

## Rapid Evaluation of *Salmonella pullorum* Contamination in Chicken Based on a Portable Amperometric Sensor

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### Abstract

In this study, anti-Salmonella polyclonal antibodies immobilized on cellulose nitrate membrane were used to capture *Salmonella pullorum* (*S. pullorum*) in biological samples. The rapid evaluation of *S. pullorum* contamination was based on the analysis of the activities of catalase, a biomarker of this bacterium. After a screen printed electrode (SPE) modified with multi-wall carbon nanotubes (MWCN)-chitosan-peroxidase was connected to a portable self-made amperometric sensor, the determination of *S. pullorum* contamination was carried out by adding the reaction product, which was obtained from the hydrogen peroxide dismutation catalyzed by the bacterial catalase, to the reacting area of the SPEs. A working potential of 0.55 V was applied in the sensing system and the current value displayed on the amperometric sensor was used as the detection signal. This method allowed the quantification of *S. pullorum* with the detection limit of 100cfu mL<sup>-1</sup> in culture media and chicken samples. The stability, reproducibility and sensitivity of the modified SPE were also investigated. Moreover, successive analysis was conveniently accomplished by replacing the one-off SPE. This portable sensing system is a rapid, cost-effective and straight-forward approach for screening *S. pullorum* contamination in food samples.

**Keywords:** *Salmonella pullorum*; Portable amperometric sensor; Catalase; Screen printed electrodes

### Introduction

*Salmonella*, one of the most frequently reported causes of food-borne illness in the world, is widely distributed in animals and environment [1-3]. Under the condition that the appliance or the environment is contaminated by the salmonella from the feces of livestock, the bacteria can be easily spread on the chain of slaughtering, transportation, processing, storage and selling. Under proper conditions, the salmonella has the ability of proliferating very quickly and food-borne toxicosis would be caused after the amount of the bacteria accumulates to certain level, imperiling the health of victims, even leading to human deaths [4-6]. Many studies have been reported on the development of new methodologies for the detection and identification of *Salmonella typhimurium*, one of the vital food-borne pathogens most likely to be found in slaughtered livestock and poultry [7-15]. However, the impairments of human health and economy caused by other serotypes of *Salmonella* cannot be neglected. *S. pullorum* and *S. gallinarum* are the major bacterial pathogens causing significant economic losses to the poultry industry in many parts of the world [16-18]. In addition, the presence of *S. pullorum* in food sample is still a public concern in China when the food quality and safety are considered. Therefore, the quantification of both pathogenic and spoilage microorganism in food samples is an essential and necessary practice. Conventional methods for the analysis of bacterial contamination were based on the isolation of the target microorganism on specific culture media and the count of viable bacterial cells. However, these methods require 3-4 days for presumptive results and 5-7days for confirmation [19]. Up to now, several rapid approaches and alternative methods have been developed for the detection of *Salmonella*, including polymerase chain reaction [20-24], immunoassay [25-28], bioluminescence [29,30] and impedimetry [31-33]. The drawbacks of these applications are the complexity and limited applicability for on-site detection. In recent years, biosensors for detection of food-borne *Salmonella* have been developed. They include piezoelectric biosensor [34,35], compact fiber-optic evanescent wave sensing system [36], immuno-optical sensor [37,38] and immune electrochemical sensor [39-42]. However, one or

some of the disadvantages were indicated in these methods including high detection limit, low regeneration of the antibody-coated surface for multiple uses and high cost of the instrument and materials.

In this study, we designed and fabricated a novel biosensor for fast detection of *S. pullorum* contamination in food samples by using MWCN-chitosan-peroxidase modified SPEs and anti-Salmonella polyclonal antibodies immobilized on cellulose nitrate membrane. We hypothesized that these techniques are very helpful to improve the sensitivity and selectivity of this sensing system. It is hoped that, in practical on-site use, the determination of *S. pullorum* can be easily carried out after a modified SPE is connected to a portable self-made amperometric sensor and successive analysis can be conveniently accomplished by replacing the one-off SPE.

