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Review

Advances in Potentiometric Biosensors for Foodborne Bacterial Detection

Mohammad Reza Rabiei,[1] Ali Hossein Rezayan,[2] Berdimurodov Elyor,[3] and Morteza Hosseini[1,*]

[1]*Nanobiosensors Lab, Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran 1439817435, Iran*
[2]*Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran 1439817435, Iran*
[3]*Faculty of Chemistry, National University of Uzbekistan, Tashkent, Uzbekistan*

*Corresponding Author, Tel.: +98-21-86093196
E-Mail: hosseini_m@ut.ac.ir

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Abstract- Due to their effect on human health, rapid, sensitive, and accurate methods for detecting foodborne bacterial pathogens are becoming increasingly important. There are more than 250 types of bacterial foodborne disease, including more than 90% of outbreaks of foodborne illness worldwide, which is considered to be one of the greatest threats to public health. Among the diagnostic methods, electrochemical biosensors have features that make them very efficient in designing and manufacturing biosensors. Potentiometric biosensors have been recognized for their effectiveness in detecting analytes with low cost, ease of use, and simple instrumentation. This article reviews key advances in potentiometric biosensors of foodborne bacterial pathogens. The categorization of different potentiometric biosensors is done on the basis of various foodborne bacterial pathogens involving *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella typhimurium*.

Keywords- Electrochemistry; Potentiometry; Biosensor; Foodborne bacterial; Pathogen

1. INTRODUCTION

The detection and identification of bacterial pathogens is of vital importance in all areas of medicine, food safety, public health, and security. There are more than 250 types of bacterial foodborne disease, including more than 90% of outbreaks of foodborne illness worldwide, which is considered to be one of the greatest threats to public health. Among the reasons for this are poor sanitation science, inadequate food handling, inadequate safety standards, and weak enforcement of food safety laws. These types of infections are most problematic in low-income countries, where medical facilities and pathways of diagnosis and treatment are lacking. In developed countries, such as the United States, foodborne pathogens are also the primary cause of health problems, causing more than 76 million diseases, 300,000 hospitalizations, and 5,000 deaths each year [1-4].

Colony counting, polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) are different methods for bacterial detection [5-7]. But these methods are time consuming, need complex preparation of sample, and dedicated operators [8,9]. An effective method of determining foodborne bacterial whole cells has been developed in recent years, which is highly sensitive, greatly selective, very fast, and inexpensive, utilizing affinity reagents such as antibodies, aptamers, antibacterial peptides, and bacteriophages.

In last decades, electrochemical and optical sensors and biosensors have attracted a lot of attention considering their long-term stability, cost-effectiveness and ease of use [10-13]. These types of sensors show remarkable advantages of simple operation, fast response time, low cost, miniaturization, and sensitivity and thus both electrochemical and optical methods are effective techniques in bio-/sensor [14-19]. The application of both optical and electrochemical sensors has been widely studied in trace analyte detections from ions to large biomolecules and from viruses to whole cells like bacteria [20-27].

Potentiometry has been proved to be one of the most sensitive electrochemical techniques. Fast response, small size, low cost, comfortable using and resistant to interferences of color and turbid are advantages of potentiometric sensors [28-33]. In potentiometry, the potential of a solution is measured and has a confined influence on the solution. This potential is measured by using electrode systems and detecting ions in solution when there is also other species present. Generally, two electrodes include the indicator/working electrode and reference electrode are placed in an analyte solution and measurements are performed without or with very little current. As the compound of the solution being analyzed is not changed, the target analyte can be quantified [34-40]. Potentiometric detection of foodborne bacterial pathogens is illustrated in Figure 1. Herein, we provide an overview of potentiometric detection of foodborne bacterial pathogens.

