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Study on the Antioxidant Capacities of Four Antioxidants Based on Oxidizing Guanine in a Composite Membrane

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The antioxidant capacities of four antioxidants (ascorbic acid, caffeic acid, coumaric acid and resveratrol) were studied herein on a guanine biosensor. The guanine was immobilized on a glassy carbon electrode (GCE) by constant potential deposition, and then a composite membrane of poly(dimethyldiallylammonium chloride) (PDDA), Fe@Fe_2O_3 core–shell nanonecklace and glucose oxidase (GOX) was formed on its surface. In glucose solution, the glucose oxidase (GOX) catalyzed glucose to generate hydrogen peroxide (H_2O_2). Then H_2O_2 reacted with Fe@Fe_2O_3 core–shell nanonecklace and produced hydroxyl radical (·OH) in the membrane to oxidize guanine. As antioxidants were introduced in this system, these antioxidants clear away •OH and alleviate the guanine oxidation degree. By recording the square wave voltammetric signal change of the guanine, the antioxidant capacities of the four antioxidants were evaluated. The sensor exhibited good reproducibility and stability for rapid assessment of the antioxidant capacity.

Keywords: Guanine; glucose oxidase; antioxidants; biosensor; antioxidant capacity

1. INTRODUCTION

Reactive oxygen species (ROS), such as superoxide radical ($O_2{\cdot}^-$), hydroxyl free radicals (·OH), hydrogen peroxide (H_2O_2) and so on, can be generated in organisms via normal aerobic metabolism. It usually keeps in a normal concentration level in organisms and has important physiological effects on transcription factors and cell proliferation and differentiation [1]. However, when the organisms are suffering from harmful environmental stress, such as ultraviolet radiation, excessive drinking alcohol, heavy metals, pollutants, etc, or the antioxidant system is in disorder, the ROS levels will increase dramatically and lead to the cell apoptosis through the cell oxidative stress reaction. If the excessive ROS could not be cleared immediately, it will cause a variety of diseases, such as aging, cancer, heart disease and arteriosclerosis [2-4]. Normally, organisms defend themselves

against ROS damage by scavenging free radicals with enzymes such as alpha-1-microglobulin, superoxide dismutases, catalases, lactoperoxidases, glutathione peroxidases and peroxiredoxins, or with small molecule antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E), uric acid, and glutathione [5, 6].

In recent years, the electrochemical DNA biosensors based on DNA damage induced by ROS have become important tools in evaluation of DNA damage and antioxidant capacity of antioxidants because the ROS can induce several types of DNA damage and antioxidants can effectively scavenge ROS [7-10]. Among of the bases of DNA, the guanine is the most easily oxidized due to it's the lowest oxidation potential. In addition, the oxidation product of the guanine, 8-oxoguanine is considered an important biomarker of DNA oxidative damage [11, 12]. Therefore, guanine is often used as a DNA model molecule to study the DNA damage by ROS and repair mechanisms [13].

One of the key factors to develop an antioxidant biosensor is the generation of the ROS which is used to oxidize biomolecules because the ROS can't be purchased from market. At present, there are five means to obtain ROS in fabricating electrochemical antioxidant biosensors. They have been summarized by the literature of [10] as follows (1) Generation via Fenton reaction [14], i.e., hydroxyl radical ($OH\cdot$) and hydroxide anion (OH^-) are generated by the reaction between transition metals, such as Fe (Ⅱ), Cu (Ⅰ), Cr (Ⅱ), with H_2O_2; (2) Using photocatalytic reaction to generate ROS. The photocatalytic reaction of TiO_2 is the most usually used among of all photocatalytic reactions to generate ROS [15]; (3) Utilizing xanthine oxidase (XOD) to catalyze the oxidation of xanthine or hypoxanthine. In this reaction, O_2 was reduced to H_2O_2, and $O_2\bullet^-$ is formed as an intermediate of the reaction [16]; (4) Adding NaOH to dimethylsulphoxide (DMSO) to generate $O_2\bullet^-$. The production of $O_2\bullet^-$ is inversely proportional to the concentration of water in DMSO solution [17]; (5) Producing $O_2\bullet^-$ by injecting KO_2 into aprotic organic solvents, especially in DMSO [18,19].