### Materials and Methods

#### Materials and apparatus

Wild-type *S. pullorum* was isolated and generously provided by Zuli Wu, professor of Shanghai Zoonosis Key Laboratory (Shanghai, China). Horseradish peroxidase (HRP) (EC 1.11.1.7, type I, activity 148U mg<sup>-1</sup> of solid), bovine serum albumin (BSA), N, N, N', N'-tetramethylbenzidine dihydrochloride (TMB) and ferrocene were purchased from Sigma (St. Louis, MO, USA). 25% Nafion was obtained from Nanjing Huiyu Energy Resource Co. Limited (Nanjing, China). Goat anti-*Salmonella pullorum* polyclonal antibody was provided by

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Ningbo Tianrun Biopharmaceutical Co. Limited (Ningbo, China). 30% H<sub>2</sub>O<sub>2</sub>, acetic acid, water-soluble Chitosan and 25% (wt.) glutaraldehyde were obtained from Shanghai Reagent Corporation (China). H<sub>2</sub>O<sub>2</sub> stock solution was standardized using the conventional titration method and serially diluted H<sub>2</sub>O<sub>2</sub> working solutions were prepared in sterile distilled water. MWCN (99.9%, -COOH, 20-30 nm) was purchased from Chengdu Organic Chemicals Co. Limited of Chinese Academy of Science (Chengdu, China). Other reagents, including nitric acid, acetate acid and phosphate, were analytical grade and purchased from Lingfeng Reagent Company (Shanghai, China). Skimmed milk powder was supplied by Shanghai Bright Dairy Corporation (Shanghai, China). Cellulose nitrate membrane (0.22 μm) was purchased from Millipore (USA). FD-1 vacuum freezing dryer was provided by Tianfeng Instrument Company (Shanghai, China). DU<sup>®</sup> 800 UV/visible spectrophotometer was obtained from Bechman Coulter, Inc. (USA). CHI 660C Electrochemistry Working Station was provided by Shanghai CHI Instrument Co. Limited (Shanghai, China).

### Modification of SPE by MWCN-chitosan-peroxidase

The SPEs used in this study were kindly provided by Shanghai Bioscan Inc (Shanghai, China). The shape of individual SPE was rectangular with the size of 7 mm×33 mm. Two parallel conductive rails were connected with two terminal posts and two electrodes areas used as working electrode (size, 2 mm×3 mm) and counter electrode (size, 2 mm×3 mm), respectively. The first layer of electric-conductive silver paste was printed on a polyvinyl chloride board based on the designed structure shown in Figure 1. The second layer was electric-conductive carbon paste which was printed exactly on the same area covered by the silver paste. An insulation layer was coated on the proper area the SPE, exposing the working area and terminal post for insertion into the slot of a portable amperometric sensor.

The new SPEs were pre-treated in 20% H<sub>2</sub>SO<sub>4</sub> solution containing 5% H<sub>2</sub>O<sub>2</sub>, followed by washing ultrasonically in ethanol and distilled water, respectively. 0.5 mg of the untreated MWCNs was added to 5 mL of nitric acid (wt. 65%) and the mixture was submitted to sonication for 2 h to obtain a relative stable suspension. 4 μL of the black suspension of MWCNs was casted onto the working area of the SPEs respectively and dried in an oven at 45°C to remove the acid, followed by washing in distilled water and drying at room temperature for the next modification. 100 mg of water-soluble chitosan was dissolved in 10 mL acetic acid (wt. 1%) and 4 μL of the mixture was added respectively to the working

area of the SPEs modified with MWCNs described above. Then the SPEs modified with MWCN-chitosan was washed in distilled water and dried at room temperature again. The last step of modification was performed by adding 4 μL of peroxidase-BSA solution to the working area of SPEs modified with MWCN-chitosan. This enzymatic solution contained 1 mg mL<sup>-1</sup> of peroxidase, 0.25% (wt.) of BSA and 0.025% (wt.) of glutaraldehyde. The MWCN-chitosan-peroxidase modified SPEs were allowed to stand at 4°C overnight and washed by 0.01 M PBS (pH 7.2). After the modified SPEs were dried by lyophilization, they were sealed in a plastic bag and stored at 4°C for future use.

### Design and assembling of portable amperometric sensor

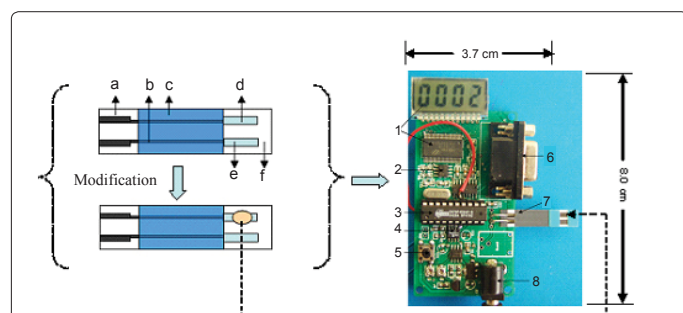
The design and assembling of the portable amperometric sensor was accomplished with the help of the postgraduates in Dr. Jingqi Yuan's laboratory (School of Electronic, Information and Electrical Engineering, Shanghai Jiaotong University, Shanghai, China). The hardware structure of the sensor mainly consisted of the following parts: (1) liquid crystal display and HT1621 driving chip (Holtek Electronic Company, Taiwan), (2) MCP3201 A/D converter (Microchip Company, USA) (3) EM78P458 single-chip microcomputer (Elong Electronic Company, Taiwan), (4) operational amplifier, (5) function key, (6) data-interface, (7) SPE slot, (8) battery (output, 6 V) and other peripheral equipments. The software of this sensor was developed based on the WicePlus translator editor (Elong Electronic Company, Taiwan).

