Chapter

Fast detection of Pathogenic *Escherichia coli* from Chicken Meats

Saloua Helali and Adnane Abdelghani

Abstract

Food is a means to sustain and enjoy life, but it is also a medium for microbial contamination, causing disease and death. Fruits, vegetables, meat, and water are the common sources of contamination. *Escherichia coli* is one of the most frequent Pathogenic Bacteria responsible for food poisoning and food-related infections. *E. coli* infection causes severe bloody diarrhea, abdominal cramps, and occasional vomiting. In the present study, electrochemical impedance spectroscopy (EIS), surface plasmon resonance (SPR), and physisorption techniques were evaluated to decrease sample preparation time and to improve the sensitivity and specificity for the detection of low levels of pathogenic *Escherichia coli* in frozen chicken meat. The electrical and optical properties of the immobilized anti-*E. coli* antibody were studied. Moreover, the developed biosensor was used for *E. coli* detection in inoculated frozen chicken meat.

Keywords: physisorption, impedance spectroscopy, SPR imaging, *E. coli*, frozen chicken meat

1. Introduction

According to the World Health Organization (WHO), foodborne illnesses are defined as diseases of infectious or toxic nature caused by consumption of contaminated foods or water. The main causes of foodborne illness are viruses, bacteria, parasites, toxins, metals, and prions where bacteria constitute 66% of the problems [1]. *Campylobacter*, *Salmonella*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *E. coli* O157:H7 are the major Pathogenic Bacteria causing different food illness [2]. The most common clinical symptoms of foodborne illnesses are diarrhea, vomiting, abdominal cramps, headache, and nausea. In many developing countries, the elderly and children under the age of 5 years are at higher risk of food infection. Among the foodborne pathogens, *E. coli* and *Salmonella* are the most common and frequent pathogens responsible for food poisoning and food-related infections. These two Pathogenic Bacteria can be transferred by poultry meat, red meat, desserts, and egg.

Nowadays, *Escherichia coli* and *Salmonella* are the most important and frequent pathogens responsible for food poisoning and food-related infections in chicken meat [2–6]. *E. coli* is a normal inhabitant of intestinal tracts and can be found in chicken feces, litter, dust, and rodent droppings [7]. Preventing food contamination and human infection from *E. coli* requires continuous control measures at all stages of the
food production: from agricultural production to processing, manufacturing, transporting, storing, and preparation of foods in both commercial establishments and the domestic environment. Recently, many analytical applications have been developed for the determination of poultry meat contamination [8–10].

Several microbiological techniques such as conventional culturing, PCR, and ELISA are still considered the oldest and the most accurate approach for bacteria detection. These techniques need traditional sample preparation, though very efficient in extracting the target analyte, which is time-consuming and produces large amount of solvent wastes. Among the various techniques, electrochemical impedance spectroscopy technique and surface plasmon resonance imaging have previously been investigated to study the detection of Pathogenic Bacteria on gold [11]. These two techniques offer several advantages: First, they are label-free and direct detection method for biomolecular interactions because the measurements are based on small electric signal and very large range of frequency (100 mHz–100 kHz) and refractive index changes [12–14]. The analyte does not require any special characteristics (scattering bands) or labels (radioactive or fluorescent) and can be detected directly without the need for multistep detection protocols (sandwich assay). Second, the measurements can be performed in real time, allowing the user to collect kinetic data, as well as thermodynamic data.

In this contribution, an innovative way for sensitive detection of E.coli bacteria based on anti-E. coli antibody immobilized onto gold surface by physisorption technique is presented. The electrical properties of the immobilization of anti-E. coli antibody were studied. Moreover, the developed biosensor was used for E. coli detection in inoculated frozen chicken meat.

2. What is Escherichia coli (E. coli)?

- *Escherichia coli* are gram-negative bacilli of the family *Enterobacteriaceae*.
- *E. coli* is commonly found in the lower intestine of warm-blooded organisms.
- *E. coli* is the most common human and animal pathogens as it is responsible for a broad spectrum of diseases.
- There are many different types (strains) of *E. coli* which cause a number of illnesses. Virulence types of *E. coli* include enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterohemorrhagic *E. coli* (EHEC) [15].