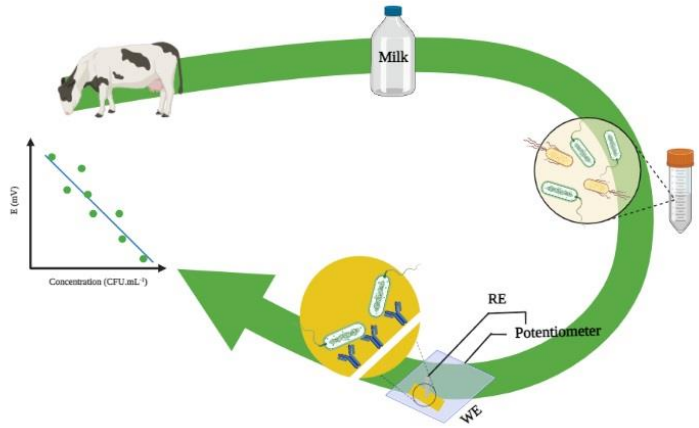

Figure 1. Schematic illustration of potentiometric detection of foodborne bacterial

2. POTENTIOMETRIC DETECTION OF *E.COLI*

In developed countries, one of the main causes of foodborne diseases is *Escherichia coli* that accounts a serious hazard for human health [41]. Shaibani et al. investigated a potentiometric sensor enabled to detect *E. coli* in real samples. They designed a novel potentiometric sensor based on nanofiber-light addressable (NF-LAPS). In this sensor, poly (acrylic acid) / poly (vinyl alcohol) (PAA/PVA) hydrogel nanofibers are utilized for the sensitive layer. The limit of detection (LOD) measured by proposed sensor was 10^2 CFU mL^{-1} and the range of bacteria determination was 10^2 CFU mL^{-1} to 10^6 CFU mL^{-1} [42]. In 2018, Hua and co-workers proposed a sensitive potentiometric aptamer-based sensor for *E. coli* detection, as can be seen in Figure 2.

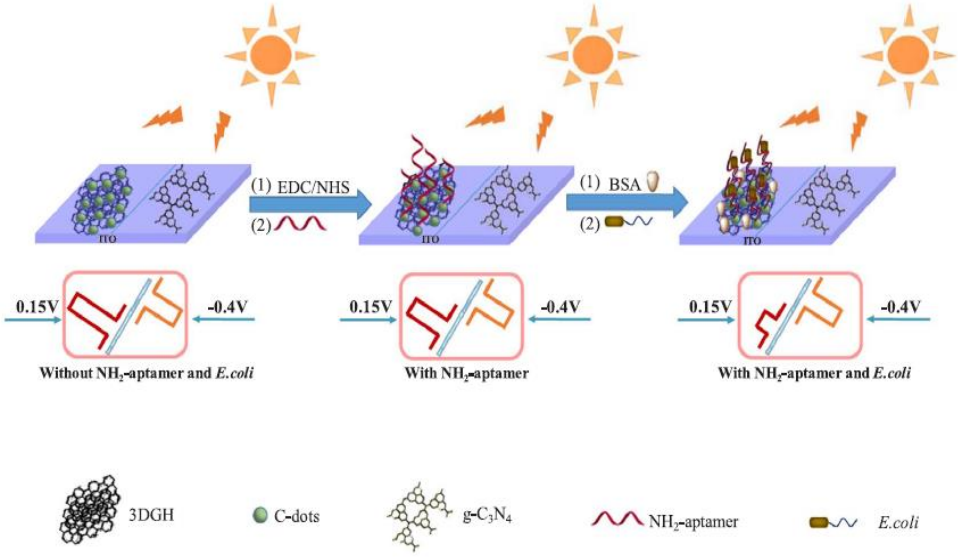

Figure 2. Schematic illustration for *E. coli* detection using a potentiometric aptasensor; reprint with permission from [43], copyright 2018, Elsevier

The fabricated biosensor was prepared by modifying ITO electrode with three-dimensional graphene hydrogel-loaded carbon quantum dots (C-dots/3DGH) and graphene-like carbon nitride (g-C_3N_4). Then the surface of C-dots/3DGH was modified by aptamer. The steric barrier was increased when bacterial cells were presented in medium. By result, the cathodic current decreased remarkably. This potentiometric biosensor was determined various concentrations of *E. Coli* in the range of 2.9 CFU mL^{-1} to 2.9×10^6 CFU mL^{-1} [43].

