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# A Sensitive and Selective Amperometric Immunosensor for Chloramphenicol Detection Based on Magnetic Nanocomposites Modify Screen-Printed Carbon Electrode as a Disposable Platform

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A sensitive and selective amperometric immunosensor for chloramphenicol (CAP, IUPAC name: 2, 2-Dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide) detection based on magnetic nanocomposites modify screen-printed carbon electrode (SPCE) as a disposable platform was fabricated. Graphene sheets (GS)-Nafion (Nf) dispersed solution was first dropped on the SPCE and then $Fe_3O_4$-Au nanoparticles (GoldMag particles, GMP) coated bovine serum albumin-CAP (BSA-CAP) conjugates was absorbed on it with the aid of external magnetic field. X-ray powder diffractometer (XRD), electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) were employed to characterize the synthesized GS and the construction processes of the modified electrode. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to study its electrochemical properties. The content of CAP was determined with a competitive immunoassay mode. When different concentration of CAP and 1.0 μg/mL anti-CAP were added to the phosphate buffer solution (PBS) containing 2.0 mmol/L $K_3[Fe(CN)_6]$, the increase ratio of the DPV current (CI%) was proportional to the concentration of CAP over the range from 2.0 ng/mL to 200.0 ng/mL after incubation for 5.0 min at 25 $^oC$. The detection limit was 0.82 ng/mL (S/N=3). The immunosensor was employed to determine CAP in milk samples and the results were consistent with high-performance liquid chromatography (HPLC) method. The proposed amperometric immunosensor is sensitive, selective, rapid, magnetic field controllable, low sample consumable and disposable. Results obtained in this study demonstrate that the immunosensor was suitable for determining trace CAP in real samples.

**Keywords:** Chloramphenicol; Magnetic nanocomposites; Screen-printed carbon electrode; Competitive immunoassay mode

## 1. INTRODUCTION

Chloramphenicol (CAP) with the IUPAC name of 2, 2-Dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide is a broad-spectrum antibiotic active against gram-positive and gram-negative bacteria. It is widely used for treating and preventing infectious diseases of cattle and poultry. However, the administration of CAP to humans, in relatively high doses has caused serious toxic effects such as agranulocytosis and aplastic anaemia. China, the European Commission, the United States and some other countries have strictly banned the use of CAP in food-producing animals. Nevertheless, due to its low price, consistent antibiotic effectiveness and driven by economic interests, illegal use of CAP still exists. Therefore, it is necessary to develop a sensitive and selective method to monitor the CAP residues in food, such as milk [1, 2].

Various methods such as liquid chromatography (LC) [3], LC-mass spectrometry (MS) [4-6], gas chromatography-MS [7], capillary zone electrophoresis [8], enzyme-linked immunosorbent assay (ELISA) [9] and chemiluminescence [10] have been applied for CAP determination in different samples. However, most of these methods need expensive apparatus/reagents and they are time-consuming. Therefore, a cheap and rapid method for analysis of CAP is still needed. From the molecular structure, it is apparent that CAP should be electrochemical active, since it contains nitro group [11], which can be oxidized on the surface of the electrode. Thus, electrochemical methods are also used for the detection of CAP and have attracted growing interests due to their high-sensitivity, portability, low cost and short analytical time. However, direct electrochemical detection of CAP is rarely. For example, the molecularly imprinted polymer (MIP) modified carbon paste electrode (CPE) [11], the cylindrical carbon fibre microelectrodes (CFMEs) [12], the monolayers of 2-mercapto-5-methylbenzimidazole (MMB) modified polycrystalline gold electrode (GE) [13], the aptamer-polymer based electrochemical biosensor [14], the single-wall carbon nanotube-gold nanoparticle-ionic liquid composite film modified glassy carbon electrodes (GCE) [15] and the nitrogen-doped graphene nanosheets decorated with gold nanoparticles (Au/N-G) modified GCE [16] were reported for the direct electrochemical detection of CAP in previous papers.