### Bacteria culture and plating method

The Regulations of Operation (2003) established in our laboratory were strictly followed for safety considerations. Wild-type *S. pullorum* was grown overnight at 37°C on a nutrient agar containing Salmonella-Shigella (S.S.) culture media to obtain a pure culture of the microorganism. Round translucent small colonies were observed on the agar. Gram staining showed that this bacterium was Gram-negative short bacillus without capsule and spore. Biochemical identification confirmed that *S. pullorum* was catalase-positive microorganism. Two or three representative colonies were transferred aseptically to 10 mL of the nutrient broth. The *S. pullorum* cultures were incubated at 37°C with aeration by shaking. After the growing stationary phase was reached, the enumeration of the colonies was carried out by inoculating the serially diluted (10-fold steps) bacterial cultures to the Luria broth agar plates and incubating overnight at 37°C. To establish a calibration graph for relating the optical density value with viable count in serial dilutions of the bacterial cultures, serial dilutions (5-10-fold depending on the requirements of the experimental protocol) were prepared in the nutrient broth and the optical density of each suspension was measured on a DU<sup>®</sup> 800 UV/visible spectrophotometer at a wavelength of 450 nm.

### Immobilization of anti-Salmonella polyclonal antibodies on cellulose nitrate membrane

Round chips of cellulose nitrate membrane were made according to the size of the wells (diameter, 15 mm) in the plastic tissue culture plate. The chips were put respectively on the bottom of 24 wells of the plate. Goat anti-Salmonella polyclonal antibodies were diluted by 100 folds using 0.01 M PBS (pH7.2). 300 μL of the antibody solution was added to the wells respectively and incubated at 37°C for 1 h, followed by washing with PBS two times. The unspecific sites on the cellulose nitrate membrane were blocked by the treatment with PBS solution containing 3% skimmed milk powder at 37°C for 20 min, followed by washing the plate in PBS three times. After the drying procedure by lyophilization, the plate was sealed in polyethylene bag and stored at 4°C for further use.



**Figure 1:** Schematic diagram of the SPE and components of the hardware of the amperometric sensor. The schematic structure of SPE consisted of following parts: (a) terminal post, (b) electric conductive rail, (c) insulation layer, (d) working electrode, (e) counter electrode and (f) polyvinyl chloride board. The hardware components of the amperometric sensor: (1) liquid crystal display and HT1621 driving chip, (2) MCP3201 A/D converter, (3) EM78P458 single-chip microcomputer, (4) operational amplifier, (5) function key, (6) data-interface, (7) SPE slot and (8) battery (output, 6 V).

## Protocols for the detection of *S. pullorum* on the portable amperometric sensor

Prior to the determination of *S. pullorum* on the portable amperometric sensor, the culture plate from the previous step 2.5. was retrieved from the polyethylene bag and 0.5 mL of bacterial samples was transferred into each of the 24 plate wells respectively, followed by incubating the plate on a shaking machine at room temperature for 20 min. After the plate was washed in PBS (0.01 M, pH 7.2) containing 0.01% Tween-20 for three times, 0.5 mL of 0.1 M acetate buffer (pH 6.0) containing 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  and 20 mM TMB was added into one of the plate wells. The mixture was allowed to react for a fixed time (8 min). After a SPE modified with MWCN-chitosan-peroxidase was connected to a portable self-made amperometric sensor, 15  $\mu\text{L}$  of the mixture from the previous step was added onto the reacting area of the modified SPE. A working potential of 0.55 V was applied in the sensing system and the currents were recorded at appropriate time (20 seconds) as the sensor signals. The successive detections of *S. pullorum* in different samples were easily performed by replacing the one-off modified SPE with a new one.

## Determination of *S. pullorum* in chicken samples

Thirty chicken samples were collected randomly from three outdoor food markets in Shanghai. All the samples were immediately subjected to the detection of *S. pullorum* on the portable amperometric sensor under the optimized methods described in Section 3. Briefly, one gram of chicken from each sample was minced in a sterile glass homogenizer. 5 mL of PBS was used to remove the homogenate from the homogenizer. Then, 0.5 mL of the homogenate was aseptically transferred into one of the 24 plate wells in which the bacterial capture and other detection procedures were followed based on the protocols described above in section 2.6.