3. History

In 1982, Riley LW et al. and colleagues were the first to recognize the EHEC serotype O157:H7 as a human pathogen associated with outbreaks of bloody diarrhea in Oregon and Michigan, USA [16]. Since then, *E. coli* O157:H7 has become one of the most important foodborne pathogens.

4. Symptoms and mode of transmission

Virulent strains of *Escherichia coli* are responsible for most diarrheal infections, meningitis, sepsisemia, and urinary tract infections in children worldwide. A person who is infected with *E. coli* O157 can pass it on to other people if there is a situation of insufficient hygiene or handwashing. Small children can still pass the infection on for a couple of weeks after they have recovered from any illness. On
the basis of World Health Organization report, the most important symptoms and mode of transmission of *Escherichia coli* are represented in the diagram (Figure 1).

5. Immunosensor conception

5.1 Working electrodes

Interdigitated microelectrodes were provided by the Microelectronics Institute of Barcelona, National Microelectronics Centre (IMB-CNM), Spain. The different steps of the fabrication of the gold interdigitated electrodes were extensively characterized as described in Ref. [17]. The electrode consists in 3 mm × 3 mm square
arrays, which consist of 108 fingers 10 μm wide, separated 10 μm from the nearest band (Figure 2A). Before modification, the gold microelectrodes were first cleaned in ethanol solution and then electrochemically activated in 0.5 M NaNO₃ solution by applying a series of potential pulses from 0 to −2 V vs. Ag/AgCl (3 M KCl). After that, a cyclic voltammetry in 1 mM potassium ferrocyanide [K₄Fe(CN)₆] was applied to check the degree of activation of the microelectrodes.

The pre-treated working microelectrodes were immersed in 100 μL goat polyclonal IgG anti-E. coli antibody solution (5 mg/mL in PBS) for 90 min (Figure 2B). The gold substrates were then rinsed with PBS buffer in order to remove the non-bonded antibody; finally the substrate was kept in bovine serum albumin (BSA) 1% for 40 min in order to block any defective areas. The excess of BSA was removed by rinsing with PBS.

5.2 Electrochemical impedance spectroscopy technique

All electrochemical measurements were performed with a three-electrode configuration using a Pt foil counter electrode, an Ag/AgCl reference electrode, and a modified gold μ-electrodes as a work electrode.

The impedance analysis was performed with a CHI604E Electrochemical Instrumentation (CH Instruments, Inc) in the frequency range of 0.1–100 kHz, using a modulation voltage of 10 mV in sterile PBS buffer.

5.3 SPR imaging technique

John Mitchell [18] has been successfully explaining the physical principles of surface plasmon resonance. The SPR is an optoelectronic phenomenon that occurs when a photon of light is incident upon a noble metal surface such as gold or silver. When the wavelength of the photon equals the resonance wavelength of the metal, then the photon couples with the surface and induces the electrons in the metal surface to move as a single electrical entity called a plasmon. This oscillation of electrons sets up an electromagnetic field that exponentially decays out from the metal surface, with significant electrical field strength typically occurring within 300 nm of the surface. When molecules with sufficient mass bind to the surface within the range of the electric field, they perturb the plasmon and change the resonance wavelength. When dealing with a fixed planar surface, this is seen as a shift in the resonance angle of the incoming photons.

In this work, the surface plasmon resonance imaging system was from GWC technologies (USA). The system is based on Charge-Couple Device (CCD) camera which can simultaneously capture all data for all the gold spots and converts the reflectivity changes to pixels data. The sensor surface was an array format with 16 gold spots (each gold spot has a surface of 0.004 cm²) deposited on glass substrate. An incident beam of excitation wavelength of 850 nm was used. At resonance condition, the variation of the reflected light was due to the refractive index variation of the external dielectric medium or immobilized thin layer. The noise of such system is equal to 0.5 pixel (Figure 3).