Lei et al. developed a potentiometric device for sensing of *E. coli O157*. This biosensor employed protamine ions that the selective electrode indicates the sigmoidal relationship between the concentration of protamine and the potential measured. In the absence of the bacteria protamine and aptamer interacted together with ion pairing and the consumption of protamine can dramatically decrease the EMF value. When bacteria presented in medium and bound with specific aptamer, protamine-aptamer complex is disrupted. Thus, a potential change can be used for detection with potentiometric approach. The biosensor can detect bacterial cells in the linear range of 10 CFU mL^{-1} to 10^4 CFU mL^{-1} and the LOD was reported 10 CFU mL^{-1} [44].

3. POTENTIOMETRIC DETECTION OF *S. AUREUS*

S. aureus is one of the main pathogenic bacteria that have a powerful ability to food contaminating and causes various diseases and infections in human [45,46]. These bacteria are dangerous to human health due to their endotoxins and other characteristics causing infections and complications [47].

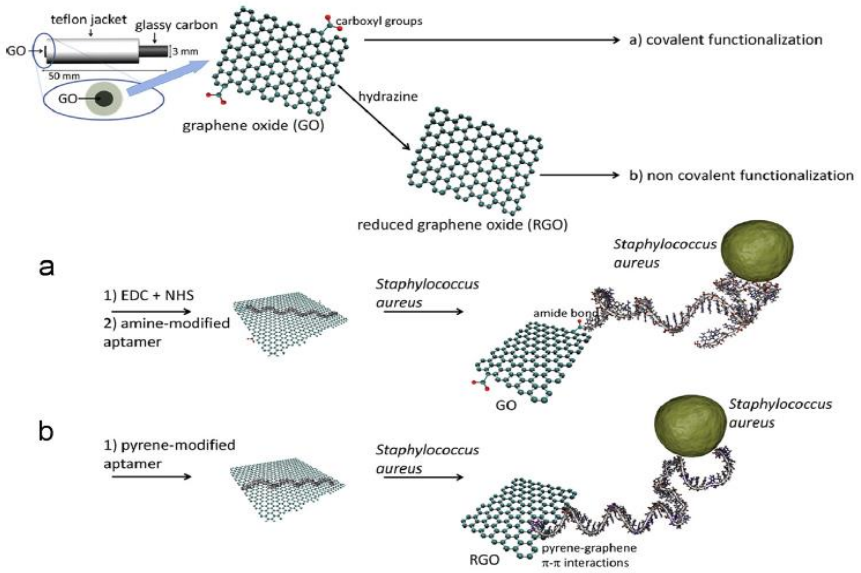

Figure 3. Schematic of the functionalization and *S. aureus* detection. (a) The covalent functionalization following carbodiimide-mediated chemistry and (b) the non-covalent functionalization π–π stacking between pyrene moieties and RGO; reprint with permission from [48], copyright 2014, Elsevier

Hernández and colleagues proposed a selective and sensitive potentiometric aptamer-based sensor for real time detection of *Staphylococcus aureus.* In this study, Graphene oxide (GO) and reduce graphene oxide (RGO) was transducer layer separately, which DNA aptamers bond covalently (in GO condition) and non-covalently (in RGO condition). In both ways, they were able to determine 1 CFU mL^{-1} *S.aureus*, that shown in Figure 3 [48].