Eight *Salmonella*-free chicken samples (confirmed by conventional plating methods) were kindly provided by Shanghai Institute of Quality Inspection and Technical Research (Shanghai, China) and five of them were used as matrix control. The other three chicken samples were also homogenized and washed using 5 mL of PBS to obtain a turbid suspension. Then 2 mL of the suspension from each sample was added respectively to three culture flasks containing 30 mL of nutrient broth. An aliquot of 1 mL of *S. pullorum* with concentration of approximate  $1 \times 10^2$  cfu  $\text{mL}^{-1}$  was inoculated in the nutrient broth and the flasks were incubated at 37°C with aeration by shaking. Media samples were collected at 1.0, 1.5, 2.0 and 2.5 h, respectively for the detection of the model bacteria. Conventional plating methods were also carried out for the confirmation determination of *S. pullorum* in the chicken from the markets and the artificially inoculated samples.

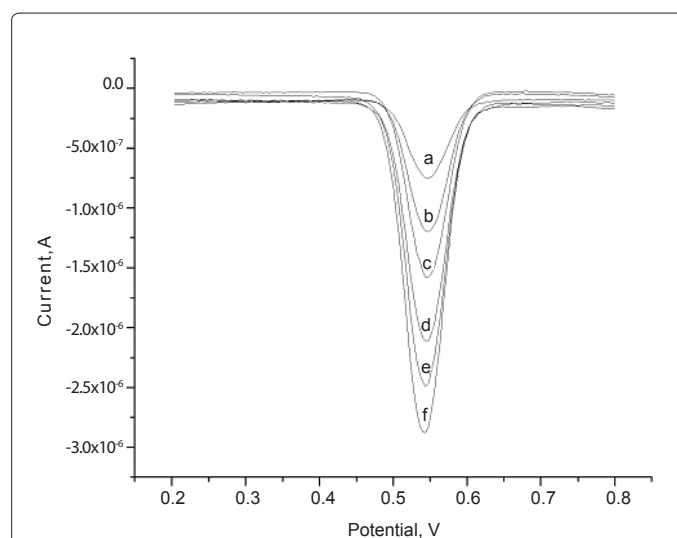
## Results and Discussion

### Electrochemical characteristics of the MWCN-chitosan-peroxidase modified SPE

A CHI 660C electrochemical working station was applied to investigate the voltammetric behavior of  $\text{H}_2\text{O}_2$  on the MWCN-chitosan-peroxidase modified SPEs. Since the detection of *S. pullorum* was based on the calculation of  $\text{H}_2\text{O}_2$  consumption in this study, it was of great value to set up reliable and sensitive methods to determine  $\text{H}_2\text{O}_2$ . We have tried other different methods of the modification of SPEs for the determination of  $\text{H}_2\text{O}_2$  using ferrocene-Nafion-peroxidase, MWCN-ferrocene-peroxidase and MWCN-Nafion-peroxidase. As expected, the best results were obtained from the MWCN-chitosan-peroxidase

modified SPEs, showing ideal stability and sensitivity. In addition, the use of the electron-transfer mediator TMB improved the sensitivity of the voltammetric determination of  $\text{H}_2\text{O}_2$  on the MWCN-chitosan-peroxidase modified SPEs (data not shown). To point out, for the determination of  $\text{H}_2\text{O}_2$  with different concentrations, the modified SPE were replaced every time by a new one. Figure 2 showed the differential pulse voltammetric graph of  $\text{H}_2\text{O}_2$  on the MWCN-chitosan-peroxidase modified SPEs using TMB as an electron-transfer mediator. The peak current at the potential of 0.55 V and was used as the voltammetric signal for the determination of  $\text{H}_2\text{O}_2$ . A linear response of the peak current to the concentration of  $\text{H}_2\text{O}_2$  was presented with the regression equation of  $y = (6.763 \pm 0.896) + (8.894 \pm 0.973)x$  (correlation coefficient equals to 0.998, for  $n=6$ ).

Because the detection of *S. pullorum* was performed on the one-off modified SPE by analyzing the consumption of  $\text{H}_2\text{O}_2$ , the stability and reproducibility were key factors affecting the reliability of the amperometric sensor. Fifty repetitive measurements of 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  were carried out on same day by replacing different one-off SPEs to evaluate the repeatability precision. For the intermediate precision, fifty replicate experiments were performed on each of ten days (five tests per day). The standard deviations for same day and intermediate days were calculated as 4.72% and 5.13%, respectively. Due to the use of the one-off SPEs for the determination, the differences of background signals among different SPEs must be considered. When the background noise was subtracted from the peak current for each determination, the standard deviations for same day and intermediate days were reduced to 3.24% and 4.06%, respectively. Thus the analysis  $\text{H}_2\text{O}_2$  consumption was based on the calculation of the increased current (current signal subtracted by baseline noise) at the potential of 0.55 V. To investigate the stability of the SPEs, fifty of them were stored at -4°C and the determination were conducted for the solutions containing 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  on the 60 and 120 day, respectively. The results showed that the relative standard deviation (RSD) was 4.62% and 4.93%, respectively. After six months, the RSD was still less 5.3%, indicating that the one-off SPEs possessed satisfactory stability.