Among of the antioxidant biosensor based on DNA damage, Fenton reaction system is one of the most common ways to produce ROS [20, 21]. Most of these sensors are conducted in solution and the unstable H_2O_2 must be used, which was inevitable to lead a bad reproducibility. To circumvent this problem, Liang et al. developed a DNA damage biosensor by embedding glucose oxidase into a composite film of sensors in which the ROS was generated in a composite membrane via the catalyzing reaction of glucose oxidase for glucose and following the Fenton reactions [22]. Wang et al. set up an electrochemical DNA damage biosensor by utilizing electro-generated H_2O_2 to react with nano $Fe@Fe_2O_3$ to generate $OH\cdot$ and damage DNA in situ in a composite membrane [23-25].

In this research, an antioxidant biosensor was developed on the basis of the works of Liang [22] and Wang [23-25]. The sensor showed good reproducibility and stability for rapid evaluation of antioxidant capabilities of ascorbic acid and phenolic compounds (caffeic acid, coumaric acid, resveratrol).

2. EXPERIMENTAL

2.1 Materials and apparatuses

Guanine (G) and poly(dimethyldiallylammonium chloride) (PDDA) were purchased from sigma. Glucose oxidase (GOX) and glucose were provided by Shanghai source biological technology

co., Ltd. (China). The GOX solution was prepared with Tris-maleate buffer solution of pH 7.0 (0.2 mol L^{-1} three hydroxymethyl aminomethane + 0.2 mol L^{-1} maleic acid + 0.2 mol L^{-1} NaOH). Fe@Fe_2O_3 core–shell nanonecklace was synthesized as our previous work [24], and scattered by 2.0 mg mL^{-1} PDDA into 2.0 mg mL^{-1} suspension. $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$, were purchased from Shanghai No. 1 Reagents Factory (China). Ascorbic acid (AA) was obtained from Xilong chemical co., Ltd. (China). Resveratrol was obtained from Sahn chemical technology co., Ltd. (China). Caffeic acid and coumaric acid were purchased from Shanghai source biological technology co., Ltd. (China); 0.1 mol L^{-1} phosphate buffer solution (PBS) was prepared with 0.1 mol L^{-1} NaH_2PO_4 and 0.1 mol L^{-1} Na_2HPO_4. All other chemicals were of analytical grade and ultrapure water was used in all the experiments.

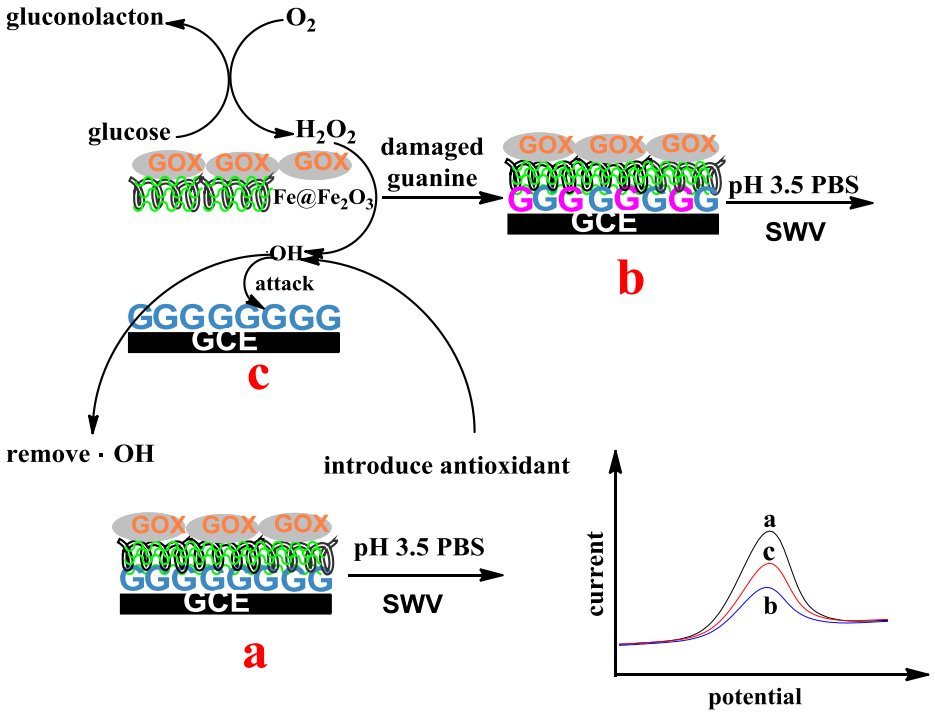
The electrochemical experiments were carried out on a CHI 660 C electrochemical workstation (Shanghai CH Instruments Co., Ltd., China) with three-electrode system a glassy carbon electrode (GCE) or modified GCE working electrode, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode.