Although all the above methods and materials showed improved performance, the sensitivity and particularly the selectivity are still the main problems with the developed CAP electrochemical immunosensor. Therefore, it is of great importance and interest to develop other novel electrochemical methods for determination of CAP. New materials, especially nanocomposites are promising novel materials which are currently used to develop highly sensitive and selective CAP sensing platform [15-17]. Because the nanocomposites have some synergistic effects compared with single nanoparticles, such as high surface area, rapid electron transport, low detection limit and good signal-to-noise ratio. Graphene sheets (GS) -based materials have been developed as a kind of advanced nano-materials for constructing CAP electrochemical immunosensor [16]. The core-shell $Fe_3O_4$-Au magnetic nanoparticles (GoldMag particles, GMP), with Au coating the magnetic nanoparticles of $Fe_3O_4$, exhibits suitable intrinsic properties of the magnetic core and Au shell. It is anticipated that

incorporation of Au coating on a magnetic core could attain both the advantages of chemical stability, biocompatibility of Au and magnetic separation of $Fe_3O_4$. The application of GMP in the immunosensors has the following advantages: (i) rapid and non-chemical damaging regeneration of the immunosensors and the electrode is easy to renewable by using an external magnetic field. (ii) high surface-to-volume ratio and biocompatibility in the presence of Au, resulting in increased sensitivity and (iii) prevents cluster aggregation when GMP are directly used as the label [18, 19]. Screen-printed carbon electrode (SPCE) is frequently used for the construction of simple portable devices for fast screening purposes and in-field/on-site monitoring, because of their low cost and easy integration into mass-production processes [20-22]. Additionally, Nafion (Nf) is a perfluorinated sulfonated cation exchanger. Nf film modified electrode used to detect CAP has been reported in the literature [23]. All of those studies demonstrated that GS and GMP based nanocomposites combined with SPCE could well meet the requirements of field detection of CAP.

To the best of our knowledge, there is no report based on GS and GMP nanocomposites for determination of CAP. In this paper, we report on a novel electrochemical immunosensor for the rapid determination of CAP by using GS-Nf film and GMP coated bovine serum albumin (BSA)-CAP conjugates modified SPCE (SPCE│GS-Nf/GMP-BSA-CAP) based on a competitive immunoassay mode. The resulting immunosensor combines the advantages of GS, Nf, GMP and SPCE. In addition, the fabrication of the modified electrode is sensitive, selective, rapid, magnetic field controllable, low sample consumable and disposable. Finally, the modified electrode has been successfully applied to analyze CAP in milk samples with good recovery and accuracy, demonstrating its potential use for real sample analysis.

## 2. EXPERIMENTAL

### *2.1. Reagents*

CAP, rabbit anti-CAP antibody (anti-CAP) and BSA-CAP conjugates were purchased from Shanghai Boyao biotechnology limited company (Shanghai, China). BSA (Sigma-Aldrich). Graphite powder was obtained from green battery material limited company (Changsha, China). GMP solution was purchased from Xi'an goldmag nanobiotech co., ltd (Particle size: 30 nm, 5.0 mg/mL, Xi'an, China). Nf solution (5% ethanol solution), 30% $H_2O_2$ solution and other chemicals were of analytical-reagent grade and used without further purification were obtained from sinopharm medicine holding co., ltd (Shanghai, China). The 0.1 mol/L phosphate buffer solution (PBS) at various pH values were prepared by mixing the stock solutions of 0.1 mol/L $NaH_2PO_4$ and 0.1 mol/L $Na_2HPO_4$ with different proportion. Doubly distilled water (18.2 MΩ resistance) was used throughout. Milk samples (Jiahui Supermarket, Huaihua). All experiments were carried out under $N_2$ protection.

### *2.2. Apparatus*

Electrochemical measurements were performed on a CHI 660D electrochemical workstation (CHI Instruments, Shanghai, China). SPCEs were purchased from DropSens corporation (Spain, the

working electrode was modified, the auxiliary and reference electrode was screen-printed carbon and Ag/AgCl electrode, respectively.). The morphology of different electrode was characterized by scanning electron microscopy (SEM, Hitachi S-4800N). The x-ray powder diffractometer (XRD, Rigaku Ultima IV, Cu Kα radiation) was used to determine the phase purity and crystallization degree of GS. The HPLC (Shimadzu, LC-10AT) was also used for the measurements of the concentration of CAP in milk samples.