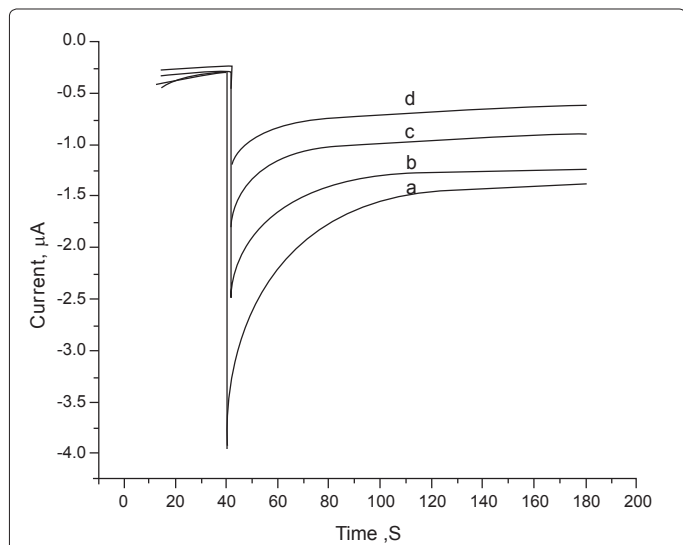


**Figure 2:** Differential pulse voltammetric graph of  $\text{H}_2\text{O}_2$  on the MWCN-chitosan-peroxidase modified SPEs using 20 mM TMB as an electron-transfer mediator. The curves from (a) to (f) were obtained respectively from 10, 50, 100, 150, 200, 250  $\mu\text{M}$   $\text{H}_2\text{O}_2$  in the acetate buffer (0.1 M, pH 5.0). The parameters set up for differential pulse voltammetry were set as follows: scanning range, 0.2–0.8 V; pulse amplitude, 50 mV; pulse duration, 20 s; pulse period, 200 s; scanning speed, 50  $\text{mV s}^{-1}$ .

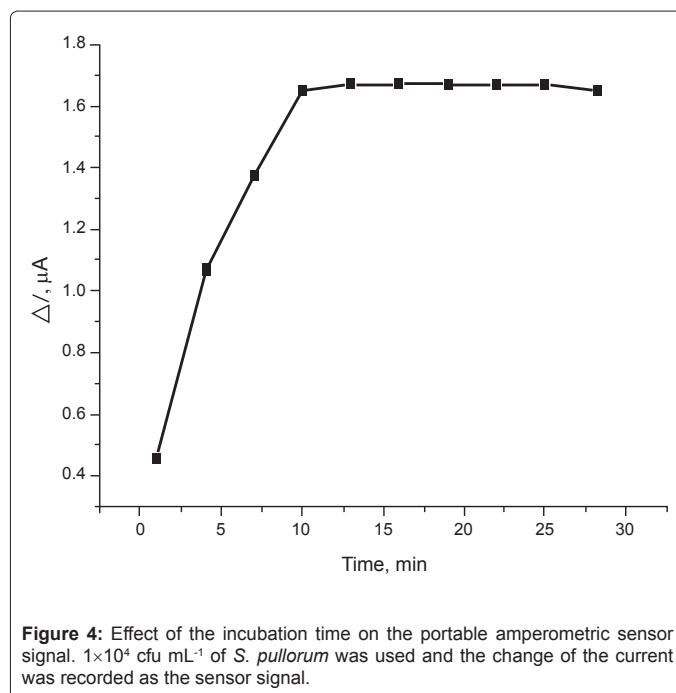
### Optimization of the experimental condition