2.2 Construction of the biosensor

The GCE was firstly pretreated to mirror as previously reported work [26], and then it was activated under the potential of +1.7 V for 160 s in PBS of pH 3.5. After that, the GCE was immersed into a 0.1 mol L^{-1} PBS of pH 3.5 containing 4 mg L^{-1} of guanine, so the guanine was immobilized onto the activated GCE surface via an adsorption step which was conducted with a potential of +0.4 V for 180 s under constant stirring [27-29]. Finally, the guanine modified electrode was sequentially covered with 2.0 mg mL^{-1} Fe@Fe_2O_3 of 4.0 μL and 3.0 mg mL^{-1} GOX of 4.0 μL. The resulted electrode was named as GOX/PDDA-Fe@Fe_2O_3/G/GCE.

2.3 Detection

In the composite membrane, the hydroxyl radical (·OH) was generated via a series of reactions between the GOX, glucose and Fe@Fe_2O_3 to oxidize guanine in situ. The mechanism of this sensor and the detection method were depicted in the Scheme 1. First, the square wave voltammetry (SWV) of the GOX/PDDA-Fe@Fe_2O_3/G/GCE was performed in PBS of pH 3.5 from 0.4 to 1.4 V with frequency of 15 Hz, step potential of 4.0 mV and amplitude of 25 mV (Scheme 1a) and the peak current was recorded as i_0. Then the modified electrode was incubated in the PBS of pH 5.0 containing 50 mmol L^{-1} glucose in the absence (Scheme 1b) or presence (Scheme 1c) of antioxidant for a certain time, after that, the SWV was performed again under the same conditions as above, and the peak current was recorded as i_t. The guanine oxidative damage and the antioxidant capacity were detected by calculating the SWV signal change of the guanine. The antioxidant capacity can be expressed as AOT % = $i_t/i_0 \times 100$.



Scheme 1. Mechanism of the biosensor and the detection method. SWV： square wave voltammetry.

3. RESULTS AND DISCUSSION

3.1 Optimal pH value for oxidizing guanine

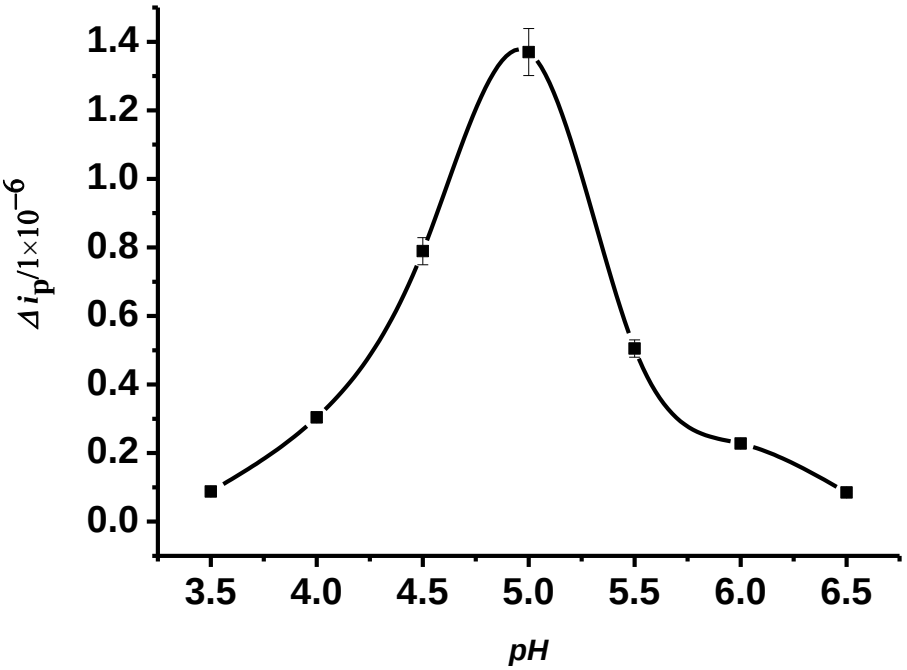


Figure 1. The influence of pH of the incubation solution on the Δi_p. Δi_p was the difference of the SWV peak currents of GOX/PDDA-$Fe@Fe_2O_3$/G/GCE before and after it was incubated in the PBS of different pH values containing 50 mmol L^{-1}glucose. Incubation time was 2.0 min.