### *2.3. Preparation of GS solution*

Firstly, the graphite oxide was synthesized according to the literature with a modified Hummers method [24]. Then, exfoliation of graphite oxide to graphene oxide (GO) was achieved by ultrasonication of the dispersion for 30 min. Finally, a bright yellow homogeneous aqueous dispersion was obtained. As a large number of carboxyl, hydroxyl hydrophilic groups have been introduced between the layers of carbon atoms, GO is soluble in water. The resulting homogeneous dispersion of GO (0.1 g) was mixed with 50.0 μL hydrazine solution. After being vigorously shaken or stirred for a few minutes, the solution was stirred for 24 h at the temperature of 80 ℃ to form GS. GS tend to form irreversible agglomerates or even restack to graphite through strong π-π conjugation and Van der waals interaction. Thus, it is difficult for GS to be directly dispersed into solvent to form a uniform dispersion. This brings about the difficulty for the construction of electrochemical sensing platforms. Herein, Nf was selected as a stabilizer to disperse GS into an aqueous solution. Due to its excellent capability for film formation, nontoxicity, biocompatibility and good water permeability, Nf is commonly used to disperse nanomaterials and immobilize enzymes for constructing immunosensors [25].

### *2.4. Preparation of the GMP-BSA-CAP conjugates*

GMP was first pretreated with Tris-HCl as coupling agent. 100.0 μL of GMP solution was suspended in 100.0 μL of 0.02 mol/L Tris-HCl solution (pH=7.5). The mixture was then allowed to react under gentle stirring, followed by collection under an external magnetic field. Subsequently, 100.0 μL of BSA-CAP solution (2.0 mg/mL) and 50.0 μL GMP solution (1.0 mg/mL) were added to 5.0 mL Tris-HCl solution. The mixture was shaken at 35 ℃ for 20 min at 180 rpm. In order to keep the formed monolayer insulating and pin-free, and to ensure a high sensitivity, BSA was used to block uncovered spaces on the GMP surface. Thus, the resulting conjugates (labeled as GMP-BSA-CAP) were treated with 1.0 mL BSA solution ($w$=0.25%) for 30 min to block non-specific binding. The final solution was centrifuged 3 times using PBS to remove any free CAP and BSA and collected by applying an external magnetic field. Finally, the prepared GMP-BSA-CAP conjugates were washed twice with PBS and stored in 1.0 mL of PBS at 4 ℃ for further use.

*2.5. Preparation of the modified electrode*

A total of 100.0 mg of GS was dispersed into 100.0 mL of 0.2% Nf solution to form a homogenous dispersion under vigorous ultrasonication for about 0.5 h. 10.0 μL of the resulting GS-Nf dispersion was dropped onto the surface of SPCE and was kept at room temperature till dry (labeled as SPCE│GS-Nf). The further modification of 10.0 μL GMP-BSA-CAP conjugates onto SPCE│GS-Nf electrode was obtained with the aid of an external magnetic field under the electrode. After modification, the modified electrode (labeled as SPCE│GS-Nf/GMP-BSA-CAP) was thoroughly rinsed with water and kept at 4 ℃ for further use.

*2.6. Preparation of the milk samples*

The milk samples obtained nearby Huaihua College were pretreated according to literature [11]. Briefly, 10.0 mL milk sample was transferred to centrifuge tubes and spiked with 1.0 mL of CAP standard solution with different concentrations. Then, 2.0 mL of trichloroacetic acid (20%) solution was added to the spiked samples for protein precipitation. The mixture was vortexed for 1.0 min, and then centrifuged at 4000 rpm for 10.0 min. The supernatant was collected and filtered through a 0.45 μm membrane filter, followed by completely evaporation in an oven (55 ℃). Finally, 1.0 mL of PBS (0.1 mol/L, pH=7.0) was added to the container to fix pH at the desired value.

*2.7. The principle of the electrochemical immunosensor for CAP*

The determination of CAP is based on the specific interaction between anti-CAP and free CAP/BSA-CAP by using a competitive immunoassay mode [26]. That is, when the modified electrode was added with the PBS containing anti-CAP, free CAP and 2.0 mmol/L $K_3[Fe(CN)_6]$, BSA-CAP conjugates immobilized on the electrode and free CAP in solution can both bind anti-CAP. There is a competition binding between them. The more free CAP in solution can bind anti-CAP, the less anti-CAP can bind BSA-CAP conjugates immobilized on the electrode. On the contrary, the less free CAP in solution, the more anti-CAP immobilized onto the electrode. $K_3[Fe(CN)_6]$ probe is a valuable and convenient tool for testing the kinetic barrier of the interface because the charge transfer between solution and the electrode must occur by tunnelling either through the barrier or through the defects in the barrier. When the electrode was added with the solution containing anti-CAP, the ability of the anti-CAP to specifically bind to BSA-CAP conjugates led to the antibody layers to immobilize onto the electrode. Formation of anti-CAP-CAP-BSA complex on the surface of SPCE led to more steric hindrance on the electrode, which decreased the electrode active area and hindered probe ions through their pathways into the electrode. Hence, the redox reaction of $K_3[Fe(CN)_6]$ was suppressed and the peak current decreased.