**Effect of horseradish peroxidase and  $H_2O_2$  concentration on sensor response:** To investigate the effect of horseradish peroxidase amount on the portable sensor response, the enzymatic solutions containing 0.1, 0.3, 1.0 and 3.0  $mg\ mL^{-1}$  of horseradish peroxidase were applied during the modification of the SPEs with MWCN-chitosan-peroxidase. Amperometric determination was performed on the portable sensor using 100  $\mu M\ H_2O_2$  as the substrate. To dynamically obtain the electrochemical signals of  $H_2O_2$  on the SPEs modified with different amount of horseradish peroxidase, the optical sensor was connected to a computer through part 6 shown in figure 1 and the signals were recorded using current/time (*i-t*) mode. The effect of HRP amount on the sensor response in time course for determination of  $H_2O_2$  was shown in Figure 3. The sensor signals from each solutions with different amounts of HRP were increased rapidly (within 5 seconds) after the addition of 15  $\mu L$  of 100  $\mu M\ H_2O_2$  solution on the reaction area of the modified SPE. These *i-t* curves for the detection of  $H_2O_2$  on the modified SPE indicated that the sensor response reached peak value and decreased rapidly to plateau phase within 20 s. Thus in this study, 20 s was selected as the detecting time for the determination of  $H_2O_2$  consumption on the portable amperometric sensor. It was clear that more sensitive response would be achieved when relatively higher amount of HRP was applied. However, the sensor signal from the SPEs modified with 3.0  $mL^{-1}$  of HRP decreased quickly after 5 s, showing the instability for the detection of  $H_2O_2$  within 20 s on the portable amperometric sensor. Thus, 1  $mL^{-1}$  of HRP solution was chosen during the modification of the SPEs with MWCN-chitosan-peroxidase.

The effect of  $H_2O_2$  concentration on the portable sensor response was also investigated and  $1 \times 10^4\ cfu\ mL^{-1}$  of *S. pullorum* was applied as the bacterial model. 50, 100, 200 and 400  $\mu M\ H_2O_2$  solutions were mixed respectively with the model bacteria and the detection signal were recorded on the sensor after the accomplishment of the catalase-catalyzed reaction step. The results showed that the  $H_2O_2$  solution with relatively lower concentration of 50  $\mu M$  displayed the instability for the detection of the bacteria in this sensing system. The deflection of  $28 \pm$



**Figure 3:** Effect of the HRP concentrations on the response of the portable amperometric sensor to  $H_2O_2$  in time course. From curve (a) to curve (d), the concentration of HRP for the modification of SPEs was 3.0, 1.0, 0.3 and 0.1  $mg\ mL^{-1}$ , respectively.



**Figure 4:** Effect of the incubation time on the portable amperometric sensor signal.  $1 \times 10^4\ cfu\ mL^{-1}$  of *S. pullorum* was used and the change of the current was recorded as the sensor signal.

4% was obtained from 100  $\mu M\ H_2O_2$  whereas the results from 50, 200 and 400  $\mu M\ H_2O_2$  showed the deflection of  $43 \pm 7\%$ ,  $36 \pm 6\%$  and  $45 \pm 7\%$ , respectively. We selected 100  $\mu M\ H_2O_2$  for all the determination in this study considering the data described above and the fact that the relatively lower concentration of  $H_2O_2$  was helpful to improve the sensitivity since the detection of *S. pullorum* (a catalase-positive microorganism) was based on the detection of  $H_2O_2$  consumption.

**Influence of the incubation time on the amperometric sensor signal:** Since the detection of  $H_2O_2$  consumption on the portable amperometric sensor took only 20 s, the testing time of this analytical technique was mainly spent at the incubation step where  $H_2O_2$  was subjected to dismutation reaction catalyzed by the catalase from *S. pullorum*. To further investigate the influence of the incubation time on the amperometric sensor signal, 15  $\mu L$  of the mixture of the dismutation reaction product was collected at an interval of 3 min.  $1 \times 10^4\ cfu\ mL^{-1}$  of *S. pullorum* was used as the bacterial model. The change of the current was recorded as the sensor signals. Figure 4 showed the sensor response to the incubation time, indicating that the response increased very quickly within 8 min, but after 8 min the change of the current reached plateau phase and even slightly decreased after 20 min. Thus 8 min was used as the optimized incubation time for the determination of *S. pullorum* in this analyzing system.

**Effect of pH:** It was obvious that two separate reactions catalyzed respectively by catalase and HRP were involved in this analytic technique using the portable amperometric sensor based on the MWCN-chitosan-peroxidase modified SPEs. In order to study the effect of pH on the activities of HRP, 15  $\mu L$  of 100  $\mu M\ H_2O_2$  solutions with different pH values in a range from 3.0 to 9.0 was added to the reaction area of the modified SPEs and the sensor responses were recorded using current/time mode. It was found that optimum sensor response was achieved at pH 5.0 though no significant response was observed in the 4.0-7.0 of pH value, suggesting that weak acid environment was more suitable for the HRP catalyzed reaction. Although the detection of the  $H_2O_2$  consumption was performed on the one-off modified SPEs, the pH value of the solution in the plate wells was the main concern