By investigating the difference of the SWV peak current ($\Delta i_p = i_0 - i_t$) of the GOX/PDDA-Fe@Fe_2O_3/G/GCE after this sensor was oxidized in PBS of different pH containing 50 mmol L^{-1} glucose, the influence of the pH value the on the guanine damage was studied (Figure 1). The experiments indicated that in the pH range from 3.5 to 6.5, the Δi_p increased with the pH values at first and the biggest value of Δi_p was obtained at about pH 5.0 and then declined. Therefore, PBS of pH 5.0 was used as optimal buffer solution for oxidizing guanine.

3.2 The influence of the volume ratio of Fe@Fe_2O_3 and GOX on the Δi_p

The volume ratio of Fe@Fe_2O_3 and GOX in fabricating the modified electrode had great influence on the Δi_p. On the premise of keeping the total volume as 8.0 μL, the influence of the volume ratio of 2.0 mg·mL^{-1} Fe@Fe_2O_3 and 3.0 mg·mL^{-1} GOX on the Δi_p was investigated (Figure 2). It was found that the Δi_p increased with the increase of $V_{Fe@Fe2O3}$ V_{GOX}, and reached the maximum value at the $V_{Fe@Fe2O3}$ V_{GOX} of 4 4 and then declined. At ratio of 0 8 or 8 0, Δi_p value was close to zero, indicating that Fe@Fe_2O_3 core-shell nanonecklace or GOX in the membrane played synergistic effect for guanine oxidation. The optimal ratio of $V_{Fe@Fe2O3}$ V_{GOX} was 4 4.

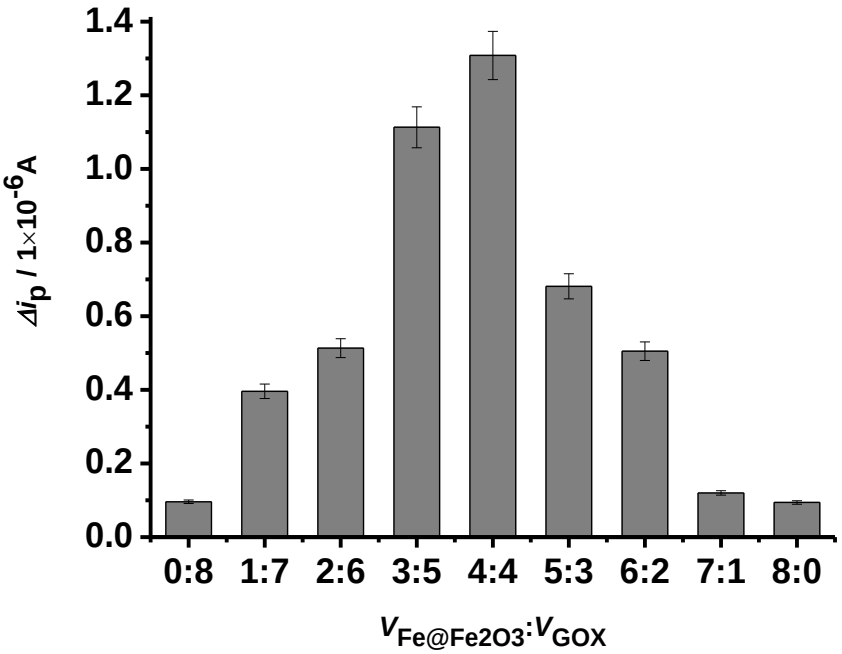


Figure 2. Effect of the ratio of $V_{Fe@Fe2O3}$ V_{GOX} in composite membrane on the Δi_p. The Δi_p has the same significance as in the figure 1. Incubation time was 2.0 min.

3.3 Detection of the antioxidant activity

The GOX/PDDA-Fe@Fe_2O_3/G/GCE was incubated in the pH 5.0 of PBS containing 50 mmol·L^{-1} glucose for different time. The relationship between the SWV peak current and the incubation time was showed in the Figure 3. The peak current decreased at first with the increase of the

time, and no more change as the incubation time was 4 min. That was to say the guanine of on the electrode surface was oxidized completely in 4 min.