The schematic diagram of the competitive immunoassay mode for CAP was shown in Fig.1. The electrochemical measurement with Cyclic voltammetry (CV) was carried out from - 0.2 V to + 0.6 V at a scan rate of 100 mV/s. Differential pulse voltammetry (DPV) measurement was set potential

between - 0.2 V to + 0.6 V. The results obtained were compared with HPLC method to confirm the reliability of this method.

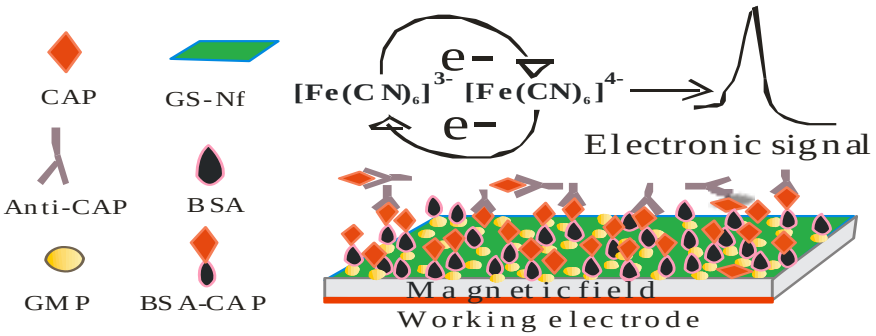


**Figure 1.** Schematic diagram of the competitive immunoassay mode for CAP determination

## 3. RESULTS AND DISCUSSION

### *3.1. Characterization of the synthesized GS*

Fig.2 shows the XRD patterns of graphite, GO and GS. It is clearly seen in Fig.2-a that the typical but strong diffraction peak (002) of graphite locates at 26° [27]. In Fig.2-b, the typical diffraction peak (002) of graphite shifts to lower angle (2θ=10.6°), which is ascribed to the introduction of various oxygenic functional groups (epoxy, hydroxyl, carboxyl and carbonyl) attached on both sides and edges of graphite sheets [28]. Fig.2-c shows the XRD of GS. The weak diffraction peak at about 2θ=23° is in good agreement with graphite. That is due to the partial removal of the oxygen-containing functional groups during reduction process, which means the partially reduction of GO to GS and restacked into a disordered crystalline structure [29].

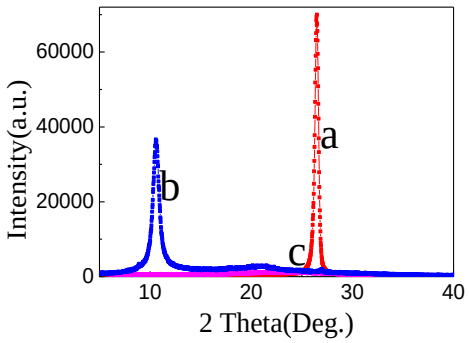


**Figure 2.** XRD patterns of graphite (a), GO (b) and GS (c)

*3.2. Electrochemical impedance study of different electrode*

The capability of electron transfer of different electrodes was investigated by electrochemical impedance spectra (EIS), shown in Fig.3. It can be seen that the EIS of the SPCE is composed of a semicircle and a straight line featuring a diffusion limiting step of the $[Fe(CN)_6]^{3-/4-}$ processes (3-a). With the GS-Nf film modified onto SPCE, the diameter of the semicircle increased (3-b). It shows the consistency with the fact that the insulation of the polymer Nf film interrupts the electron transfer with increased Rct [26]. Compared with the EIS of the SPCE | GS-Nf electrode, the SPCE | GS-Nf/GMP-BSA-CAP electrode (3-c) is somewhat higher than SPCE | GS-Nf electrode, owing to the insulation of BSA-CAP conjugates [30]. The diameter of the semicircle of SPCE | GS-Nf/GMP-BSA-CAP electrode increased remarkably after incubated with 1.0 μg/mL anti-CAP (3-d). All above indicate that GS-Nf and GMP-BSA-CAP conjugates can successfully immobilize on the surface of SPCE.

