1.3.
Table 1.3: Parameters that affect the properties of electrospun fibres
(Bhardwaj and Kundu, 2010; Teo and Ramakrishna, 2006; Ramakrishna et al., 2005; Yang et al., 2005)

<table>
<thead>
<tr>
<th>Influencing Factors</th>
<th>Effect on Fibre Properties</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity of solution</td>
<td>Low viscosity produces beads, alternately with high viscosity beads disappears and fibre diameter increases</td>
<td>Zhao et al., 2005; Zhang et al., 2005; Jiang et al., 2004; Huang et al., 2003</td>
</tr>
<tr>
<td>Concentration of polymer in solution</td>
<td>Fibre diameter increases with higher concentration</td>
<td>Kim et al., 2005; Jun et al., 2003</td>
</tr>
<tr>
<td>Conductivity of solution</td>
<td>Decrease in fibre diameter occurs with an increase in conductivity</td>
<td>Jiang et al., 2004; Koski et al., 2004; Jun et al., 2003</td>
</tr>
<tr>
<td>Surface Tension of solution</td>
<td>Relationship with fibre morphology is inconclusive: high surface tension causes instability of polymer jets.</td>
<td>Zuo et al., 2005; Zhang et al., 2005; Mituppatham et al., 2004; Hohman et al., 2001</td>
</tr>
<tr>
<td>Applied voltage</td>
<td>Decreased fibre diameter when voltage is increased</td>
<td>Kim et al., 2005; Jun et al., 2003; Demir et al., 2002</td>
</tr>
<tr>
<td>Distance between spinneret and collector</td>
<td>Uniform fibre is produced when a minimum distance is maintained, beads produced are too small and too large distance</td>
<td>Geng et al., 2005; Ki et al., 2005; Zhao et al., 2005; Zhang et al., 2005; Buchko et al., 1999;</td>
</tr>
<tr>
<td>Flow rate</td>
<td>Reduction of fibre diameter when flow rate is low, generation of beads with too high a flow rate.</td>
<td>Sill and Recum, 2008; Zuo et al., 2005; Zhang et al., 2005</td>
</tr>
<tr>
<td>Humidity</td>
<td>High humidity leads to circular pores on the fibres.</td>
<td>Casper et al., 2004; Li and Xia, 2004; Mit-</td>
</tr>
</tbody>
</table>
Temperature | Fibre diameter Increases at higher temperature | Mit-Uppatham et al., 2004 ; Reneker and Chun, 1996

1.2.3.1. Applications of nanofibres

Nanofibres can be used in a broad array of fields including: biomedical applications, healthcare, biotechnology, environmental engineering, defense and security, energy storage and generation (Bhardwaj and Kundu, 2010; Vaseashta, 2008).

In regards to agriculture and food applications, there are not many significant commercial uses as the use nanofibre is comparatively new in this sector. Much research has been conducted and nanofibres have been introduced to develop sensor to detect microorganisms, toxins or enzymes in food (Ligler et al. 2003). Nanofibres could be a potential material for food packaging applications.

1.2.4. Conducting polymers

A polymer is a large molecule which is composed of repetitive monomer units, joined covalently in a chain and its conductivity is defined by the number of charge carrier units or electrons mobility (Janata and Josowicz, 2003). Conducting polymers are those with metallic and semiconductor characteristics (Heeger, 2001). Conducting polymers show unusual electrochemical properties (high electrical conductivity, low ionization potential, high electronic affinities, and
semi-conductor properties) because of the presence of one unpaired electron, i.e., presence of a \( \pi \)-electron per carbon atom in the backbone of the polymer (Ahuja et al., 2006; Malhotra et al., 2006; Janata and Josowicz, 2002). Although conducting polymers were first described in the mid 1800’s, by Letheby through the color changing property of acidic polyaniline solution upon addition of an oxidant, the use of conducting polymer in research did not become popular until 1975 (Gerard et al., 2002). The conductivity of conducting polymers can be tuned by chemical manipulation of the polymer backbone with a dopant and by blending with other polymers (Angelopoulos, 2001).

Generally, based on the degree of conductivity, conducting polymers can be classified into four groups (Kumar and Sharma, 1998). The first and the most widely used conducting polymeric system is the composite polymers. These polymers are highly conductive because they have an insulating polymer matrix which is filled with fibrous conductive fillers such carbon or metal. Applications for such composites are wide spread, including: interconnections, printed circuit boards, encapsulations, die attachment, heat sinks, conducting adhesives, electromagnetic interference (EMI) shielding, electrostatic discharge (ESD), and aerospace engineering. The second group of polymers is known as ionically conducting polymers, for example, polyethylene oxide. The source of electrical conductivity is a result of the movement of ions present in the system. These types of polymers are used in the battery industry. The third group of polymers is
known as redox polymers. These polymers contain immobilized redox centers (electroactive centers). Since these centers are not connected to each other, electrons transfer from one center to another through the well-known “hopping” mechanism (Bhadra et al., 2009). As a result, these polymers conduct charge. The fourth group of conducting polymer is conjugated polymers. These polymers consist of alternating single and double bonds, creating an extended π-network. The movement of electrons within this π-framework is the source of conductivity. However, a dopant is required to increase the level of conductivity for this type of polymers. The structure of different conducting polymers is presented in Figure 1.4. PANi has better stability and processability compared to the other conducting polymers (Park and Lee, 2005). Therefore, polyaniline was selected for this existing study.
Figure 1.4: The structure of various conducting polymers (collected from Park and Lee, 2005)
1.2.4.1. Production of conducting polymers through polymerization methods

Conducting polymers can be produced either by chemical oxidation or electrochemical oxidation because the π-bonds in conjugated polymers are highly susceptible to chemical or electrochemical oxidation or reduction. Chemical oxidative polymerization is the oxidation of monomers to form cation radicals followed by coupling to form di-cations. Electrochemical oxidation is popular because the reaction can be carried out at room temperature and a polymer film can be produced on any electrode surface. As well, a free standing film can be obtained using this method. The thickness of the film can be controlled by changing polymerization time (Ahuja et al., 2006; Gerard et al., 2002).

Electrochemical polymerization is well known for producing nanostructures. In this process a potential is applied to oxidize the monomer, and consequently, the polymer chain elongates from the nucleation sites on the electron surface. In addition, direct immobilization of bioagents, e.g., enzymes or antibodies on the surface of electrodes is possible during electrochemical polymerization (Vidal et al., 2003).
1.2.4.2. Applications of conducting polymers

The use of conducting polymers has increased because of their conducting and semiconducting properties, as well as their versatility and ease of production and handling (Malhotra et al., 2006; Bruno, 1994). Since polyaniline, polypyrrole and polythiophene have sufficiently low redox potential to avoid oxidative degradation they are may be used for sensor applications (Ahuja et al., 2006; Malhotra et al., 2006). These conducting polymers provide enormous opportunities for binding biomolecules, modifying their bio-catalytic properties, rapid electron transfer and direct communication to produce a range of analytical signals and new analytical applications (Prathap et al., 2012; Dhand et al., 2010).

1.2.5. Polymers used in electrospinning

Electrospinning is a versatile method to produce nanofibres, nanowires and nanotubes (Li and Xia, 2004; Frenot and Chronakis, 2003; Reneker et al., 2000). The electrospinning process can be used to control the deposition of polymer fibres onto a target substrate due to its inherent properties (Frenot and Chronakis, 2003). As a result, nanofibres with complex and seamless three-dimensional shapes could be formed. When the diameters of polymer fibres are reduced from micrometers (e.g., 10–100 µm) to sub-micrometers or nanometers (e.g. 10×10^{-3}–100×10^{-3} µm) (Huang et al., 2003), desirable characteristics appear such as large surface area to volume ratio (10^{3} times of those for microfibres), flexibility in surface functionalities, and sometimes superior mechanical
performance (e.g., stiffness and tensile strength) compared with any other known form of the material. Because of these characteristics electrospun fibres have been used in many sectors including food safety diagnosis (Huang, Jiaxing et al., 2003; Huang, Zheng-Ming et al., 2003).

Electrospinning is applicable to a wide range of polymers (synthetic, natural, and blends) including nylon, polyolefin, polyamides, polyester, aramid, acrylic, proteins (polypeptides), DNA, electron conducting and photonic polymers (Angammana and Jayaram, 2011; Burger et al., 2006; Ohgo et al., 2003; Frenot and Chronakis, 2003; Fang and Reneker, 1997). Polyamides, often used to produce electrospun fibres (Li et al., 2006; Stephens et al., 2004), include nylon 6 (Zhang et al., 2009, Fong et al., 2002), nylon 6/SiO$_2$ (Ding et al., 2009), nylon-11 (Dhanalakshmi and Jod, 2008), nylon 6, 66, 1010 terpolymer (Li et al., 2006), nylon 6 (PA-6) /montmorillonite (Mt) (Fong et al., 2002). Besides polyamides, other polymers can be successfully electrospun into nanoscale fibres, for instance: polyaniline (PANI) /PEO blend (MacDiarmid et al., 2001), polysulphones (Yuan et al., 2004), polyaniline (PANI)/ polystyrene (PS)(nylon-4,6, PA-4,6 (Bergshoef and Vancso, 1999), poly(ethylene oxide) (PEO) (Yarin et al., 2001) and many others.