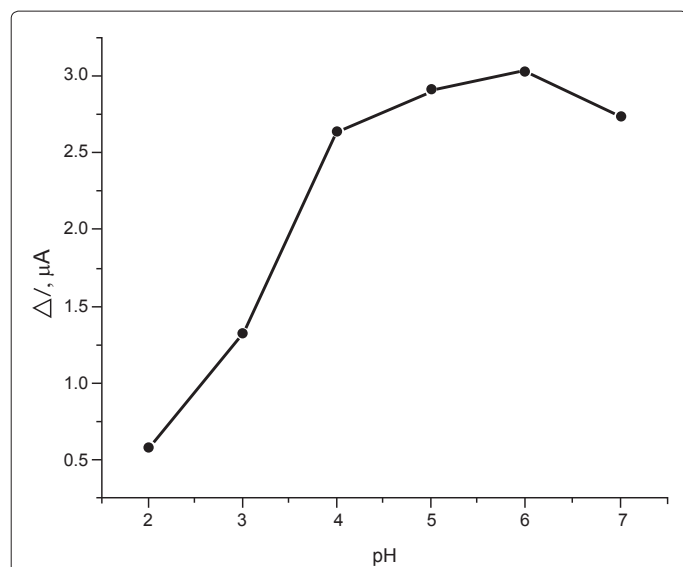


Figure 5: Effect of pH on the response of the portable amperometric sensor.

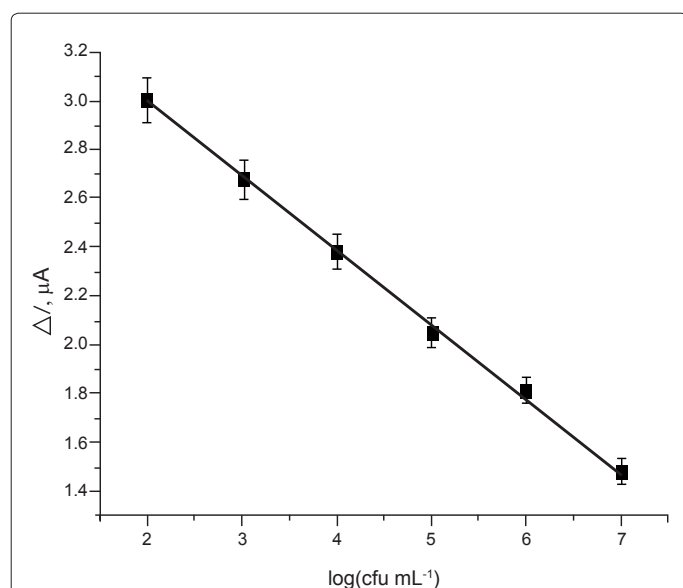


Figure 6: Calibration plot for the determination of *S. pullorum* on the amperometric sensor based on the MWCN-Nafion-peroxidase modified SPEs.

affecting the activities of both HRP and catalase since the supporting electrolytes added on the one-off modified SPEs were obtained from the mixture of the bacterial sample and acetate buffer. With the purpose of investigating the effect of pH on the sensor response, prior to the incubation step, the pH value of the mixture (equal volume of bacterial sample and 0.1 M acetate buffer containing 100  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$ ) was adjusted from 2.0 to 7.0. The sensor responses for the mixtures with different pH values were recorded by the methods described above. It was observed that no significant change in sensor response was obtained in the 4.0-8.0 range of pH values. However, a maximum response was achieved at pH 6.0 (Figure 5). Therefore, a pH value of 6.0 was selected for the determination of *S. pullorum* in this analytical technique.

### Calibration curves for the determination of *S. pullorum*

In order to establish the calibration curves for the determination of *S. pullorum*, serial dilutions of the bacteria were prepared and the current changes were recorded for each dilution sample in a triplicate way under the optimized conditions described above compared with a negative control solution (nutrient broth). It was found that sensor signal decreased linearly with the logarithm value of *S. pullorum* concentration in the range from  $1.75 \times 10^2$  cfu  $\text{mL}^{-1}$  to  $2.63 \times 10^7$  cfu  $\text{mL}^{-1}$  (Figure 6). The calibration curves could be represented by the linear regression equation  $y = (3.763 \pm 0.652) - (0.299 \pm 0.0537) \log(C, \text{cfu mL}^{-1})$  (correlation coefficient equals to 0.998, for  $n=6$ ). The detection limit was calculated as the bacterial concentration which produced an analytical signal (current change) three times the background noise. This technique allowed the quantification of *S. pullorum* in culture media with the detection limit of 100 cfu within 10 min.