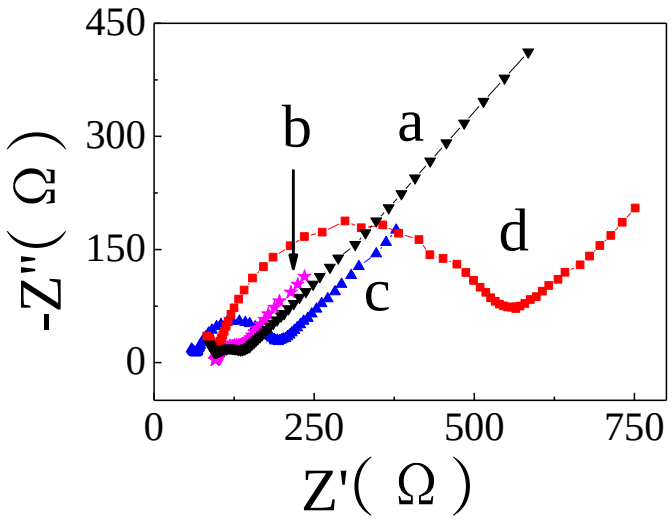


**Figure 3.** EIS of SPCE (a), SPCE | GS-Nf (b), SPCE | GS-Nf/GMP-BSA-CAP (c) and (c) electrode incubated with 1.0 μg/mL anti-CAP in 0.1 mol/L KCl solution containing 10 mmol/L $[Fe(CN)_6]^{3-/4-}$(1:1)

*3.3. Characterization of different electrodes' surface*

Fig.4-a appears the flake graphite on the surface of SPCE. As indicated in the magnified SEM image, the surface of SPCE | GS-Nf electrode exhibits a few thin wrinkles (Fig.4-b), the observation for the edge of the nanosheets confirms the layered structure of GS. While the conjugates of GMP-BSA-CAP was absorbed on the surface of SPCE | GS-Nf electrode with the aid of an external magnetic field to fabricate SPCE | GS-Nf/GMP-BSA-CAP electrode, uniform BSA-CAP conjugates of about 100 nm in average diameter formed randomly on the GS (Fig.4-c). Obviously, the GMP-BSA-CAP conjugates could homogenously distribute onto the GS-Nf matrix.

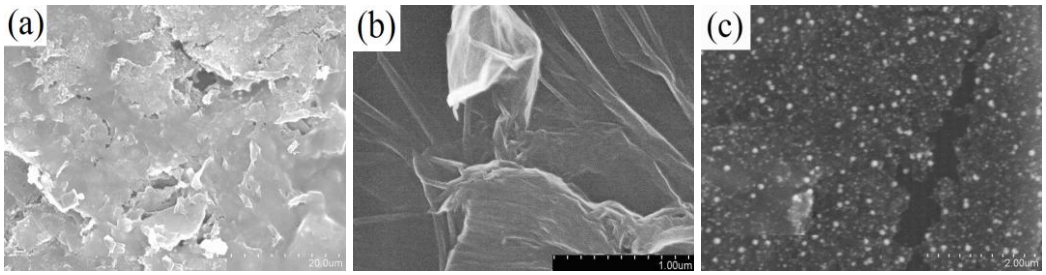


**Figure 4.** SEM images of SPCE (a), SPCE | GS-Nf (b) and SPCE | GS-Nf/GMP-BSA-CAP (c) electrode

### *3.4. Electrochemical behaviors of different electrode*

Fig.5 shows the CVs of different electrode in 0.1 mol/L PBS containing 2.0 mmol/L $K_3[Fe(CN)_6]$ at a scan rate of 100 mV/s. The response of SPCE electrode (5-a) is obvious with a pair of redox peaks corresponding to the redox of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$. In contrast, the response of the SPCE | GS-Nf electrode (5-b) is remarkably enhanced than that of the SPCE electrode, which can be interpreted as that the nano-sized GS can effectually accelerate the transfer rate of electron [31]. However, the response of SPCE | GS-Nf/GMP-BSA-CAP (5-c) electrode is lower than the SPCE | GS-Nf electrode but still higher than the SPCE electode. It may deduce that the synergistic effect of GS-GMP nanocomposites to current amplification is higher than electron hinder effect of BSA-CAP conjugates to current reduction [26]. There is a significant decrease in overall voltammetric signal after the SPCE | GS-Nf/GMP-BSA-CAP electrode incubated with 1.0 μg/mL anti-CAP (Fig.5-d). The result illustrates that the anti-CAP-CAP-BSA complex further insulates the electrode and hinders the diffusion of the redox marker toward the electrode surface, and also shows that the BSA-CAP conjugates successfully immobilize on the electrode surface and can be reacted with anti-CAP well.