Ren et al. (2006) developed a sensor by immobilizing glucose oxidase enzyme onto the polyvinyl alcohol electrospun fibres. A urea biosensor was developed based on electrospun fibres by Sawicka et al. (2005). They prepared
nanocomposite fibers of urease and polyvinylpyrrolidone (PVP) by the electrospinning and established a biosensor to detect urea in medical diagnoses, environmental and bioindustrial analyses (Sawicka et al., 2005). The fibres produced by electrospinning a solution of PEO, fluorescent poly((p-phenylene ethynylene)-alt-(thiénylene ethynylene)) and chloroform were used as chemosensor material (Frenot and Chronakis, 2003). Zaung et al. (2003) reported that electrospun nanofibres from a mixture of PAA-PM (polyacrylic acid – poly(pyrene methanol)), polyurethane and dimethylformamide were used as optical sensor.

1.2.5.1. Nylon

Nylon is one of the most commonly used polymers and was the first commercially successful synthetic thermoplastic polymer. Nylon is also known as aliphatic polyamide because it is made of repeating units linked by amide bonds (Palmer et al., 2001). Nylon was first produced on February 28, 1935, by Wallace Carothers. Chemical elements of nylon are carbon, hydrogen, nitrogen and oxygen. Nylon is formed by reacting equal parts of a diamine and a dicarboxylic acid. Molecules with an acid (-COOH) group on each end are reacted with molecules containing amine (-NH₂) group on each end. The resulting nylon is named on the basis of the number of carbon atoms separating the two acid groups and the two amines, for instance: nylon 6, nylon 6,6, nylon 6,9, nylon 6,10, nylon 6,12, nylon 11, and nylon 12. Nylon 6,6 is the most common
commercial grade of nylon, and nylon 6 is the most common commercial grade of molded nylon. Nylon 6 is obtained by polycondensation of polycarprolactam and nylon 6,6 is produced by reacting adipic acid with hexamethylene diamine (Brandrup et al., 1999; Nair et al., 2006). Figure 1.5 represents chemical structures of nylon 6 and nylon 6,6.

![Chemical structure of nylon 6,6 (a) and nylon 6 (b)](image)

**Figure 1.5:** Chemical structure of nylon 6,6 (a) and nylon 6 (b)

There is wide range of application for nylon fibres, membranes and films in the areas of affinity chromatography, filtration, molecular biology, molecular imprinting, biosensor matrices etc because nylon fibre is inexpensive, mechanically strong (Ohe et al., 2007), non-toxic and can be modified into different forms. Various research has been performed using nylon to produce affinity membranes and electrospun fibres. Shi et al. (2005) used nylon
membrane modified with polyhydroxyl containing materials to bind polylysine ligand in order to detect bilirubin.

1.2.5.2. Polyaniline

In 1960, after the discovery of conducting polymers (CPs), a promising subject of research was initiated because of the interesting properties and numerous application possibilities of CPs (Guimard, 2008). Polyaniline (PANi) is an organic polymer which can be converted to a conducting state by appropriate oxidation or doping (Wanekaya et al., 2007; Gerard et al., 2002). Among all conductive polymers, polyaniline has gained more attention due to its suitability to perform in aqueous conditions, its redox properties and high thermal stability (Sapurina and Shishov, 2008). In addition, polyaniline is nontoxic and stable in aggressive chemical environments (Sapurina and Shishov, 2008). The discovery of polyaniline (PANi) can be traced back 180 years to the experiments of Runge. In 1834, he heated the mixture of copper (II) chloride and aniline nitrate on a porcelain plate at 100 °C; a dark green color material was produced which afterward changed to a black color (Gordana, 2013). Polyaniline and its derivatives are synthesized through two general routes: (a) electrochemical; and (b) chemical methods (Bhadra et al., 2007). The most common method for fabrication of polyaniline in large quantities is the chemical method. In conventional chemical methods, the aniline is polymerized in water based solution in the presence of an oxidant and a dopant. Polyaniline can be found in
one of three idealized oxidation state (Bhadra et al., 2009) (Figure 1.6):
leucoemeraldine – white/clear & colorless \((\text{C}_6\text{H}_4\text{NH})_n\), (per)nigraniline –
blue/violet \((\text{C}_6\text{H}_4\text{N})_n\), and emeraldine – green for the emeraldine salt, blue for the
eameraldine base \([\text{C}_6\text{H}_4\text{NH}]_2[\text{C}_6\text{H}_4\text{N}]_2)_n\).

**Figure 1.6:** The three oxidation states of PANI: (a) a fully reduced
leucoemeraldine base (LEB); (b) a fully oxidized pernigraniline base (PNB);
and (c) a half oxidized/half reduced emeraldine base (EB) state (Bhadra et al.,
2009).

The four most important factors which influence the conductivity of PANi are: (i)
molecular weight; (ii) percentage of crystallinity and inter-chain separation; (iii)
oxidation level and molecular arrangement; and (iv) percentage of doping and
type of dopant. Although, the potential applications of polyaniline have been
limited due to its poor conductivity at neutral pH, it has been used in
multidisciplinary fields. A few possible applications of PANI are presented in
Table 1.4.
Table 1.4: Properties and possible applications of Polyaniline and its composites on biosensors

<table>
<thead>
<tr>
<th>Special properties of Polyaniline</th>
<th>Related applications of Polyaniline</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrically conductive in nature</td>
<td>Conductive adhesive, ink, paint</td>
<td>Hino <em>et al.</em>, 2006; Yoshioka and Jabbour, 2006; Barros <em>et al.</em>, 2005; Roth and Graupner, 1997;</td>
</tr>
<tr>
<td></td>
<td>Antistatic textile</td>
<td>Bowman and Mattes, 2005;</td>
</tr>
<tr>
<td>Viscosity in solution increases under electric field</td>
<td>Electro-rheological (ER) material</td>
<td>Lee <em>et al.</em>, 2005; Cho <em>et al.</em>, 2004; Cho <em>et al.</em>, 2003; Choi <em>et al.</em>, 1997;</td>
</tr>
<tr>
<td>Electrical conductivity or color changes upon exposure to acidic, basic and some neutral vapors or liquids</td>
<td>NH₃, CO₂, NO₂, CO, Cl₂, O₃, gas sensor</td>
<td>Bai <em>et al.</em>, 2007; Yan <em>et al.</em>, 2007; Irimia-Vladu and Fergus, 2006; Ando <em>et al.</em>, 2005; Dixit <em>et al.</em>, 2005; Jain <em>et al.</em>, 2005;</td>
</tr>
<tr>
<td></td>
<td>Volatile organic compound, toxic gas sensor</td>
<td>Kim <em>et al.</em>, 2005; Hosseini and Entezami, 2001;</td>
</tr>
<tr>
<td></td>
<td>Petroleum, H₂O₂, humidity, chemical sensor</td>
<td>Joshi <em>et al.</em>, 2007; Zou <em>et al.</em>, 2007; Nohria <em>et al.</em>, 2006; Huang <em>et al.</em>, 2003;</td>
</tr>
<tr>
<td></td>
<td>Mercury, pH sensor, biosensor</td>
<td>Arora <em>et al.</em>, 2007; Muthukumar <em>et al.</em>, 2007; Talaie <em>et al.</em>, 2000;</td>
</tr>
<tr>
<td></td>
<td>Bacteria, vitamin C detector</td>
<td>Ren <em>et al.</em>, 2007; Andreu <em>et al.</em>, 2005;</td>
</tr>
<tr>
<td>Feature</td>
<td>Material / Property</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Color changes upon change in the pH of the medium due to protonation–deprotonation</td>
<td>‘Acid–base’ indicator</td>
<td>Syed and Dinesan, 1990;</td>
</tr>
<tr>
<td>Variation of oxidation states on charging and discharging, with concomitant diffusion of ions into and from the polymer</td>
<td>Ion-exchange material</td>
<td>Bidan and Ehui, 1989; Dobhalhofer and Armstrong, 1988;</td>
</tr>
<tr>
<td>Shows very high capacitance values</td>
<td>Capacitor</td>
<td>Lu et al., 2007; Gupta and Miura, 2006</td>
</tr>
<tr>
<td></td>
<td>Energy storage devices</td>
<td>Meng et al., 2009; Sung et al., 2004</td>
</tr>
<tr>
<td>Ability to absorb and reflect electromagnetic radiation</td>
<td>Electromagnetic interference shielding</td>
<td>Bhadra et al., 2008; Joo et al., 1995; Epstein et al., 1994;</td>
</tr>
<tr>
<td>Response to an electromagnetic field in the optical regime</td>
<td>Medium for erasable optical information storage</td>
<td>Epstein et al., 1994; Coplin et al., 1993; Leng et al., 1992; McCall et al., 1991;</td>
</tr>
<tr>
<td></td>
<td>Non-linear optics (NLO)</td>
<td>Chen et al., 2003; Petrov et al., 1995; Osaheni et al., 1992;</td>
</tr>
<tr>
<td></td>
<td>Digital memory device</td>
<td>Tseng et al., 2005</td>
</tr>
<tr>
<td>Can be converted from highly conducting to almost insulating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ability to accumulate and transform energy (including optical frequencies), and hence, to memorize (erase) information</td>
<td>Electrode for rechargeable batteries</td>
<td>Desilvestro et al., 1992; Koga et al., 1989; Somasir and MacDiarmid, 1988; MacDiarmid et al., 1987;</td>
</tr>
<tr>
<td></td>
<td>Anode for microbial fuel cell</td>
<td>Qiao et al., 2007;</td>
</tr>
</tbody>
</table>
Emits color under various excitations
Organic or polymer light emitting diodes