Zelada-Guillén et al. were detected *Staphylococcus aureus* in skin using label-free strategy based on carbon nanotubes and aptamers. They were studied non-covalent adsorption of pyrenil modified aptamer and covalent bond amine-aptamer approaches, as shown in Figure 4. Finally, they were found the minimum concentration detected with covalent functionalization was 8×10^2 CFU mL^{-1}, that lower than with non-covalent method [49].

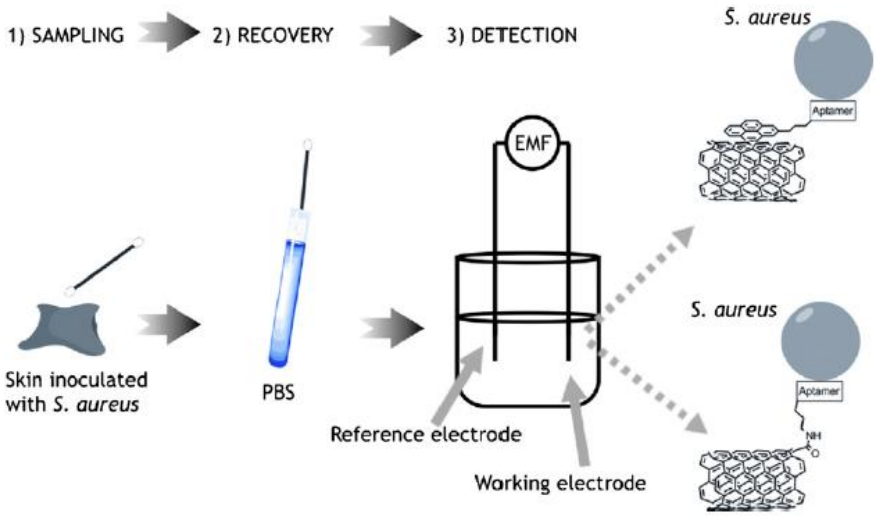

Figure 4. Schematic diagram for preparation steps needed to analyze human skin using the potentiometric biosensor; reprint with permission from [49], copyright 2012, Elsevier

4. POTENTIOMETRIC DETECTION OF *SALMONELLA TYPHIMURIUM*

Among other bacteria pathogens, *Salmonellal typhimurium* is one of the leading reasons of foodborne diseases, resulting in many hospitalizations and deaths each year [50,51]. In 2019, a paper-based potentiometric immunosensor developed by Silva and colleagues for *Salmonellal typhimurium* real-time detection. For developed paper-strip electrode, two interfaces' methods were assembled; The first method was simpler than second method, which relied on direct immobilization of the antibody to the polymer membrane and the secondary method was based on an intermediate layer of a polyamidoamine dendrimer, with an ethylenediamine core from the fourth generation. The detection limit was reported 5 CFU mL^{-1} in optimized conditions [52]. The sensing platform based on gold nanoparticle polymer inclusion membrane (AuNPs-PIM) was allowed Silva et al. to use a potentiometric approach for *S. typhimurium* detection. The blocking effect of the ionic flux on potentiometric

measurements is caused by the conjugation of antigens with antibodies (Figure 5). The proposed biosensor was showed high sensitivity and achieved to 6 CFU mL^{-1} as limit of detection [53].