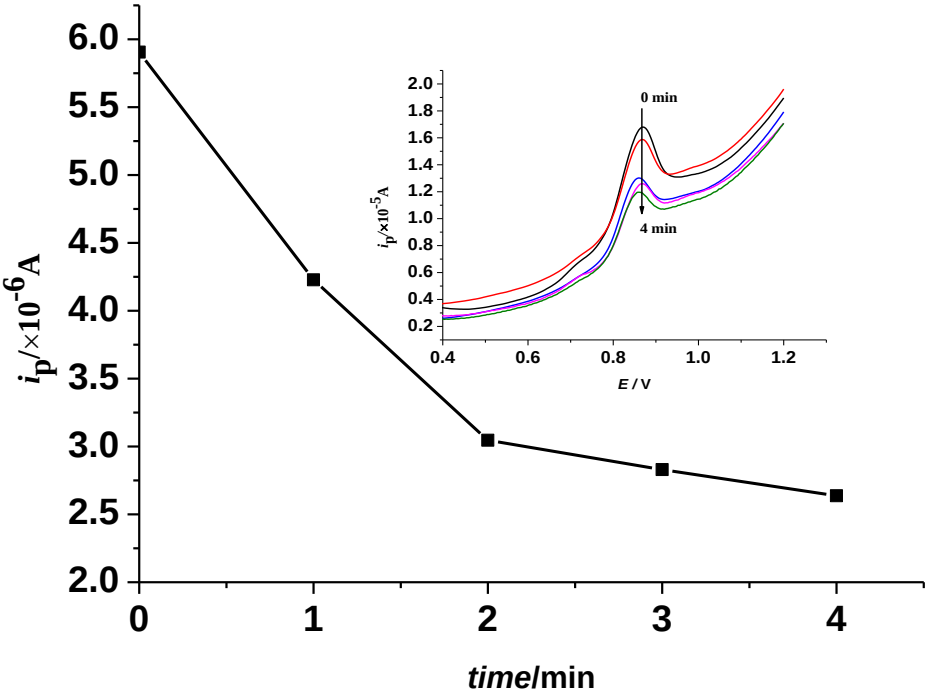


Figure 3. The relationship between i_p and the incubation time. Inset: SWV of GOX/PDDA-Fe@Fe_2O_3/G/GCE in pH 3.5 of PBS after it was incubated in pH 5.0 of the PBS containing 50 mmol L^{-1} glucose for different time.

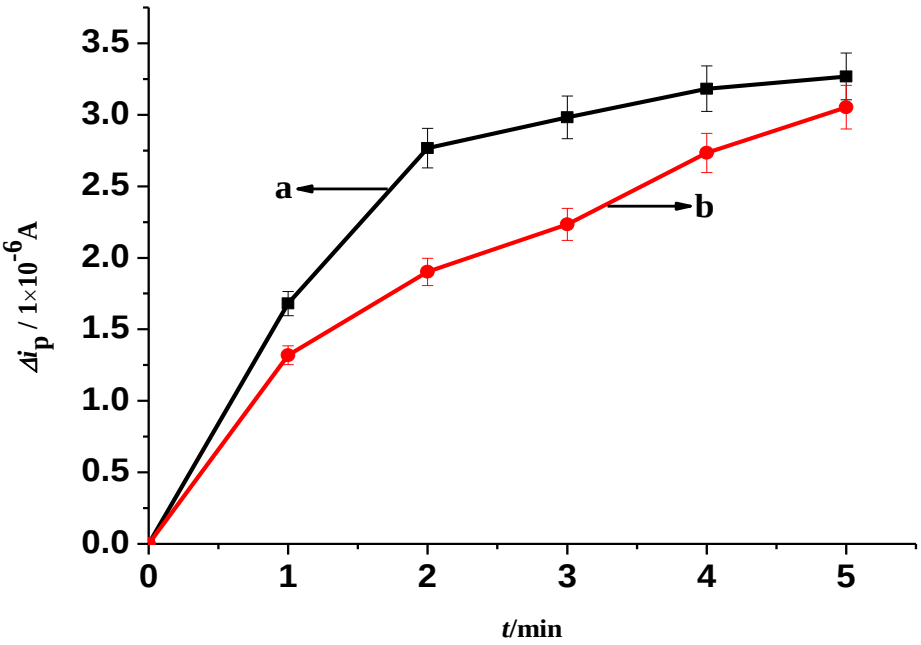


Figure 4. The relationship between Δi_p in the absence of AA (a) and presence of AA (b) with the incubation time.