1.2.6. Biosensors

Biosensors are chemical sensing devices which combine a biological recognition mechanism with a physical transduction technique (Wieczorek and Pace, 2008). The working principle of a biosensor is based on the idea that interaction between the specific group of analytes and the biorecognition element (enzymes, antibody, nucleic acid, tissue, and cells), results in specific change in one or more of the physio-chemical properties such as pH, electron transfer, heat or mass. The transducer part of biosensors detects this change and converts it into an electronic signal which is proportional to the concentration of a specific analyte or target analyte. Recently, IUPAC (International Union of Pure and Applied Chemistry) has given the specific definition of a biosensor: “it is a self-contained integral device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological element” (Thevenot et al., 1999).

The history of biosensors began in 1962 with the development of the first enzyme based glucose sensing device by Clark and Lyons (Clark and Lyons, 1962). Since then various research has been conducted to establish more rapid
and sensitive detection methods and in recent years, biosensors have become very popular. Due to their adaptability, specificity, simple use in relatively complex samples and faster fabrication of portable sensors, biosensors (Cosnier, 2005; Gerard et al., 2002) have been used in diagnosis, food technology, biotechnology, genetic engineering, and environmental monitoring. Figure 1.7 shows the schematic diagram of a typical biosensor.

**Figure 1.7:** Schematic of a simple biosensor

Biosensors based on an enzyme as the catalytic recognition element, are very popular (Zhang and Li, 2004). Besides this category, there are affinity sensors—immunosensors, DNA sensors and receptor sensors. Affinity sensors are used
for the detection and measurement of antigenic proteins such as hormones, drugs, viruses, bacterial antigens, as well as the main type of mammalian antibodies, e.g., immunoglobulins and they can be effective at extremely low concentrations (Skladal, 2003). Affinity sensors display sensing capabilities at the picomolar and even at the femtomolar level. On the other hand, enzyme sensors are mainly focused on the determination of small molecules, such as, glucose from the millimolar to the micromolar range (Estep et al., 2013; Cosnier, 2005).

1.2.6.1. Advantages of using conducting polymer in biosensors

The use of a conducting polymer in biosensor technology has become popular because conducting polymers improve the sensitivity (by preventing interference from reaching the active parts of the sensors), speed, and the versatility of the biosensor in detection of microorganism or any other contaminants. Moreover, conducting polymers have always been a suitable matrix for binding or immobilizing antibodies or enzymes (Sung et al., 2004; Sung and Bae, 2000; Adeloju and Wallace, 1996; Bartlett and Cooper, 1993). These polymers create a suitable condition for localization of any biologically active molecule because they are available in very flexible chemical structures and can be modified or synthesized as required. In addition, electrical conducting polymers are self-modifiable to make them available to bind with protein (Situmorang et al., 2000; Mulchandani and Wang, 1996; Lu et al., 1995). One of the main benefits of using
conducting polymers is that biologically active molecules can be simultaneously immobilized or trapped in the polymer matrix while polymers are being synthesized by electro-polymerization.

In recent years, many biosensors or sensing methods, which can be classified into sensing methods for labeling the target substances via an auxiliary reaction using a labeling compound (Ramanavicius et al., 2006), have been designed. Fluorescence or luminescent markers and high-sensitivity methods such as surface plasma resonance (SPR), quartz crystal microbalance (QCM), carbon nanotube field-effect transistors (CNT FET) and nanowire FET for detecting the bonding to the target substances (Murphy, 2006) are some of the examples of labeled immunosensor. The labeled immunosensors format belongs to indirect analytical signal detection methods. Among all indirect electrochemical immunoassays, amperometric transducers are used much more frequently than others (Ramanavicius et al., 2006). Many researchers have reported amperometric detection of foodborne pathogens such as E. coli O157:H7 (Chemburu et al., 2005; Varshney et al., 2005; Ruan et al., 2002; Abdel-Hamid et al., 1999), Salmonella (Yang et al., 2001; Abdel- Hamid et al., 1999b; Che et al., 1999; Brooks et al., 1992), L. monocytogenes (Chemburu et al., 2005; Crowley et al., 1999) and C. jejuni (Chemburu et al., 2005). Indirectly labeled immunosensors need additional immunochemicals labeled by electrochemical labels which add extra procedures mainly based on incubation with labeled
antibodies, making these methods more expensive and time consuming. Moreover, due to the application of broad-range-selectivity exhibiting secondary antibodies, these methods often decrease selectivity (Ramanavicius et al., 2006). To improve the reading of molecular signals and to remove the faults of these labeled immunosensor technologies, researchers have used the semiconductor properties of conducting polymers to provide a label-free sensor to facilitate signal reading. Such sensors allow real-time measurement without any additional hazardous reagents (Warsinke et al., 2000). The majority of label-free electrochemical immunoassays do not need any auxiliary electrochemical reaction for the detection procedure. They are based on changes in charge density or conductivity for transduction.

Nowadays, the majority of biosensors are based on one or more enzymes used in conjunction with an electrode (Dhand et al., 2010; Murphy, 2006). These biosensors use the concept of redox reaction. They use a small mediator species that shuttles between the biomolecule and the electrode or in some cases by direct electron transfer between the biomolecule redox site and the electrode (Murphy, 2006). Since the redox site of a biomolecule is often hidden deep inside the biomolecule, direct electron transfer can be difficult to achieve. Various studies have been done to facilitate direct electron transfer. Conducting materials have been used to modify biomolecules or electrode surfaces to achieve direct electron transfer (Hartmann, 2005). Thus, highly conductive organic transducers
are gradually emerging for the development of the next generation biosensor designed for highly reliable, stable and robust field-based diagnostic devices.

1.2.6.2. Detection of a pathogen using the electrochemical transduction method

Several transduction methods have been developed in the past decade for the detection of foodborne pathogens. For instance, optical, electrochemical and mass based transduction systems are the most popular and common (Velusamy et al., 2010). There are new types of transducers constantly being developed for use in biosensors.

Electrochemical based detection methods are one of the means of transduction that has been used for identification and quantification of foodborne pathogens. Based on the observed parameters such as current, potential, impedance and conductance respectively, electrochemical biosensors can be classified into amperometric, potentiometric, impedimetric and conductometric (Velusamy et al., 2010). Table 1.5 summarizes different modes of electrochemical based foodborne pathogen detection (Velusamy et al., 2010).
Table 1.5: Different modes of electrochemical based foodborne pathogen detection

