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Development of an aptasensor using reduced graphene oxide chitosan complex to detect *Salmonella*

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ABSTRACT

The development of accurate and rapid biosensors to detect pathogenic *Salmonella enterica* is an active area of interest with significant impact towards public health. An electrochemical aptasensor was developed using electrochemically-reduced graphene oxide-chitosan (rGO-CHI) composite as a conductive substrate to detect whole-cell *Salmonella enterica* serovar *Typhimurium*, a common serovar that causes foodborne infections in humans. A thiol-functionalized aptamer specific to *Salmonella* outer membrane protein was selected as the biorecognition element and was immobilized on rGO-CHI using glutaraldehyde as the crosslinker. The sensitivity and selectivity of this aptasensor against *S. Typhimurium* was investigated using cyclic voltammetry and differential pulse voltammetry techniques. The rGO-CHI composite formed a conductive coating (4.5 A m^{-2}) which was stable to accommodate the buildup of activating agents without degrading. The developed aptasensor is specific to *Salmonella* and could distinguish between *Salmonella enterica* cells and non-*Salmonella* bacteria (*S. aureus, K. pneumonia* and *E. coli*). The aptasensor exhibited a low limit of detection of 10^1 CFU mL^{-1} for *S. Typhimurium*. The system was tested with artificially spiked raw chicken samples and the results were consistent with the sensitivity results obtained using with pure cultures. This shows the potential of the developed aptasensor in direct *Salmonella* detection in contaminated food.

1. Introduction

Salmonellosis is a foodborne disease caused by *Salmonella enterica*, an ubiquitous pathogen that is commonly found in poultry, eggs and vegetables [1]. *Salmonella* has over 2500 serovars, among these, *Salmonella Enteritidis* and *Salmonella Typhimurium* are the most common non-typhoidal serovars associated with human illnesses [2]. These *Salmonella* serovars are responsible for gastrointestinal diseases and can cause severe illness in immunocompromised people such as the elderly, children or generally people with low immune function [3]. In the United States, the annual cases of salmonellosis were about 140 million people, including 55 million children under the age of 5 years [4]. In Malaysia, an increase in food- and water borne *Salmonella* cases reported in the past decade (48.51 cases per 100,000 people) [5,6]. These reports showed that strains of *S. Typhimurium* are ubiquitous and multidrug resistant [5]. Hence, there is a need for the development of a point-of-care biosensor device for rapid and accurate detection of *Salmonella*. Many molecular methods have been developed for *Salmonella* detection, for instance PCR, multiplex PCR, real-time PCR, and NASBA [7,8]. These methods are commonly used for detection but pose several setbacks which could be improved to make detection easier. Generally, these methods require skills to handle nucleic acid fragments, purification steps, and have a complex working protocol [9,10]. Thus, biosensors could offer an alternative, a simpler and more affordable method for pathogen detection [11]. Electrochemical biosensors detect an ana-

Abbreviation: rGO, reduced graphene oxide; CHI, chitosan; GLU, glutaraldehyde; MB, methylene blue; *S. Typhimurium*, *Salmonella Typhimurium*; ssDNA, single stranded deoxyribonucleic acid; CV, cyclic voltammetry; DPV, differential pulse voltammetry; CFU mL^{-1}, colony forming unit per mL.

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