5.4 Physisorption of polyclonal antibody on interdigitated gold microelectrodes

Physisorption is defined as weak electrostatic interactions including Van Der Waals interactions, dipole-dipole, and London forces. This physical interaction resulting from nonspecific was forming on substrate have energy range from 0.2 to 4 kJ/mol. The binding energy depends on the polarizability and on the number of atoms involved of the molecules. It takes place on all surfaces provided that temperature and pressure conditions are favorable.

Random physisorption is the easiest and fastest strategy for biomolecule immobilization onto substrates. Mainly, physisorption does not depend on multistep, long
experimental procedures and is easily reversed [19]. In addition, physisorbed phages have been described to promote bacteria-specific capture, infection, and lysis, when monitored by SPR [20, 21]. This work was carried using physisorption functionalization based on its simplicity. First, the gold microelectrodes were modified with anti-\textit{E. coli} antibody, followed by washing with PBS then physical blocking with BSA. Blocking prevented nonspecific adsorption of unwanted nontarget components during subsequent incubations. Then, in this work, a fast and suitable immunosensor for \textit{E. coli} bacteria detection, using physically adsorbed antibodies, SPR and EIS, is developed.

6. Electrochemical impedance measurement

The rapid and specific detection of Pathogenic Bacteria has become an increasingly demanding field in recent years for ensuring the safety of human health. EIS is a sensitive technique, which monitors the electrical response of the system studied after the application of a periodic small amplitude AC signal [22]. With this aim, the gold microelectrode surface and antibody coverage are of high importance for ensuring high reactivity and stability of the immunosensor.

The typical response of electrochemical impedance spectra of gold, “gold/Antibody /BSA” interfaces was illustrated in Figure 4(I). The curve shows the typical Nyquist plots presented as a combination of the real, Zre, and imaginary, Zim, components originating mainly from the resistance and capacitance of the cell, respectively. The impedance spectra corresponding to each step were fitted with computer-simulated spectra using Randles circuit in Figure 4(II) by Zview modeling program (Scriber and Associates, Charlottesville, VA).
This equivalent circuit includes the ohmic resistance of the electrolyte solution, $R_s$, at 100 kHz; the Warburg impedance, $Z_w$, from the diffusion; the constant phase element, CPE, which was introduced into the circuit instead of a capacitance in order to depict the nonhomogeneous quality of the deposited layer, respectively [23, 24]; and the charge transfer resistance, $R_{ct}$. The constant phase element impedance (CPE) was introduced into the circuit instead of a capacitance:

$$Z_{\text{CPE}} = \frac{1}{K(j\omega)^n}$$  \hspace{1cm} (1)

where $\omega = 2\pi f$ is the angular frequency and $K$ and $n$ are the experimental parameters. When $n$ approaches 1, the CPE acts as an ideal capacitor. The CPE can be viewed as a heuristic method to incorporate the effects of surface heterogeneity both along and through the electrochemical interface. Data was fitted to the Randles circuit shown in Figure 4(II). The plot shows the expected increases in the charge transfer resistance after immobilization of anti-\textit{E. coli} antibody from 0.14 to 1.22 MΩ. This increase could be attributed to a rearrangement in the structure of the antibody and showed that the grafted layer becomes more insulating, whereas the gradual decrease in capacitance was related to the positive change in thickness after immobilization of anti-\textit{E. coli} antibody and BSA. This behavior is consistent with the successful immobilization of anti-\textit{E. coli} antibody and BSA molecules.

7. Surface plasmon resonance measurement

In the last few years, surface plasmon resonance was used as a sensitive method and as a label-free detection method for biomolecular interactions.

The immunosensing protocols exposing sensor surface to the PBS buffer as the baseline, followed by injecting anti-\textit{E. coli} antibody, which is allowed to flow over the sensor surface, leading to binding. This binding alters the mass of the surface layer which translates into refractive index variation and resonance angle shift. Figure 5 shows the resulting response obtained from the injection of 5 mg/mL.