<table>
<thead>
<tr>
<th>Mode of detection</th>
<th>Analyte</th>
<th>Limit of Detection (CFU/mL)</th>
<th>Assay time</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amperometric</td>
<td><em>E. coli</em> O157:H7</td>
<td>81</td>
<td>6 min</td>
<td>Muhammad-Tahir and Alocilja, 2004;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amperometric</td>
<td><em>E. coli</em> O157:H7</td>
<td>7.8 \times 10^1</td>
<td>10 min</td>
<td>Muhammad-Tahir and Alocilja, 2004;</td>
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<tr>
<td>Amperometric</td>
<td><em>E. coli</em> O157:H7</td>
<td>6 \times 10^2</td>
<td>2 h</td>
<td>Ruan <em>et al.</em>, 2002</td>
</tr>
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<tr>
<td>Amperometric</td>
<td><em>S. typhimuriumphi</em></td>
<td>1.09 \times 10^3</td>
<td>2.5 h</td>
<td>Tang <em>et al.</em>, 2001</td>
</tr>
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<tr>
<td>Amperometric</td>
<td>Salmonella</td>
<td>1-5</td>
<td>6 h</td>
<td>Brooks <em>et al.</em>, 1992;</td>
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<tr>
<td>Amperometric</td>
<td><em>E. coli</em> O157:H7 without any enrichment</td>
<td>1.6 \times 10^1-7.2 \times 10^7</td>
<td>15 min</td>
<td>Varshney <em>et al.</em>, 2005;</td>
</tr>
<tr>
<td></td>
<td>after enrichment</td>
<td>8 \times 10^{0-1}</td>
<td></td>
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<tr>
<td>Amperometric</td>
<td><em>E. coli</em>, <em>L. Monocytogenes</em>, <em>C. jejuni</em></td>
<td>50 cell/mL 10 cells/mL 50 cells/mL</td>
<td>6 h 30 min</td>
<td>Chemburu <em>et al.</em>, 2005;</td>
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<tr>
<td>Amperometric</td>
<td><em>E. coli</em> O157:H7 Salmonella</td>
<td>50 cells/mL 50 cells/mL</td>
<td>35 min</td>
<td>Abdul_Hamid <em>et al.</em>, 1999b8;</td>
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<tr>
<td>Amperometric</td>
<td><em>E. coli</em> O157:H7</td>
<td>100 cells/mL</td>
<td>30 min</td>
<td>Abdul_Hamid <em>et al.</em>, 1999a;</td>
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<tr>
<td>Photentiometric</td>
<td><em>E. coli</em> O157:H7 heat-hided Live</td>
<td>7.1 \times 10^2 cells/mL</td>
<td>45 min</td>
<td>Gehring <em>et al.</em>, 1998;</td>
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<tr>
<td>Photentiometric</td>
<td><em>E. coli</em></td>
<td>10 cells/mL</td>
<td>30 min</td>
<td>Ercole <em>et al.</em>, 2003;</td>
</tr>
</tbody>
</table>
To perform selective and efficient bioanalyte detection using a biosensor, it is very important to immobilize biorecognition elements such as enzymes, antibodies, whole cell or DNA on the transducer surface (Arshak et al., 2009). There are various types of immobilization methods, such as physical adsorption, electrochemical adsorption, and covalent attachment. Table 1.6 summaries electrochemical biosensors that use conducting polymers for the detection of various foodborne pathogens and immobilization methods used during the detection process.
Table 1.6: Conducting polymers systems used for foodborne pathogen detection

<table>
<thead>
<tr>
<th>Conducting Polymers</th>
<th>Biomolecules</th>
<th>Immobilization methods</th>
<th>Pathogen</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypyrrole</td>
<td>Antibody</td>
<td>Covalent attachment</td>
<td>L. monocytogenes</td>
<td>Minett et al., 2003;</td>
</tr>
<tr>
<td>Polypyrrole</td>
<td>DNA</td>
<td>Entrapment</td>
<td>B. cereus</td>
<td>Velusamy et al., 2009;</td>
</tr>
<tr>
<td>Polyaniline</td>
<td>Antibody</td>
<td>Biotin-avidin</td>
<td>L. monocytogenes</td>
<td>Tully et al., 2008;</td>
</tr>
<tr>
<td>Polyaniline</td>
<td>Antibody</td>
<td>Absorption</td>
<td>E. coli 0157:H7</td>
<td>Muhammad-Tahir and Alocilja, 2003;</td>
</tr>
<tr>
<td>Polyaniline</td>
<td>Antibody</td>
<td>Absorption</td>
<td>B. cereus</td>
<td>Pal et al., 2008;</td>
</tr>
<tr>
<td>Polypyrrole</td>
<td>DNA</td>
<td>Entrapment</td>
<td>E. coli</td>
<td>Rodriguez and Alocilja, 2005;</td>
</tr>
<tr>
<td>Polyaniline</td>
<td>DNA</td>
<td>Biotin-avidin</td>
<td>E. coli</td>
<td>Arora et al., 2007;</td>
</tr>
</tbody>
</table>

1.2.7. Enzyme-linked immunosorbent assays (ELISA)

ELISA is an analytic assay to detect the presence of an analyte in a liquid sample by using the antibody and enzyme immunoassay technique resulting in a color change. It has been reported that different kinds of ELISA are used in detecting microorganism e.g. E. coli O157 (Dylla et al., 1995; Johnson et al., 1995). ELISA is one of the most frequently used methods for immunoassay
because of its good sensitivity, selectivity, and ease in use (Ramanavicius et al., 2006).

The basic design of the standard ELISA for antibody detection firstly requires reaction of the sample with a solid-phase-fixed antigen, and secondly is followed by a reacting with an enzyme-conjugated anti-immunoglobulin (Kendall et al., 1983). Since ELISA can evaluate or detect the presence of antibodies or antigens in the sample, it has been used in various sectors of food industries and medical science. In the food industry, potential allergens (dairy, nuts, and eggs) have been detected by using ELISA. ELISA-based biosensors have been successfully used for accurate detection of a wide variety of antigens. The original ELISA method, however, is a complicated multistage process and is tedious and expensive, which has led to the development of easy, time saving and inexpensive methods to identify the presence of a microorganism.

Since ELISA-based biosensors are mainly capture-based, the sensitivity of such biosensor can be significantly improved by increasing the surface area to volume ratio of the binding surface. In current study, a method to capture and detect biohazards was developed. The concept was to develop a label free detection method to detect the presence of microorganism and because of their large surface area to volume ratio electrospun fibres were considered as a means of label free detection of antigen binding.
1.3. Research Hypothesis

The underlying hypothesis of the study is that conducting electrospun fibres modified with affinity agents can be used to capture and detect biohazards from sample matrices.

1.4. Objectives

The specific objectives of this study are:

- Optimization and preparation of conductive fibres
- Immobilization of antibody onto conductive fibres
- Evaluation of capture efficiency of model pathogens on immobilized fibres
- Detection of captured pathogens using electrochemical transduction
Chapter 2

2.1. Materials and Methods

2.1.1. Materials

Nylon 6 films were purchased from DuPont, Canada. Formic acid (99% pure), hydrochloric acid, ammonium persulphate and aniline (ACS reagent 99.5%) were purchased from Sigma Aldrich, Oakville, ON, Canada. Teflon tubes and stainless steel needle spinnerets were purchased from Cole-Palmer Canada Inc., QC, Canada and a 10 mL glass syringe was bought from SGE Analytical Science Pty Ltd., Ringwood, Victoria, Australia. Sodium chloride (≥ 99.5%) and Tween-20 (enzyme grade) were obtained from Thermo Fisher Scientific, Ottawa, Ontario, Canada. All other reagents were analytical grade.

Antibody goat polyclonal to *E. coli*, mouse monoclonal [BSA-7G10] to bovine serum albumin (BSA) and substrate p-nitrophenyl-phosphate were purchased from ABCAM, Cambridge, USA. A carbo free blocking solution (10x concentration sp-5040) and an animal free blocking reagent (5X concentration sp-5030) were purchased from Vector Laboratories Inc. Burlingame, Ca.

Other chemicals used to make PBS, 0.85% (w/v) saline solution, wash buffer, 10% (w/v) NaCl and tris buffer, were obtained from Thermo Fisher Scientific, Canada.
2.1.2. Preparation of nylon 6 electrospun fibres

2.1.2.1. Sample preparation

Nylon 6 polymer solutions of various concentrations (15%, 16%, 18%, 20%, 22%, and 24% w/v) were prepared by dissolving nylon 6 film (DuPont, Canada) in 5 mL of formic acid (99% pure) and stirring for 24 h at room temperature (21°C ± 2°C).

Figure 2.1: Electrospinning apparatus set-up (packaging Lab, University of Guelph). Arrow indicates the collector and the spinneret.
2.1.2.2. Electrospinning of nylon 6 fibres

The nylon 6 solution was electrospun in a Plexiglas enclosure (Figure 2.1). A 10 mL glass syringe was filled with the nylon 6 solution. This solution was passed from the syringe through a teflon tube which was adapted with a 16-gauge blunt end stainless steel needle spinneret on the end. An infusion pump (Kd scientific, model 780200) was set to control the flow rate of the polymer solution and to keep a constant flow rate of 0.5 mL/h. In a typical experimental setup, the spinneret was attached to the positive electrode and the nylon 6 solution was spun in a vertical position onto a stainless steel disc covered with aluminum foil (25 cm diameter) which was grounded. A contact voltage of 18 KV was applied to the positive electrode using a DC supply (Gamma high voltage research, Ormond Beach, FL32174). The distance between the collector and the spinneret was 15 cm. Electrospinning was conducted at room temperature (21° ± 2 °C).

