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Detection of bacterial endotoxin in food: New planar interdigital sensors based approach

Mohd Syaifudin Abdul Rahman [a,d], Subhas Chandra Mukhopadhyay [a,*], Pak-Lam Yu [a], Javier Goicoechea [b], Ignacio R. Matias [b], Chinthaka P. Gooneratne [c], Jűrgen Kosel [c]

[a] School of Engineering and Advanced Technology, Massey University, Palmerston North, New Zealand
[b] Department of Electrical and Electronic Engineering, Public University of Navarra, Pamplona, Spain
[c] Sensing, Magnetism and Microsystems Group, King Abdullah University of Science and Technology, Saudi Arabia
[d] Malaysian Agricultural Research and Development Institute, Serdang 43300, Selangor, Malaysia

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ABSTRACT

Food poisoning caused by endotoxins or Lipopolysaccharide (LPS) are associated with Gram-negative bacteria. Two major food-borne pathogens, *Escherichia coli* and *Salmonella* are examples of Gram-negative bacteria which cause a large number of outbreaks of food poisoning. New types of planar interdigital sensors have been fabricated with different coating materials to assess their response to endotoxins. A carboxyl-functional polymer, APTES (3-Aminopropyltriethoxysilane) and Thionine were chosen to be coated onto FR4 interdigital sensors. The chosen coating materials have carboxylic or amine functional groups, which were optimized to be stable in water. All coated sensors were immobilized with PmB (Polymyxin B) which has specific binding properties to LPS. The sensors were tested with different concentrations of LPS O111:B4, ranging from 0.1 to 1000 μg/ml. Analyses of sensors' performance were based on the impedance spectroscopy method. The impedance spectra were modeled using a constant phase-element (CPE) equivalent circuit, and a principal component analysis (PCA) was used for data classification. Sensor coated with APTES has shown better selectivity for LPS detection. The experiments were repeated by coating APTES and immobilizing PmB to a new improve designed of novel interdigital sensors (thin film silicon based sensors). These sensors were observed to have better sensitivity and selectivity to the target biomolecules of LPS. Further experiments were conducted to study the effect of different coating thickness on sensor sensitivity, selectivity and stability. Different food samples contaminated with endotoxin were also tested to verify that the interdigital sensing approach is able to be used for endotoxin detection.

1. Introduction

Bacterial endotoxins are toxins associated with some Gram-negative bacteria (Wagner, 1989). *Escherichia coli* and *Salmonella* are examples of Gram-negative bacteria which have been identified as two major food-borne pathogens (Lazcka et al., 2007). These bacteria are commonly found on many raw foods. In order to protect the consumers, all food needs to be tested for endotoxins, which places a heavy workload on the laboratories and is extremely expensive to the industry. The culturing technique is a conventional method of endotoxin detection which is capable of giving accurate results because of high selectivity and sensitivity. This technique is conducted by enriching the food sample and performing various media-based metabolic tests (agar plates or slants) (Leoni and Legnani, 2001). However, this method of detection is tedious, time-consuming and also expensive. One of the most popular methods of pathogen detectionuses polymerase chain reaction (PCR) (Fratamico, 2003), which is based on the isolation, amplification and quantification of a short DNA sequence. However, PCR methods are expensive and need trained personnel to operate. Enzyme linked immunosorbent assays (ELISA) and culture techniques for determining and quantifying pathogens in food have been established (McMeekin, 2003). The ELISA technique also needs to be confirmed using conventional tests. Biosensors have been widely used to detect pathogens in food (Alocilja and Radke, 2003); the transduction methods used in biosensors are optical fiber (Baeumner, 2003), electrochemical (Mubammad-Tahir and Alocilja, 2003), interdigitated array microelectrodes (Varshney and Li, 2009), and surface plasmon resonance (SPR) (Morris and Sadana, 2005).

Planar interdigital sensors, also known as interdigitated electrode structures, have been used in biosensor applications (Radke and Alocilja, 2005; Varshney and Li, 2009), and are capable of characterizing near-surface properties, such as conductivity, permeability and dielectric properties. This research work is based on

* Corresponding author.
E-mail addresses: saifudin@mardi.gov.my (M.S. Abdul Rahman), S.C.Mukhopadhyay@massey.ac.nz (S.C. Mukhopadhyay).

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these sensors, which have also been used to estimate the property of dielectric materials for milk (Mukhopadhyay et al., 2006), meat inspection (Mukhopadhyay and Gooneratne, 2007), assessment of pelt quality in leather (Mukhopadhyay et al., 2008) and detection of contaminated seafood with domoic acid (Syaifudin et al., 2009). Impedance spectroscopy (IS) is a popular technique to evaluate electrical properties of food (Li et al., 2011; Wu et al., 2008), materials and their interfaces with surface-modified electrodes (Barsoukov and Macdonald, 2005; Olson and Buhlmann, 2010). The combinations of planar interdigital sensors, or interdigitated array electrodes, with impedance spectroscopy measurements have been reported (Koep et al., 2006; Ramon-Azcon et al., 2008). Principal component analysis (PCA) has been utilized as a method for characterization of food properties (Probola and Zander, 2007), sensor sensitivity (Barko and Hlavay, 1998; Kerschen et al., 2005), material properties (Yang et al., 2009) and others (Olivati et al., 2009). The research work presented in this paper discusses the development of a new structure of planar interdigital sensors and their impedance spectroscopy measurements for endotoxin detection. Data were analyzed using principal component analysis correlating the impedance parameters of the test sample.