Due to the bacterial proliferation in culture media and food samples, the evaluation of the microorganism contamination at different time is of great value. To monitor the bacterial growth in the culture media using the amperometric sensor and investigate the reliability of the portable amperometric sensor, the determinations of *S. pullorum* at different time (1, 2, 4, 6, 8, 10, 12 and 24 h) were carried out under the optimized conditions described above. The results showed that the current change decreased linearly with the logarithm value of the total count of the bacteria confirmed by the conventional plating methods. The linear regression equation was represented by  $y = (3.539 \pm 0.848) - (0.393 \pm 0.0726) \log(C, \text{cfu mL}^{-1})$  (correlation coefficient equals to 0.996, for  $n=8$ ).

### Determination of *S. pullorum* in chicken samples

The safety of the chicken sold in free market is still a public concern due to the lack of efficient monitoring and quality control system. Therefore, rapid technique for the evaluation of bacterial contamination in food samples is very necessary. To investigate the dependability of the portable amperometric sensor for monitoring *S. pullorum* contamination in food, thirty chicken samples collected from the free markets were used and subjected directly to the detection of *S. pullorum* on the portable amperometric sensor under the optimized methods described in Section 3. The results indicated that *S. pullorum* was detected in three out of the thirty chicken samples without bacterial enrichment. The total count of the *S. pullorum* in the three samples was  $1.7 \times 10^3$ ,  $5.3 \times 10^2$  and  $2.6 \times 10^4$  cfu  $\text{mL}^{-1}$ , respectively. To study the effect of the bacterial enrichment time on the detection of *S. pullorum* in chicken samples and compare the consistence of this technique with the conventional methods, three *S. pullorum*-free chicken samples were inoculated with the model bacteria and the detection was performed on the sensor at 1.0, 1.5, 2.0 and 2.5 h, respectively. Conventional plating methods were also conducted for the same samples. Table 1 demonstrated the results from the portable amperometric sensor and the conventional methods, indicating the satisfactory consistency between these two techniques. It was obvious that the bacterial sample with initial concentration of 60-100 cfu  $\text{mL}^{-1}$  would be detected after 1.5-2 h of enumeration. Therefore, with the combination of the sensing system and bacterial enrichment, the detection limit would be decreased to 60 cfu  $\text{mL}^{-1}$ , demonstrating the favorable sensitivity.

### Conclusion

In this experiment, a portable amperometric sensor was established for the rapid evaluation of *Salmonella pullorum* contamination in chicken samples. The specificity of this technique was based on the

Samples	Incubation time (h)	Number of <i>S. Pullorum</i> detected, log (C, cfu mL <sup>-1</sup> )	
		Amperometric sensor methods	Conventional methods
S1	1.0	- <sup>a</sup>	1.82 ± 0.37
	1.5	3.13 ± 0.56	3.42 ± 0.28
	2.0	3.55 ± 0.49	3.67 ± 0.31
	2.5	4.15 ± 0.52	4.46 ± 0.46
S2	1.0	1.90 ± 0.71	2.15 ± 0.43
	1.5	2.97 ± 0.63	3.13 ± 0.52
	2.0	3.28 ± 0.65	3.85 ± 0.62
	2.5	3.96 ± 0.46	4.23 ± 0.74
S3	1.0	2.12 ± 0.56	2.37 ± 0.49
	1.5	3.05 ± 0.37	2.96 ± 0.52
	2.0	3.78 ± 0.41	3.52 ± 0.57
	2.5	4.15 ± 0.53	3.97 ± 0.62

<sup>a</sup>Not detectable.

**Table 1:** Comparison of the amperometric sensor with the conventional method for the detection of *S. Pullorum*.

capture of the target bacteria on the cellulose nitrate membrane through specific polyclonal antibodies. Due to the use of the MWCN-chitosan-peroxidase modified SPEs, this sensing system showed favorable sensitivity, allowing the quantification of *S. pullorum* in culture media with the detection limit of 100 cfu mL<sup>-1</sup> within 10 min for each sample, without bacterial enumeration. The stability, reproducibility and sensitivity of the modified SPE were reliable for the determination of hydrogen peroxide consumption. In addition, the determination of *S. pullorum* was easily carried out by adding the reaction product, which was obtained from the hydrogen peroxide dismutation catalyzed by the bacterial catalase, to the reaction area of the modified SPEs. Successive analysis was conveniently conducted by replacing the modified SPE with a new one. By the combination of the portable sensing system and bacterial enumeration, the detection limit could be decreased to 60 cfu mL<sup>-1</sup> within two hours. Furthermore, no labeled secondary antibody was applied, shortcutting the analyzing procedures compared with conventional immunoassays. In summary, this portable sensing system is a rapid, cost-effective and straight-forward approach for monitoring *S. pullorum* contamination in food samples.

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