2.1.3. Synthesis of nylon 6 / Polyaniline (PANi) conducting fibres by polymerization

Aniline was distilled twice before use and diluted in 0.35 M HCl aqueous solution. The polymerization was done according to the procedure mentioned in Hong et al., 2004 with some modification. After electrospinning, the nylon 6 electrospun fibres were immersed in the aniline solution (0.3, 0.45, 0.5 and 0.6 M) at 40°C for 30-120 min. The aniline coated fibres were then placed in 0.35 M HCl and the reaction was initiated by adding ammonium persulphate (0.3, 0.45, 0.5 and 0.6
M) as the ratio of monomer to oxidant (aniline : ammonium persulphate) should be 1:1 (Hong et al., 2004). The reaction was allowed to proceed for 1 h at 4°C. After 1 h, the fibres were taken out of the oxidative solution and rinsed with distilled water. The fibres were then dried under vacuum for 24 h. PANi (emeraldine salt form) was produced on the surface of the electrospun fibres as a result of oxidative polymerization of aniline and was protonated with HCl present in the reaction mixture. The mechanism of the polymerization reaction is presented in Figure 2.2.

![Mechanism of oxidative polymerization of Aniline](image)

**Figure 2.2:** Mechanism of oxidative polymerization of Aniline (recreated from Nicolau and Beadle, 2001).

### 2.1.4. Scanning Electron Microscopy (SEM)

Samples of nylon 6 fibres and polyaniline coated nylon 6 fibres were examined by SEM after mounting a 1 cm² section onto an aluminum platform followed by the deposition of a gold layer (20 nm) using a sputter coater (Model K550; Emitech, Ashford, Kent, England). The samples were examined using a SEM (Hitachi S-570; Hitachi High Technologies Corp., Tokyo, Japan) at an
accelerating voltage of 10 kV. The samples were observed at a low magnification to observe the fibre morphology. Higher magnifications were used to observe each of the corners and the center of the square sample and images of the fibre were captured by using Quartz PCI (version 7, Quartz Imaging Corp. Vancouver, BC). To observe *E. coli* K-12 captured by antibody immobilized nylon 6/PANi conducting fibre membranes were also examined by SEM (an accelerating voltage of 15 kV). Samples were prepared by capturing *E. coli* K-12 on to the antibody immobilized fibres and they were dried at room temperature.

### 2.1.1. Fourier Transform Infrared Spectroscopy (FTIR)

To ensure the development of polyaniline on the fibre surface FTIR spectroscopy was done. FTIR spectroscopy was performed by placing electrospun fibres onto a diamond crystal held within an attenuated total reflectance (ATR) cell. IR spectra were collected at room temperature for PANi coated nylon 6 electrospun fibres using 30 averaged scans at 4 cm$^{-1}$ resolution. Scans were collected from 400 to 1800 cm$^{-1}$ using an infrared spectrophotometer (IRPrestige-21; Shimadzu Corp., Tokyo, Japan). Before testing each sample, the background measurement was recorded.

### 2.1.2. Measurement of conductivity using impedance spectroscopy

Conductivity of nylon-PANi composite fibres was measured by impedance spectroscopy. Impedance spectroscopy was performed using a Solatron
frequency response analyzer (Model 1260, Solartron, London Scientific, London, Ontario, Canada) connected to a potentiostat (Model 1287 Solartron) using a 4-electrode configuration. The sensing part of the electrode probe consisted of 4 gold electrode pins (2 mm diameter) with each being separated by 4 mm. The Solatron 1260 analyzer (London Scientific) was used in conjunction with Z plot software (Scribner Associates Inc., Southern Pines, NC, U.S.A.) with the generated data being analyzed using Z view software (Scribner Associates Inc., Southern Pines, NC, U.S.A.). A 0.1 N NaCl solution (0.58% w/v was dissolved in 500 L of distilled water) and was used as a supportive electrolyte to facilitate electron transport throughout the composite fibre.

The 4-electrode system was connected to the Grain-phase analyser to make an electric circuit. A 1 cm × 1 cm nylon/PANi composite fibre membrane was placed on top of a glass slide with 1 µL of 0.1 N NaCl. Charge transfer resistance was measured by using software Zplot with its companion data display and analysis program ZView. Charge transfer resistance was obtained from a Nyquist plot which is a two-dimensional presentation of impedance data. Electrochemical impedance was measured by applying an AC potential (50 mV) to composite nylon/PANi conducting fibers and measuring the current through the cell. The impedance scan was performed over a frequency limit from 50 Hz to 100 000 Hz (100 KHz). The results were summarized in terms of an equivalent circuit by the software and this circuit also had identical impedance to the real physical
system. The real impedance is plotted on the X axis and the imaginary impedance (impedance of the equivalent circuit) on the Y axis of a chart; a Nyquist plot (Figure 2.3) is the result.

Figure 2.3: Nyquist plot for nylon 6/PANi electrospun fiber. The fibre sample was mounted onto a 4-electrode cell and 0.1 N NaCl was used as the supportive electrolyte to ease the electrotransfer. Impedance was run when initial frequency was 100 KHz, final frequency was 50 Hz, AC amplitude was 50 mV, and DC potential was 0 V.

On the Nyquist plot, the impedance is represented as a vector of length $|Z|$; a semi-circle is formed. The charge transfer resistance of conducting nylon 6/PANi composite fibres was calculated from the diameter of that semi-circle. The most conductive composite fibre was found by comparing the charge transfer resistance among the nylon 6/PANi composite fibres where the concentration of
aniline and the polymerization time were different for each sample. Frequency (initial frequency 100 KHz and final frequency 50 Hz), AC potential (50 mV) and DC potential (0 V) were initially fixed in the Solatron 1260 analyzer. To ensure the correct measurement, ten measurements from different spots were recorded for the same sample.

2.1.3. ELISA- direct ELISA procedure

In the current study, direct ELISA was conducted to test the binding efficiency of antibody to *E. coli* K-12. ELISA includes four sequential steps: 1) coating the antigen (*E. coli* K-12) to the microplates; 2) use of a blocking reagent to block unbound ends; 3) incubation with the antibody; and 4) use of the ALP substrate to change the color which indicates the amount of antibody present and bound to the fibres. A schematic diagram of direct ELISA process is presented in Figure 2.4. The methodology of ELISA was followed as per the method mentioned in ABCAM, 2010 with some modifications (http://www.abcam.com/index.html?pageconfig=resource&rid=11388; accessed on October 3, 2010).
PBS, pH 7.4 was prepared by adding 0.232% (w/v) of Na$_2$HPO$_4$, 0.02% (w/v) of KCl, 0.02% (w/v) of K$_3$PO$_4$ and 0.8% (w/v) of NaCl in distilled water. 0.05% (v/v) Tween 20 was added to the PBS (pH 7.4) to make a wash buffer.

*E. coli* K-12 was cultivated in the laboratory in brain heart infusion (BHI) broth at 37°C for 24 h. The cells were harvested by centrifugation (5 min) and re-suspended in 0.85% saline to obtain an optical density (at 600 nm) of 0.2 ($10^8$ CFU/ 8 log CFU). A serial dilution was performed with $10^8$ CFU *E. coli* K-12 suspension in distilled saline.
2.1.3.1. Coating the antigen to the microplate

The *E. coli* K-12 suspension was diluted with distilled saline (0.85% NaCl) to obtain a serial dilution from $10^8$ CFU to $10^1$ CFU in $10^1$ increments. An aliquot of 50 µL of this diluted suspension was pipetted onto the top wells of a PVC microtiter plate. A control was used without any *E. coli* K-12 suspension. Three experimental replicates were performed for each dilution.

2.1.3.2. Blocking

An aliquot (200 µl) of carbo free blocking solution (1X) was added to wells to block the remaining protein binding site in the coated wells. After covering with adhesive plastic, the plate was incubated for 2 h at room temperature. The plate was then washed three times with the wash solution (PBS with 0.05% v/v Tween 20).

2.1.3.3. Incubating with the antibody

Antibody goat polyclonal to *E. coli* was diluted with sterile saline at a ratio of 0.5 mg/mL. Diluted antibody of 100 µL was placed in each well and the plate was incubated at room temperature for 2 h. After 2 h, the plate was washed four times using the wash solution.
2.1.3.4. Detection

A substrate solution was prepared using sterile saline according to the manufacturer's instruction. 100 µL of p-nitrophenyl-phosphate substrate solution was dispensed per well using a micro pipette. The plate was incubated in a dark environment for 30 min. 100 µL of 0.75 M NaOH solution was added to stop the reaction. The yellow color of p-nitrophenyl-phosphate was measured using a plate reader at 405 nm.