Ascorbic acid (AA) is well-known radical scavenger. Its antioxidant capacity for against guanine damage was studied first. As can be seen from the Figure 4, curve a and b showed the effect of incubation time on Δi_p in the absence of AA and presence of 10 mg·L^{-1} AA, respectively. It was found that the slope of curve b was smaller than that of curve a, indicating as 10 mg·L^{-1} AA was added into the incubation solution, the increase trend of Δi_p was slowed. This phenomenon indicated that AA, had some effects on the protection guanine from oxidation.

Then antioxidant capacity of 10 mg L^{-1} caffeic acid was studied by SWV and the results were showed in Figure 5. In pH 3.5 of PBS, GOX/PDDA-Fe@Fe_2O_3/G/GCE had a sensitive SWV peak of guanine at about 0.82 V (curve a). It was the oxidation peak of guanine. After it was incubated in pH 5.0 of the PBS containing 50 mmol L^{-1} glucose for 2 min, the SWV peak decreased apparently (curve b), indicating that partial guanine has been oxidized. However, when the modified electrode was incubated in pH 5.0 of the PBS containing 50 mmol L^{-1} glucose and 10 mg L^{-1} caffeic acid for 2 min, the SWV peak (curve c) was bigger than that of curve b. The results indicated that caffeic acid could effective against the guanine oxidation.

Under the same concentration (10 mg L^{-1}) condition, the antioxidant capacity of ascorbic acid, caffeic acid, coumaric acid and resveratrol was investigated respectively (Figure 5 inset). The AOT % of ascorbic acid, resveratrol, coumaric acid and caffeic acid were calculated as 73.5%, 71.6%, 64.4% and 60.7%, respectively. The results showed that different antioxidants have different antioxidant capacity. The highest values was obtained for ascorbic acid and followed by resveratrol. Caffeic acid has the lowest antioxidant capacity. The conclusions are consistent with the reference of [30]. Compared with the other electrochemical biosensors [27, 28, 31], the guanine damage processes and the protection of antioxidants for it in this biosensor were not conducted directly in Fenton solutions, but via a series of biochemical reactions in a composite membrane. These processes are more similar with those processes in vivo. The proposed biosensor had potential use in determination of the total antioxidant capacities in fruit juices.

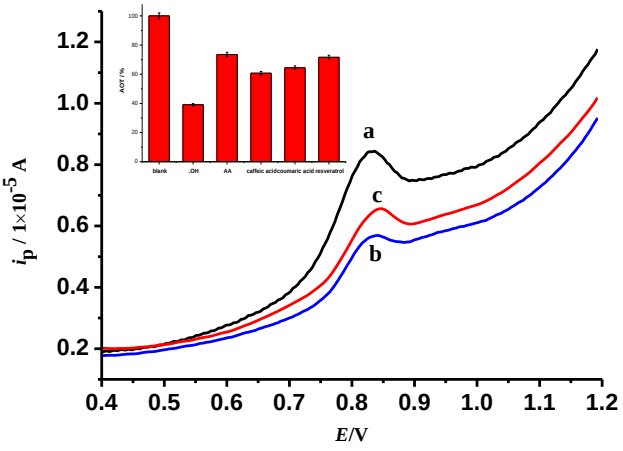


Figure 5. SWV of GOX/PDDA-Fe@Fe_2O_3/G/GCE in pH 3.5 of PBS before (a) and after it was incubated in pH 5.0 of the PBS containing 50 mmol L^{-1} glucose in the absence (b) or presence of 10 mg L^{-1} caffeic acid (c). Inset Comparison of the efficiency of radical scavenging of four antioxidants.

3.4 Repeatability and stability of the biosensor

The repeatability and stability of the biosensor was investigated by measuring the SWV peak current before and after it was incubated in the incubation solution. For a batch of five biosensors fabricated in the same way, the relative standard deviation (RSD) of Δi_p was 5.6%, suggesting that that the sensor shows good repeatability. By comparing the difference of Δi_p between a new fabricated biosensor and a stored biosensor in the air for 7 days at room temperature, the results showed that the mean deviation was less than 3% (n = 5), showing the good stability of the biosensor.

4. CONCLUSION

The antioxidant capacity of ascorbic acid, resveratrol, coumaric acid and caffeic acid was investigated using a guanine electrochemical biosensor. The antioxidant capacities of the four antioxidants were detected by recording the SWV signal change and results showed that ascorbic acid had the highest antioxidant capacity and followed by resveratrol. Caffeic acid had the lowest antioxidant capacity. The sensor showed good reproducibility and stability for rapid evaluation of antioxidant ability of scavenging free radicals.

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