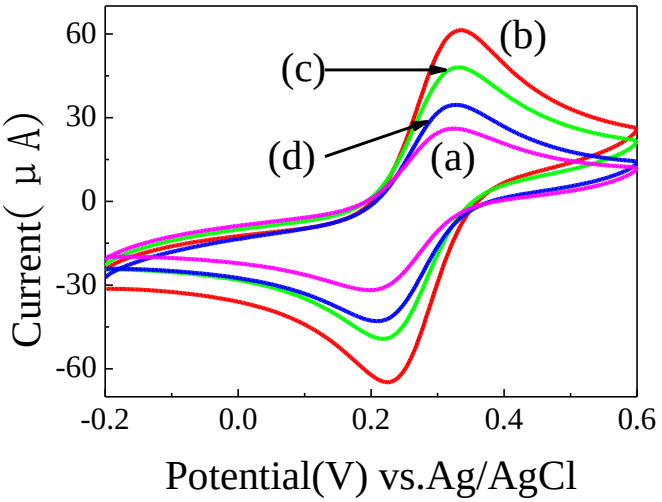


**Figure 5.** CVs of SPCE (a), SPCE | GS-Nf (b), SPCE | GS-Nf/GMP-BSA-CAP (c) and (c) electrode incubated with 1.0 μg/mL anti-CAP (d) in PBS containing 2 mmol/L $K_3[Fe(CN)_6]$

### *3.5. Optimization of the electrode modification*

#### *3.5.1. The effect of the amount of GS-Nf solution*

The amount of GS-Nf solution on the SPCE is a critical parameter for electrochemical performance of CAP. Experiments were carried out to choose the amount of GS-Nf solution used. A series of SPCE│GS-Nf electrodes were prepared with different volume of GS-Nf solution, and then modified these electrodes with the same amount of GMP-BSA-CAP conjugates to obtain a series of SPCE│GS-Nf/GMP-BSA-CAP electrode. The peak current of 2.0 mmol/L $K_3[Fe(CN)_6]$ increased with the volume of GS-Nf solution modified onto the electrode at first up to 10 μL and then decreased. Higher amount of GS-Nf solution caused broadening of voltammogram and decreased the intensity of peak current. So, 10 μL of GS-Nf solution was used to prepare the modified electrode.

#### *3.5.2. The effect of concentration of anti-CAP in the incubation solution*

To prepare the incubation solution, 50 μL of CAP standard solution with a series of concentrations was mixed with 50 μL of anti-CAP solution (4.0 μg/mL) to a total volume of 100 μL. Then, in order to block possible remaining active sites of the electrode, an additional blocking agent BSA was introduced in the system to block uncovered spaces on the electrode surface. Then the incubation solution was added to the reaction area of modified electrode. The CAP in solution competed with the BSA-CAP conjugates immobilized on the surface of the electrode to bind the anti-CAP in the incubation solution. After incubated for 5 min at 25 ℃, the electrochemical workstation recorded the DPV responses at different concentrations of CAP. As expected, the DPV peak current increased with an increasing CAP concentration (the current defined as I when $C_{CAP} > 0$) in the incubation solution compared with no CAP (the current defined as $I_0$ when $C_{CAP}=0$). That is, the higher concentrations of free CAP, the less amounts of anti-CAP to bind the BSA-CAP conjugates immobilized on the SPCE. The increase ratio of the DPV current ($CI\%=[(I-I_0)/I_0]\times100\%$) defined by our previous report [26] was proportional to the CAP concentrations in the incubation solution.