2.1.4. Elution of *E. coli* from the conducting fibres

*E. coli* K-12 was eluted from antibody modified electrospun conducting fibre with 10% (w/v) NaCl solution. This process was optimized using BSA and 10% (w/v) NaCl solution, tris buffer solution and phosphate buffer solution were used to elute BSA from anti-BSA modified electrospun fibres. In this assay, BSA was used as the antigen.

2.1.4.1. Optimization of the elution process

Appropriate buffer conditions for the elution step in the affinity biosensor depend on salt content and/or the pH of the buffer solution. A high salt buffer would compete for the positive and negative charges and would result in the disruption of interactions between the conducting composite fibre and the antibody causing the antibody to elute. In this study, there were two buffer solutions: tris buffer
solution - pH 8 and phosphate buffer solution- pH 8, as well as 10% (w/v) NaCl solution used to elute BSA from conducting fibres.

Mouse monoclonal antibody [BSA-7G10] to BSA was diluted with animal free blocking reagent (1x). 1 cm × 1 cm area of fibres were taken and added to 1 mL anti-BSA (0.5 mg/mL). Fibre samples were incubated for 30 min at room temperature. Conducting fibres were taken out and washed three times with the wash solution. Antibody modified electrospun fibres were then transferred to 1.5 mL of 10 mg/mL BSA and incubated overnight at 4 °C. At the end of incubation time, conducting fibres were washed three times to remove any unbound BSA. Conducting fibres, with captured BSA, were placed consecutively into 1.5 mL of 10% NaCl solution, tris buffer solution- pH 8 and phosphate buffer solution-pH 8. Control samples were produced where there was no anti-BSA present.

Conducting fibres were kept in elution buffers for 30 min at room temperature. To accelerate the elution process, fibres with elution buffers were vortexed for 10 sec. All the experiments were performed in triplicate.

After 30 min, conducting fibres were taken out of the elution buffers and the BSA concentration in solution was determined by using Nanodrop 8000 (proteinA-280).
2.1.4.2. Elution of *E. coli* K-12

Immobilization of the antibody was performed on 1 cm × 1 cm nylon 6/PANi conducting fibre mesh (fibre membrane) using 300 µL of diluted (0.5 mg/mL) Antibody goat polyclonal to *E. coli*. Figure 2.5 presents an illustration of the immobilization of antibody onto the nylon 6/PANi fibres. The immobilized conducting fibres were washed three times using the wash buffer (pH 7.4) to wash out any unbound antibody. For this procedure, several concentrations of *E. coli* K-12 suspensions (from 0 to $10^6$ CFU) were used. Required concentrations of *E. coli* K-12 suspensions were achieved by using the procedure mentioned before. Antibody modified conducting fibres were added to 1.5 mL of *E. coli* K-12 suspensions and incubated overnight at 4 °C. After the incubation period, conducting fibres were taken out and washed four times with the wash buffer. *E. coli* K-12 was captured by antibody modified nylon 6/PANi electrospun fibre mesh (Figure 2.4).
Figure 2.5: Illustration of antibody immobilization on the electrospun conducting fibre (a) and capture of *E. coli* K-12 by antibody modified electrospun fibre.

Fibres with captured *E. coli* K-12 were then added to 1.5 mL 10% NaCl solution for 30 min to elute *E. coli* K-12 from the conducting fibre. Samples were vortexed for 10 sec to accelerate the elution of *E. coli* K-12. After 30 min, the conducting fibres were taken out and eluted *E. coli* K-12 in 10 % NaCl solution was plated on MacConkey agar to observe the colony count (CFU) of *E. coli* K-12. The principle of elution of *E. coli* K-12 is presented in Figure 2.6.
2.1.4.3. Calculation of % recovery and % yield

The following equations were used to calculate % yield and % recovery

Equation 2—1:

\[
\text{% Yield} = \left( \frac{\text{Initial Loading (CFU/mL)}}{\text{Initial Loading (CFU/mL)}} \right) \times 100
\]

Equation 2—2:

\[
\text{% Recovery} = \left( \frac{\text{Amount eluted from conducting fibres (CFU/mL)}}{\text{Initial Loading (CFU/mL)}} \right) \times 100
\]
2.1.5. Detection of bound *E. coli* K-12 by antibody modified nylon/PANi sensor membrane

Detection of *E. coli* K-12 through antibody modified nylon 6/PANi conducting fibre was carried out by measuring the changes in conductivity of the fibre. Changes in conductivity of the nylon 6/PANi fibre were measured by using a 4-electrode configuration. Impedance spectra were collected until stable spectra were attained.

2.1.5.1. Immobilization of the antibody

Electrospun conducting fibres were cut into square pieces (1 cm × 1 cm). Antibody goat polyclonal to *E. coli* was diluted with the blocking reagent (1X) at a concentration of 0.5 mg/mL. Diluted anti-*E. coli* of 300 µL was pipetted into a sterile 1 mL vial. A square piece of the electrospun conducting fibre mesh (1 cm × 1 cm) was added to the antibody (anti-*E. coli*). The conducting fibre membrane was then incubated with diluted anti-*E. coli* at room temperature for 30 min. The antibody was immobilized through an absorption method of immobilization. Antibody was adsorbed at the polymer/solution interface due to electrostatic interactions between the polycationic matrix of the oxidised polyaniline and the total negative charge of the antibody. The incubated conducting fibre piece was then washed three times with the wash solution (PBS with 0.05% v/v Tween 20) to remove the unbound antibody. Conducting fibre pieces were dried at room temperature for 15 min.
2.1.5.2. **The capture of E. coli K-12 on to the antibody modified electrospun fibres**

$10^8$ CFU of E. coli K-12 was obtained by following the procedure mentioned above. An aliquot of 1 mL of E. coli K-12 was removed and serially diluted in distilled saline to $10^{-7}$. An aliquot of 5 mL was dispensed into sterile 10 mL tubes. The antibody modified conducting fibres were allowed to react with the E. coli K-12 suspension concentration from 0 to $10^6$ CFU for 5 to 60 min at 37°C. The fibre membrane was then removed from the E. coli K-12 aliquots and rinsed with the wash solution to remove the unbound E. coli from the fibre surface. Three experimental replicates were performed for each dilution.

2.1.5.3. **Evaluation of biosensor response**

In this study, the developed biosensor was evaluated based on the conductivity change of nylon 6/PAni fibre mesh before and after capturing E. coli K-12. Initial charge transfer resistance of antibody-modified fibre was measured using a 4-electrode configuration. The charge transfer resistance of the fibre was then remeasured after reacting with E. coli K-12. The difference between the initial reading and final reading constituted the response. Charge transfer resistance values for fibres were calculated from an average of ten measurements.
2.1.6. Statistical analyses

Data were analysed statistically by using SPSS20.0 software to check statistics, skewness and kurtosis at a 95% confidence level. One way ANOVA with Tukey was performed to check the significance of differences observed. Differences were statistically significant at $p < 0.05$. 
Chapter 3

3.1. Result and discussion

3.1.1. Optimization of nylon 6 electrospun fibre

Different percentages of nylon 6 solutions (15-24% w/v) were electrospun to determine the optimal concentration for generations of homogenous fibres. It was observed that 15% and 16% (w/v) nylon 6/formic acid solutions were too low in concentration for electrospinning; beads and drops were observed. According to Ryu et al., (2003) the characteristics and diameter of electrospun fibres depended on the concentration of polymer. They prepared polymer solutions by dissolving nylon 6 in formic acid. Ryu and team observed a beads-on-a-string structure caused by the formation of beads and drops when the concentration of nylon 6/formic acid solution was below 15% (w/v). Doshi and Reneker (1995) also reported that higher viscosity, higher net charge density and lower surface tension favoured the formation of fibres without beads. Moreover, the presence of beads was observed following electrospinning of 18% (w/v) nylon 6/formic acid solution (Figure 3.1-a). The presence of beads and drops with the fibres did not provide continuous fibre structure which was the primary objective of this study.
Figure 3.1: Scanning Electron micrographs of nylon 6 electrospun fibres of different concentrations of w/v nylon 6/formic acid solution: (a) 18% showing presence of beads and fibres (b) 20% and (c) 22% not showing any beads
On the other hand, electrospinning of high concentrations (23 and 24 % w/v) of nylon 6 solution resulted in disrupted fibre formation due to rapid drying of the solvent, formic acid, as the polymer solution left the spinneret. Consequently, the electrospinning process was discontinued because of formation of a clump at the tip of the spinneret. The distance between spinneret and the collector was set at 15 cm when the electrospinning process was run at room temperature (21 ± 2°C).