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**Figure 4.**

(I) [A] Nyquist impedance plots for gold microelectrode and [B] Nyquist impedance plots after physisorption of anti-\textit{E. coli} and BSA on gold microelectrodes. (II) equivalent circuit used to model impedance data.
anti-\textit{E. coli} antibody solution. The real-time sensorgram showed a gradual increase in the response with antibody immobilization onto the sensor chip. The highest response obtained was equal to 87 pixels.

8. Detection of \textit{E. coli} bacteria in inoculated frozen chicken meat sample

Although chicken is the most consumed meat in the world and is one of the most important sources of good-quality proteins, it is highly susceptible to microbial contamination and often implicated in foodborne disease. Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning [25]. According to Osman Albarri (2017) [26], in turkey the highest percentage (93.75\%) of \textit{E. coli} was isolated from chicken, while the lowest percentage (56.25\%) was isolated from meat. Therefore, ensuring the microbial safety of chicken meat products is an important issue in the context of increasing consumption and production [24]. This moved us to develop rapid, easy, simple, sensitive, and non-time-consuming physical adsorption methods for the detection of \textit{E. coli} bacteria in frozen chicken meat.

With this aim, two samples of fresh chicken meat were kept in freezers at $-18^\circ$C during 45 days [27]. The first sample (S1) was inoculated with \textit{E. coli} with a concentration of $10^5$ CFU/ML in PBS, and the second sample (S2) was kept in PBS buffer (reference). These samples were characterized by EIS as previously described.

\textbf{Figure 6} shows Nyquist plots for gold microelectrode (curve A), gold electrode with immobilized anti-\textit{E. coli} antibody with BSA blocking layer (curve B), gold electrode with immobilized anti-\textit{E. coli} antibody with BSA blocking layer without inoculated bacteria (sample S2, curve C), and gold electrode with immobilized anti-\textit{E. coli} antibody with BSA blocking layer with inoculated bacteria (sample S1, curve D). Using the same equivalent circuit model described in Figure 4 (II), the best-fit equivalent circuit parameters for each step are given in Table 1. The injection of sample S1 and S2 on the microelectrode induce an increase in the charge transfer resistance proving the detection of \textit{E. coli} bacteria in the chicken meat sample.

It is obvious that the chicken was initially contaminated by the \textit{E. coli} bacteria and the concentration of the bacteria is about $10^5$ CFU/ML.
In this work, we describe an approach of detecting *Escherichia coli* bacteria by Electrochemical Impedance Spectroscopy technique and surface plasmon resonance imaging technique. The physisorption method used for immobilization of anti-*E. coli* into interdigitated microelectrode is rapid and easy. Furthermore, *Escherichia coli* bacteria detection was also possible in frozen chicken meat. This method can be used for real-time detection of meat contamination.

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**Table 1.**
The electrical parameters of Randle’s circuit.

| Parameters               | Gold microelectrode | BSA/anti-*E. coli* / gold | Sample S2 | Sample S1 |
|--------------------------|----------------------|---------------------------|-----------|-----------|
| Capacitance, CPE (F)     | 8.34E-7              | 7.46E-7                   | 5.76E-7   | 5.128E-7  |
| n                        | 0.93                 | 0.86                      | 0.88      | 0.9       |
| Resistance, R_s (Ω)      | 110                  | 74.74                     | 70.7      | 71        |
| Resistance, R_w (Ω)      | 1.43E5               | 1.225E6                   | 4.68E6    | 5.01E6    |
| Warburg, Z_W (Ω)         | 45,364               | 1452                      | 132       | 120       |

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**Figure 6.**
[A] Nyquist impedance plots for gold microelectrode, [B] nyquist impedance plots for gold microelectrode with anti-*E. coli* and BSA, and [C and D] nyquist impedance plots for gold microelectrode with anti-*E. coli* and BSA after injection of sample S2 and S1, respectively.
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Author details
Saloua Helali¹,²* and Adnane Abdelghani³

1 Faculty of Science, Department of Physics, University of Tabuk, Tabuk, Kingdom of Saudi Arabia
2 Centre de Recherche et Technologies de L’Energie, Tunisia
3 Carthage University, National Institute of Applied Science and Technology, Tunisia

*Address all correspondence to: s.helali@ut.edu.sa

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