To investigate the influence of concentration of anti-CAP in the incubation solution, the SPCE│GS-Nf/GMP-BSA-CAP electrode was added with different concentrations of anti-CAP at 25 ℃ for 5 min. The $K_3[Fe(CN)_6]$ peak currents decreased significantly when the concentration of anti-CAP in the solution was changed from 1.0 to 1.5 μg/mL. With further increase in the anti-CAP concentration, the decrease of the current response was nearly not observed. The results suggest that an anti-CAP concentration higher than 1.5 μg/mL would not increase the amount of absorbed anti-CAP. It indicated that the concentration saturation of the binding sites was basically reached. For a competitive immunoreaction mode, we would choose a concentration that gives a high enough signal but is well below the saturation concentration [32]. Thus, the concentration of 1.0 μg/mL was chosen as the optimal anti-CAP concentration.

*3.5.3. The effect of electrolyte and pH*

The SPCE│GS-Nf/GMP-BSA-CAP electrode has different electrochemical behaviors in different electrolytes. The effects of some electrolytes, such as 0.1 mol/L NaAc-HAc, $NH_3 \cdot H_2O$-$NH_4Cl$ and PBS on stripping peak currents were studied. The results showed that the best electrochemical responses were obtained in PBS. When the measurements were performed in this electrolyte, the largest stripping peak current, the lowest background current and the best shape of peak was obtained. The influence of pH on the determination of CAP was also investigated. At first, the peak current decreased as the pH from 5.0 to 7.0. Then the peak current reached a minimum value around pH=7.0. Continuous increase of pH led to increase of the peak current, consistent with the reported literature [11]. Taking account of the factors above, 0.1 mol/L PBS (pH=7.0) was used as the electrolyte.

*3.5.4 The effect of incubation temperature and time*

Since the reactivity of anti-CAP and CAP is strongly affected by temperature. The reaction of CAP and anti-CAP was examined at 15, 20, 25, 30, and 35 ℃. The results showed that the voltammetric responses were decreased from 15 to 25 ℃, and then increased when the temperature was higher than 25 ℃. It was showed that anti-CAP kept the activities best and exhibited the highest affinity towards CAP at 25 ℃. Thus, we employed 25 ℃ as the optimum incubation temperature in this study. Using the same anti-CAP concentration, the incubation times were 0, 2.0, 5.0, 10.0 and 15.0 min in incubation solution respectively. The DPV peak current of modified electrode to the redox marker $K_3[Fe(CN)_6]$ in solution decreased rapidly with incubation time up to 5.0 min and then decreased slightly. Longer incubation time had no significantly effect on the current response. Thus, 5.0 min was adopted as the optimal incubation time for the immunoassay in the subsequent work.

*3.6. The detection of CAP on SPCE│GS-Nf/GMP-BSA-CAP electrode*

The linear range and limit of detection (LOD) were studied by using DPV under the optimized experimental conditions. Fig.6 shows the DPV responses of the SPCE│GS-Nf/GMP-BSA-CAP electrode toward CAP at different concentrations. Well-defined CI% proportional to the concentration of CAP ($C_{[CAP]}$) was obtained in the ranges from 2.0 ng/mL to 200.0 ng/mL with the linearization equation: CI%=[3.36+0.14$C_{[CAP]}$]% (ng/mL, $R$=0.997). The LOD was 0.82 ng/mL obtained with the calculation based on signal-noise ratio equal to 3 ($S/N$=3).

As seen in Tab.1, compared with other CAP electrochemical immunosensors using direct electrochemical detection reported previously, the present modified electrode displays an excellent comprehensive performance. Firstly, it has the advantages of $Fe_3O_4$ magnetic nanoparticles. The proposed electrode can be easily prepared in a rapid and simple procedure, and the surface of electrode is also easily renewable with the aid of an external magnetic field. Secondly, it shows low detection limit. Those may be due to the high surface-to-volume ratio and good electrochemical activities of GS and GMP nanocomposites with a lot of electroactive sites and large surface area for CAP and anti-

CAP to absorb or react. Therefore, the modified electrode could be used for the preparation of a CAP electrochemical immunosensor.