After analysing the morphology based on SEM images of nylon 6 fibres, continuous fibre formation (without any bead formation) was observed following electrospinning 20 and 22% (w/v) of nylon 6/formic acid solution (Figure 3.1-b and c). Therefore, nylon 6 solutions of 20 - 22% (w/v) were electrospun to test suitability of the fibre mesh as a base for addition of polyaniline to produce conductive fibre membranes.

### 3.1.2. Optimization of the polymerization of aniline

Polyaniline was produced by dissolving aniline in a 0.35M hydrochloric acid solution and this process was used to coat the electrospun nylon 6 fibres. The nylon 6/formic acid solutions of 20 and 22% (w/v) were electrospun, and oxidative polymerization of aniline was conducted by immersing nylon 6 electrospun fibres in a 0.35 M hydrochloric acid solution containing aniline. Polyaniline was produced and used to coat the electrospun nylon 6 fibres. Thus, composite fibres of nylon 6 electrospun fibres coated with polyaniline have
conductivity to serve as a self-standing functional unit or a transducer in a biosensor.

The polyaniline was adsorbed on nylon 6 fibre mesh (20-22% w/v) and then the polyaniline was polymerized in the ammonium per sulphate (APS-HCl). Thus composite fibres of nylon 6 electrospun fibres coated with polyaniline are conductive and served as a self-standing functional unit or transducers in a biosensor. A baseline study by Hong et al., (1999, 2004) optimized the polymerization temperature at 40°C and the ratio of monomer to oxidant, Aniline: Ammonium per sulphate (APS) = 1:1.

In the current study, nylon 6 fibre membranes were immersed in the aniline solutions for 30 - 120 min. Various concentrations of aniline and concentration of ammonium per sulphate (APS) ranging from 0.3 to 0.6 M were used in the oxidative polymerization protocol. From the SEM images, the polyaniline coating seemed to be uniform; nylon 6 electrospun fibres were completely coated with polyaniline (Figure 3.2).
Figure 3.2: Scanning Electron micrographs of nylon 6/ PANi electrospun fibres following the oxidative polymerization of aniline with different concentrations: (a) 0.3 M (b) 0.45 M (c) 0.5 M (d) 0.6 M for 60 min.

To measure the conductivity of nylon 6/PANi conducting fibres, impedance analysis was performed using a 4-electrode probe to measure the charge transfer resistance. The impedance scan was performed over a frequency limit from 50 Hz to 100000 Hz (100 KHz). From impedance analysis, it was observed
that the charge transfer resistance of the nylon 6 (22% and 20% w/v)/PANI composite fibres was significantly (p < 0.05) lower when polymerized for 60 min (average charge transfer resistance around 2.9 MΩ for 20% and 1.5 MΩ for 22% w/v nylon/ PANi fibres), whereas, the composite fibres (both 22% and 22% w/v nylon/PANI fibres) were highly resistant (significantly lower conductivity, p < 0.05) as the polymerization process continued for 90 min and 120 min. It is presumed that due to the longer polymerization time, aniline formed a insulation layer around the nylon 6 electrospun fibre mesh. In addition, it was shown by Bhadra and team (Bhadra et al., 2007) that after a specific period of reaction PANi obtained structures with good doping which means more conductivity but after reaching to a certain period of reaction the conductivity of PANi decreased as the polymerization time increased. Consequently, the charge transfer resistance is higher when the polymerization time was 120 min. Table 3.1 and 3.2 represents the mean charge transfer resistance for both 20% (a) and 22% (b) w/v nylon/PANI conducting electrospun fibres when polymerization of aniline (0.3 M, 0.45 M, 0.5 M and 0.6 M) was performed for 30-120 min.
Table 3.1: Comparison of charge transfer resistance of 20% w/v nylon/PANi electrospun fibres with different monomer concentration (M) and polymerization time (min)

<table>
<thead>
<tr>
<th>20% w/v nylon</th>
<th>Monomer (aniline) concentration/charge transfer resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymerization time (min)</td>
<td>0.3 M</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>30</td>
<td>4623±13Aa</td>
</tr>
<tr>
<td>60</td>
<td>3527±18Ba</td>
</tr>
<tr>
<td>90</td>
<td>4132±19Ca</td>
</tr>
<tr>
<td>120</td>
<td>4479±17Da</td>
</tr>
</tbody>
</table>

Means followed by the same capital letter within columns are not significantly different (P>0.05) Means followed by the same lower case letter within rows are not significantly different (P>0.05) (Tukey’s HSD)

Table 3.2: Comparison of charge transfer resistance of 22% w/v nylon/PANi electrospun fibres with different monomer concentration and polymerization time

<table>
<thead>
<tr>
<th>22% w/v nylon</th>
<th>Monomer (aniline) concentration/charge transfer resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymerization time (min)</td>
<td>0.3 M</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>30</td>
<td>4650 ±19Aa</td>
</tr>
<tr>
<td>60</td>
<td>2905 ±10Ba</td>
</tr>
<tr>
<td>90</td>
<td>3149 ±15Ca</td>
</tr>
<tr>
<td>120</td>
<td>4940±23Da</td>
</tr>
</tbody>
</table>
Means followed by the same capital letter within columns are not significantly different (P>0.05) Means followed by the same lower case letter within rows are not significantly different (P>0.05) (Tukey's HSD)

It can be said from the table 3.1 and 3.2 that the charge transfer resistance of the nylon 6 (22% and 20% w/v)/PANi composite fibres was significantly (p < 0.05) higher while using 0.3 M aniline during polymerization, whereas, the composite fibres were less resistant (significantly higher conductivity, p < 0.05) while using 0.45 M and 0.5 M of aniline in the polymerization process. It was also noticed that the charge transfer resistance, as shown in table 3.1 and 3.2, increased significantly (p < 0.05) at 0.6 M aniline concentration for both 20% and 22% w/v nylon/ PANi electrospun fibres. It was presumed that the increase in aniline concentration affected the molar percent of HCl in reaction mixture. Consequently, less conductive polyaniline was developed.

It was observed that charge transfer resistance increased as the aniline concentration decreased from 0.3 M to 0.5 M for both 20% and 22% w/v nylon/ PANi conducting fibres (table 3.1 and 3.2). Similarly, charge transfer resistance was significantly less when the polymerization process continued for 60 min and after that, charge transfer resistance increased as the polymerization time increased. As a result, aniline of concentration of 0.45 M and 0.5 M and 60 min polymerization time were considered for further oxidative polymerization which was performed according to the method mentioned earlier in this section.
Figure 3.3: Impedance spectroscopy measurement of the change of charge transfer resistance in 20% w/v and 22% w/v conducting nylon/PANi composite fibres with polymerization time 60 min and aniline concentration (0.45 M and 0.5 M). Values of bars with the different letter above them are significantly different for the given attribute at $p \leq 0.05$.

Figure 3.3 shows the charge transfer resistance of nylon 6/PANi composite fibres; it is clearly shown that 22% w/v nylon/PANi composite fibres have significantly lower resistance for both 0.45 M and 0.5 M aniline concentration ($p < 0.05$). Polymerization was performed for 60 min. Therefore, 22% w/v nylon 6/formic acid solution was used for producing electrospun fibres for subsequent studies. Furthermore, the charge transfer resistance of the nylon 6/PANi composite fibres was significantly lower ($p < 0.05$) when using 0.5 M aniline at the 60 min polymerization time (Figure 3.3) in comparison to the charge transfer
resistance of the composite fibres when polymerized with aniline concentration of 0.45 M for 60 min.

Based on the aforementioned results and explanation, the nylon 6/PANI conducting fibres which were used for present study, were electrospun from 22% w/v nylon 6/formic acid solution. The polyaniline coating was produced by polymerization of 0.5 M aniline in an acidic solution of HCl (0.35 M) and ammonium-per-sulphate for 60 min.

![FTIR spectra](image)

**Figure 3.4:** FTIR spectra of 22% w/v nylon 6 electrospun fibre (control) and 22% w/v nylon 6/PANI composite fibre.

The presence of polyaniline (PANI) in the electrospun nylon 6 fibre was observed in SEM Images and confirmed by FTIR spectra (Figure 3.4). To ensure the corrected peak area values, the FTIR spectra were recorded three times in different places on the same sample. The 1626 cm\(^{-1}\) peak was attributed to C=C
stretching in aromatic nuclei. A peak at 1570 cm\(^{-1}\) was caused by the quinonoid structure of PANi. Another sharp peak at 1493 cm\(^{-1}\) corresponded to the benzenoid structure of PANi. 1314 cm\(^{-1}\) represented C-N stretching of secondary aromatic amine (Arasi et al., 2009).

3.1.3. Capture efficiency of the nylon/PANi biosensor and elution of \textit{E. coli} K-12

In addition to using the antibody modified electrospun fibres as sensors, additional trials were undertaken to evaluate the capture efficiency of the pads.