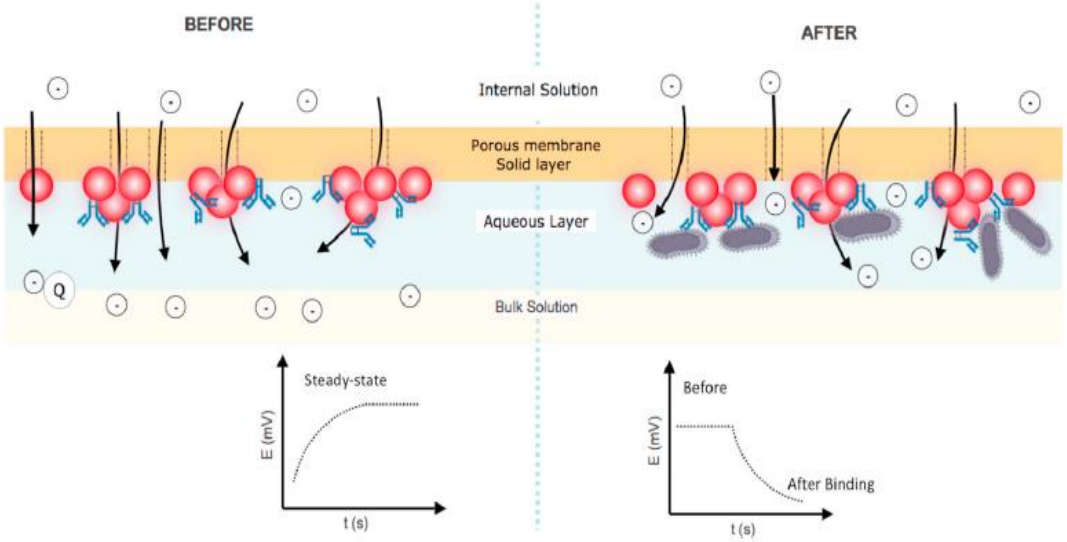

Figure 5. Schematic display of surface blocking influence detection mechanism in the fabricated immunosensing interface; reprint with permission from [53], copyright 2019, Elsevier

5. POTENTIOMETRIC DETECTION OF *LISTERIA MONOCYTOGENES*

The *Listeria monocytogenes (LM)* bacterium is a Gram-positive bacterium which is the cause of listeriosis and one of the most infectious foodborne pathogens found in sediments, seafoods, and groundwater [54-59]. Ding and colleagues proposed potentiometric aptamer-based biosensor for *L. monocytogenes* detection using protamine as an indicator. In presence of target, aptamers bind to internalin A protein in the surface *L. monocytogenes* cells. This event prevents the aptamer from electrostatically interacting with protamine, then determined using a sensitive membrane electrode. The fabricated aptasensor could detected bacteria sensitively because LOD was attained 10 CFU mL^{-1} [60]. In 2018, Enguang Lv et al. demonstrated potentiometric biosensor for *L.monocytogenes* detection by a pair-based sandwich assay with short antimicrobial peptide. They used magnetic beads to form the sandwich structure and horseradish peroxidase as a label. The oxidation of 3,3′,5,5′-tetramethylbenzidine with H_2O_2 is catalyzed by this enzyme to oblige a potential shift on a polymeric membrane ion-selective electrode. After optimal conditions have been reached, bacteria can be determined in a range from 10^2 to 10^6 CFU mL^{-1} with 10 CFU mL^{-1} as limit of detection [61]. Several potentiometric biosensors have been proposed for detection of foodborne bacteria. Table 1 lists different potentiometric biosensors for foodborne bacteria.

Table 1. Various potentiometric biosensors for foodborne bacteria

Bacteria	**Range of detection (CFU mL^{-1})**	**Detection Limit (CFU mL^{-1})**	**Time (min)**	**Reference**
E. Coli	4-10^4	4	1	[62]
E. Coli	10^6-10^8	9×10^5	10	[63]
S. aureus	0.2-10^6	0.2	1	[64]
S. aureus	800-10^8	800	30	[49]
S. typhimurium	10-10^8	20	75	[65]
S. typhimurium	12–12×10^3	5	< 60	[52]
L. monocytogenes	10-500	10	40	[60]
L. monocytogenes	10^2-10^6	10	60	[61]

6. CONCLUSION

As one of the most effective methods in electrochemical biosensors, potentiometric biosensors can be applied to detect different targets like foodborne bacteria. Nowadays, simple pathways and real time methods are attracting attentions in the determination fields. Also, several approaches have been reported to designed potentiometric biosensors. In this review, we illustrated how potentiometric biosensors enable to detect foodborne bacteria with high sensitivity and less time consuming.

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