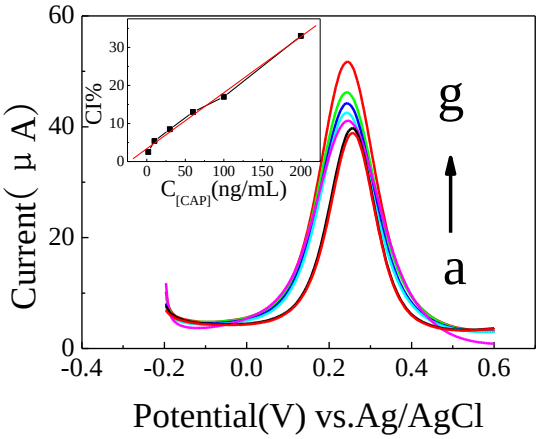


**Figure 6.** DPVs of the immunosensor incubated in PBS containing (a) 0, (b) 2.0, (c) 10.0, (d) 30.0, (e) 60.0, (f) 100.0 and (g) 200.0 ng/mL of CAP, 1.0 μg/mL anti-CAP and 2 mmol/L $K_3[Fe(CN)_6]$ at 25 ℃ for 5.0 min. Inset: The calibration curve of the CI% *vs* $C_{[CAP]}$

**Table 1.** Comparison of several CAP electrochemical immunosensors

| Electrochemical immunosensor | Renewable | Linear range (ng/mL) | Detection limit (ng/mL) | References |
|---|---|---|---|---|
| MIP-CP | No | 2.6-323.1 | 0.65 | 11 |
| MMB/GE | No | 16.2-323.1 | 14218 | 13 |
| $OMIMPF_6$-GNP-SWNT/GCE | No | 3.2-1938.8 | 1.62 | 15 |
| Au/N-G/GCE | No | 646.3-25850.4 | 190.65 | 16 |
| SPCE\|GS-Nf/GMP-BSA-CAP | Yes | 2.0-200.0 | 0. 82 | This work |

*3.7. Reproducibility, stability and selectivity of the modified electrode*

The reproducibility of the immunosensor was valued at a CAP concentration of 100.0 ng/mL with the same modified electrode. The relative standard deviation (*R.S.D.*) was 3.6% for five successive assays. The electrode-to-electrode reproducibility was determined in the presence of 100.0 ng/mL CAP with five different SPCE|GS-Nf/GMP-BSA-CAP electrodes prepared independently. It showed an acceptable reproducibility with *R.S.D.* of 3.0%.

The stability of the modified electrode was also examined during storage in a drying state at 4 ℃. It was found that the current response to 100.0 ng/mL CAP remained nearly 93.2% of its initial value after one month. The long-term stability can be attributed to good biocompatibility of GMP and strong interaction between BSA-CAP and GMP.

The selectivity of the modified electrode was examined in presence of 100.0 ng/mL CAP when the relative error less than ±5%. The addition of 30-fold concentration of nitrobenzene and para-nitrophenol, 40-fold concentration of glucose, fructose, tyrosine, glutamic acid and glycine, 500-fold concentration of $Na^+$, $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Ca^{2+}$, $Cl^-$ and $SO_4^{2-}$ did not cause observable interference. These results indicate that the developed sensor have a good selectivity for CAP.

### *3.8. Application on real milk samples*

In order to test the validity of this method, the proposed procedures were applied for determination of CAP in real milk samples. Three samples were treated as that described in section 2.6 and spiked with CAP at 50.0, 100.0 and 150.0 ng/mL levels. Recovery tests and HPLC method were used to examine the reliability and accuracy of this method. The recoveries were 96.0%, 105.2% and 104.2% respectively. The results confirmed that it is feasible to determine the CAP concentration in real milk samples using the proposed method (Tab.2).

**Table 2.** Determination results of CAP in real samples (n=3, concentration unit: ng/mL)

| Samples | Added | This method | HPLC method | *R.S.D*/% | Recovery/% |
|---|---|---|---|---|---|
| 1 | 50.0 | 48.0 | 49.8 | 2.5 | 96.0 |
| 2 | 100.0 | 105.2 | 101.4 | 3.8 | 105.2 |
| 3 | 150.0 | 156.3 | 155.5 | 2.0 | 104.2 |

## 4. CONCLUSIONS

In summary, a novel electrochemical immunosensor for rapid determination of CAP by using GS-Nf film and GMP-BSA-CAP conjugates to modify SPCE based on a competitive immunoassay mode was fabricated. The proposed modified electrode has high surface activity due to being modified with nanocomposites, which could amplify the response current. It also shows the merits of disposable design, renewable and long-term stability compared with other CAP electrochemical immunosensors. The practical analytical application of the CAP immunosensor is assessed by measurement of the real samples and the results are consistent with HPLC method. It may open up a new approach to explore GS-GMP nanocomposites materials for food or drug residues analysis. Moreover, the techonology platform is general and could be used in the determination of other food or drug residues.

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