2. Materials and methods

A new structure, which is different than the conventional interdigital structure, has been adopted for higher sensitivity. The sensors were designed to have the same effective area of 4750 by 5000 μm, and a pitch length of 250 μm. The positive and negative electrodes have the same length and width of 4750 and 125 μm, respectively. The configuration of the electrode structure chosen was 1_5 configuration (five negative electrodes between two positive electrodes) as shown in Fig. 1. The analysis of this configuration was reported in (Syaifudin et al., 2011). In this research work these sensors were tested with different coating materials. A new type of silicon based interdigital sensor, which has a basic structure of 1_5 configuration, was fabricated and tested. The details of which will be discussed further in Section 3.

2.1. Coating of sensors

Different coating materials and coating techniques have been chosen and tested. Fig. 2 shows interdigital sensors fabricated on FR4 coated with different materials. The optical micrograph images of different coating materials on interdigital sensors are shown in Fig. 3. The carboxyl-functional polymer, APTES (3-Aminopropyltrietoxysilane) and Thionine (a blue organic dye) were introduced as coating materials. The objective is to study if these coatings could improve the performance and the selectivity of the sensors. All coating materials were selected and optimized to be stable in water, as the measurements were taken place in water media. Moreover, the coatings have been designed to have available carboxylic or amine functional groups. The use of these functional groups is a widely used technique to specifically bind some kind of bio-molecules. Different coating techniques were applied in order to study how different techniques contribute to the sensor performance. If the simplest coating technique gives a significant result, it can be used for future experiments without the necessity of sophisticated machines and procedures. The electro-spinning method and spin coating method are examples of coating procedures which need specific machines and technical procedures to operate, while the dip-coating method is a manual procedure, not requiring such machines or procedures, used widely in coating materials. The thickness of the coating materials were observed in between 800–1000 nm on the electrode surface.

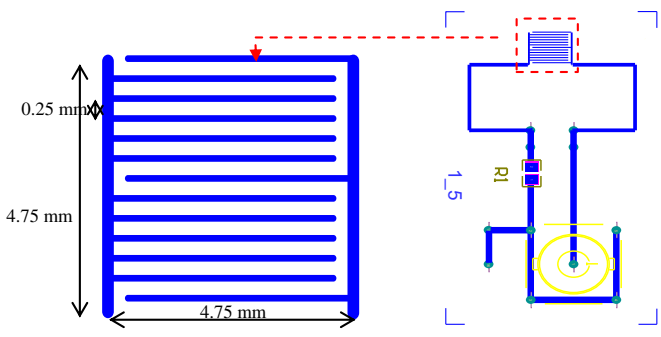

Fig. 1. Interdigital sensor design: 1_5 configuration.

2.1.1. Electro-spining method

A polymeric solution was placed into a syringe and a high voltage (23 kV) was applied between the needle and a cathode screen. The polymer was then accelerated towards the cathode, and the electric field pulls the materials, forming a thin polymer fiber. The substrates were placed at the cathode, where the fibers are collected, forming a mat. Various diameter fibers, as thin as 50 nm, can be obtained by controlling the conditions of the polymeric solutions and the voltage and distance from the needle and the cathode. In this particular case (Coating A), a carboxyl- functional polymer was used. The carboxyl-terminated functional groups are widely used in biological applications for immobilization of amine-molecules, such as proteins, etc. The experimental conditions were adjusted to form sub-micron diameter fibers. The final fibers were water insoluble, and their constituent material can be considered a hydrogel. This kind of material has a high affinity for water and ionic solvents.

2.1.2. Spin-coating method

The spin-coating technique is based on a rotation of the substrate at high speeds (from 1500 to 5000 rpm). If the desired solution is placed onto the substrate, and the sample is rotated, the centrifugal forces push the excess solution away. Equilibrium is reached between the centrifugal forces from the rotation, and the viscous forces inside the solution. In the final step, the solvent evaporates away, leaving a uniform thin solid film. This technique makes it easy to obtain uniform coatings as thin as 40 nm. In this particular case (Coating B), a carboxylic functional polymer, which is stable in water and has a hydrogel nature, was spin-coated over the interdigitated electrodes. Accurate measurement of the thickness of the coating is difficult due to the non-uniform profile of the substrate, however, it is within 800 nm. In this case the polymer was doped with a small amount of silver nanoparticles, with the objective of achieving more impact in the capacity variations of the coating.

2.1.3. Dip-coating Method

The dip-coating technique uses a similar physical phenomenon as spin-coating, but without spinning the sample. In this case the thinning of the fluid layer is achieved by reaching the equilibrium between the viscous forces inside the film and gravity (instead of the spinning force). Firstly, the substrate was immersed into the desired solution, and subsequently impregnation was carried out by lifting the substrate at a very controlled speed. During this step the fluid layer was thinned by the effect of gravity until the solvent was partially evaporated. Finally the substrate was hold, to allow the solvent to evaporate completely. This process can be repeated several times to ensure stabilization of the film at the end of each step. The initial solution was prepared from a chemical precursor of silica. The PCB substrates were immersed into the