To select an appropriate buffer solution for the elution process, there were three buffer solutions (10% NaCl solution, tris buffer solution pH-8 and phosphate buffer solution pH-8) were used to elute BSA (mg/mL), which was used as the antigen, from anti-BSA modified nylon 6/PANi conducting fibres. It was observed that the concentration of the eluted BSA was significantly higher (p < 0.05) (6.3 mg/mL) when using 10% NaCl. On the other hand, the concentrations of BSA were 2.4 mg/mL and 1.8 mg/mL while using tris buffer and phosphate buffer respectively as the elution buffer. Figure 3.5 presents a summary of the results. A control sample was established that was not treated with anti-BSA. Since 10% NaCl solution eluted a significantly higher amount (6.3 mg/mL) of BSA from the sensor membrane, 10% Nacl was used to elute \textit{E. coli} K-12 from the antibody modified electrospun fibres during further assays.
Figure 3.5: Elution volumes measured using different buffers to elute Bovine Serum Albumin (BSA) from antibody modified nylon 6/PAni sensor membrane. As elution buffer 10% NaCl solution, tris buffer solution and phosphate buffer solution were used. Control fibres weren’t treated with anti-BSA.

In the current study, the fabricated nylon 6/PAni electrospun fibres were modified with antibodies with affinity towards *E. coli* K-12. The bacteria binding ability of the antibody modified electrospun fibres was observed by SEM (Figure 3.6).
Figure 3.6: Scanning electron microscopy images of electrospun fibres after bacteria detection (a) *E. coli* K-12 was captured onto antibody modified nylon 6/PANi electrospun fibre and (b) no bacteria was observed on the electrospun fibre without antibody treatment.

Figure 3.6 (a) shows that the target antigen (*E. coli* K-12) can be effectively captured on the electrospun fibres, modified with affinity antibody. On the other
hand, the control (without antibody) fibre mesh didn't contain any organism after rinsing, Figure 3.6 (b). It was observed that the average % recovery of *E. coli* K-12 was < 80% for an initial loading of $10^5$-$10^7$ CFU (equivalent to $3 \times 10^8$ CFU/mL - $1.6 \times 10^4$ CFU/mL). While eluting with 10% NaCl solution, the %recovery was above 80% for all concentration of *E. coli* K-12 (Figure 3.7). The efficiency measurement of bacterial cell release from nylon 6/PANi conducting electrospun fibre membrane may be confounded by cell losses during processing such as aggregation, adhesion to other surface (e.g., tube walls, pipette tips) and/or viability loss.

![Figure 3.7](image-url): % Recovery of *E. coli* K-12 after elution with 10% NaCl solution. % recovery was calculated from equation 2-2.
3.1.4. Detection of *E. coli* K-12 using the antibody modified conducting fibre biosensor

The sensitivity and response of the antibody modified electrospun biosensor was demonstrated by an assay using *E. coli* K-12 for various concentrations (0 – 10⁶ CFU). The response of the biosensor was based on the difference (ΔR) between charge transfer resistance (R₀) before and the charge transfer resistance (Rᵢ) after antibody-antigen binding. Figure 3.8 represents the relationship of the response of the biosensor membrane with time duration. The biosensor responses show a parabolic pattern instead of the linear relationship that was anticipated (Figure 3.8).

![Figure 3.8](image)

**Figure 3.8**: Biosensor response verses test time for different target concentration of *E. coli* K-12 bacteria sample. Detection time was established at around 20 minutes and the detection limit was from 1-5 log CFU (1.6×10⁴ CFU/mL-3×10⁸ CFU/mL).
It was observed that around 20 min duration the difference between initial (before treatment with *E. coli* K-12) and final (after treatment with *E. coli* K-12) was greater for every concentration of bacteria. Therefore, the charge transfer resistance increased. After 20 min of exposure the response decreased. This can be explained by the fact that equilibrium adsorption of antibody modified electrospun fibres was achieved after 20 min. It was observed that after 20 min of treatment with $10^5$ CFU ($3 \times 10^8$ CFU/mL) *E. coli* K-12, the conductance was significantly higher ($p<0.05$) than that of other concentrations of bacteria. The control sample was established without treatment with the antibody.

In Figure 3.8 (presented with yellow broken line), the response of the control sample (0 CFU) was near to zero. On the other hand, around 20 min a significant response drop from 6.86 at $10^5$ CFU *E. coli* K-12 suspension to 1.94 at $10^6$ CFU *E. coli* K-12 was observed. This could be because of higher concentration (above $10^1$ - $10^5$ CFU). At higher concentration (above 1- 5 log CFU), the binding site can be over-occupied with antigen, e.g., bacteria, hence obstructing the charge transfer within the conductive polymer fibre mesh. It can be anticipated that at higher concentration of *E. coli* K-12, the excess proteins, which are unbound antigen, may obstruct the charge transfer within the conductive polymer structure (Luo *et al.*, 2012). German Cavelier also concluded (Cavelier, 1996) that protein molecules served as a barrier against conduction by interfering with electron and charge transfer.
The linear detection range for \textit{E. coli} K-12 was obtained from $10^{1}$ to $10^{5}$ CFU (figure 3.9) using antibody modified electrospun fibre. It can be assumed that the detection range of \textit{E. coli} K-12 by antibody modified nylon 6/PANi biosensor membranes is from $10^{1}$ to $10^{5}$ CFU ($1.6 \times 10^{4}$ CFU/mL-3$\times$10$^{8}$ CFU/mL).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.9.png}
\caption{Charge transfer resistance of antibody modified electrospun fibre after treating with \textit{E. coli} K-12 ($10^{1}$- $10^{5}$ CFU) for 20 min.}
\end{figure}

The lower detection limit of this proposed biosensor is equivalent (if not higher) to that of conventional detection methods, e.g., IMS (between $10^{3}$ and $10^{4}$ CFU/mL) and the most used rapid methods, e.g., ELISA and PCR (ranges from $10^{1}$ to $10^{6}$ CFU/mL). Also, the current study has minimized one hurdle by demonstrating that the biosensor developed from polyaniline coated nylon 6 electrospun fibre is capable of detecting the target biosensor in 20 minutes which is significantly less than that the detection time of ELISA (10-24 h), PCR (4-6 h)
and other conventional biosensors (around 2 h) (Ivnitsk et al., 2000; Su and Li, 2004).
Chapter 4

4.1. Conclusion and future work

4.1.1. Conclusions

The purpose of the current research was to fabricate electrospun fibres modified
with conducting polymers to produce a homogenous, conductive surface for the
immobilization of capture antibodies. When modified with anti-\textit{E. coli} K-12
antibodies, it was possible to achieve recoveries of up to 82% that in
comparative terms is equivalent if not superior to conventional extraction
methods based on immune capture technology. The interaction of \textit{E. coli} K-12
with immobilized antibodies could be monitored via measuring the change in
impedance (charge transfer resistance) with a linear range between 1 – 5 log CFU.

The final conclusions of the study are:-

1) Polyaniline can be coated onto the surface of electrospun nylon fibres with
the concentration of monomer and polymerization time governing the
conductivity, in addition to thickness, of the conducting polymer layer.

2) The optimal conditions for the deposition of polyaniline layers onto
electrospun fibers were 0.5 M aniline polymerized for 60 min.

3) Anti-\textit{E. coli} K-12 antibodies could be immobilized onto the surface of
conducting polymer modified electrospun fibres and upon optimization
could recover 80% of *E. coli* K-12 from suspensions containing 1 – 5 log CFU of the bacterium.

4) The binding of *E. coli* K-12 to immobilized antibody resulted in a charge transfer resistance of the supporting conducting polymer that was linear within the range of 1 – 5 log CFU *E. coli* in saline cell suspensions.

### 4.1.2. Future Work

The capture-sensor membrane based on conducting polymer modified electrospun fibres has provided high capture efficiencies of *E coli* K-12 and sensitive detection. However, it must be noted that simple buffered solutions, saline and monocultures were applied in the current study. In reality, samples whether in enrichment media or food matrices, will provide more of a challenge because interference from sample constituents on the conductivity of sensor membrane and non-specific binding would likely be encountered. Therefore, future research should be directed towards evaluating sensor performance in relevant sample matrices such as ground meat homogenate and enrichment cultures. Additional trials should be undertaken to assess the inclusivity and exclusivity of the sensor to establish the specificity. In the event that a high number of false-positives are generated it can still be envisioned that the membrane can still find utility as a means of high recovery of bacterial targets over detection performed by conventional diagnostic platforms such as real Time-PCR.
In the near future, research can focus on using different types of conducting polymers to develop biosensors. Other conducting polymers might improve the conductivity of electrospun fibres.

As different foods have different pH value, the study can be expanded to observe the performance of biosensor with various pH values.
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