

Fabrication of polyglutamic acid-based sensor for electrochemical determination of a phenicol antibiotic in water environment

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Abstract. In this study, a graphite electrode (GrE) modified with polyglutamic acid was used as a sensor for the determination of chloramphenicol (CAP) - a phenicol antibiotic - in water environment using adsorptive stripping linear sweep voltammetry. Polyglutamic acid (pGA) was successfully electropolymerized onto graphite electrode surface using a cyclic voltammetric method in glutamic acid/0.1 M phosphate buffer solution with pH 7. The electrochemical active area of the pGA/GrE was 1.5 times higher than that of the initial GrE. The highest CAP signal was obtained on the electrode fabricated by scanning 50 cycles in the potential range from -1.2 V to +2.0 V. The CAP signal recorded on the pGA/GrE electrode was nine times higher than that on the original GrE; that is due to the larger electrochemical active area (1.5 times) of the pGA/GrE and its good adsorption capacity towards CAP. Some effects of analytical conditions such as pH of the electrolyte solution and accumulation time were optimized. Under the optimal conditions, the calibration curve was built with two linear regions in the concentration ranges of 0.5 - 20 $\mu\text{mol L}^{-1}$ ($R^2 = 0.987$) and 20 - 100 $\mu\text{mol L}^{-1}$ ($R^2 = 0.996$), and the limit detection of CAP was 0.28 $\mu\text{mol L}^{-1}$.

Keywords: chloramphenicol determination, graphite electrode, electropolymerization, polyglutamic acid.

Classification numbers: 2.5.3, 3.2.1.

1. INTRODUCTION

Chloramphenicol (2,2-dichloro-N-[[1,3-dihydroxy-1-(4-nitrophenyl) propan-2-yl] acetamide (CAP), is a broad-spectrum antibiotic drug that has been widely used to treat diseases caused by both gram-negative and gram-positive bacteria on humans and livestock. However, it is shown that benzene-containing substances with a nitro-group connected to the ring are toxic [1]. So, CAP has serious adverse effects which can cause some illnesses such as leucopenia, anaemia, and aplastic anaemia. The abuse of antibiotics for a long time in some countries has been a primary reason for *Escherichia coli* and *Salmonella* drug resistance [2]. Moreover, other bacteria such as *Shigella* sp and *H influenza* are known as the chloramphenicol resistance [3]. Therefore, it is essential to develop a rapid, selective and sensitive method to detect CAP residues in the environment.

Nowadays, liquid chromatography [4], gas chromatography-mass spectroscopy [5], ultra V-visible, high-performance liquid chromatography [6], and electrochemical techniques have been commonly used for detecting CAP. In which, the electrochemical method has attracted extensive attention due to its simplicity, low cost, high sensitivity, and selectivity. The working electrodes modified with different materials, such as three-dimensional reduced graphene [7], multi-walled carbon nanotube-cetyltrimethyl ammonium bromide-poly(diphenylamine) [1], AuNPs/GO [8], the nanocomposite of molybdenum disulfide (MoS_2) and polyaniline (PANI) [9], some metal organic frameworks such as BUT-128, BUT-129 [10] have been applied for the determination of CAP. Besides, some research groups in Viet Nam have also tested modified materials such as rice husks derived activated carbon [11] and $\text{Fe}_3\text{O}_4/\text{Au}$ [12] to achieve good efficiency in CAP analysis. These researches show that the advantage of modified materials on the electrode surface, such as high sensitivity, stability, and good reproducibility, plays an important role in the enhancement of CAP.

Recently, a new class of bio-friendly polymer, poly (amino acid), has attracted great attention for biosensor fabrication. Amino acids are green organic compounds, which are favorably electropolymerized onto the electrode surface. The thickness of the poly (amino acid) can be controlled by adjusting the cycle number in cyclic voltammetry method. Polyglutamic acid (pGA) can be synthesized through linkages between the α -amino and δ -carboxylic acid in the structure of glutamic acid, one of twenty prevalent amino acids, and contains a free protonated carboxylic group in each unit in its chain. Thus, pGA has been a promising candidate for the electrode modification that was used for the determination of various substances such as norepinephrine, ascorbic acid, and uric acid [13] and amoxicillin [14]. In these studies, the pGA-modified electrode showed high biocompatibility, stability, reproducibility, sensitivity, a non-toxic nature, and biomedical activity.

Herein, we report a new electrochemical sensor to determine CAP based on the modification of a pristine graphite electrode by polyglutamic acid. The electropolymerization of glutamic acid was controlled by cyclic voltammetric technique to obtain high performance for CAP analysis. The polyglutamic acid layer with suitable functional groups in the structure facilitated the adsorption of CAP onto the electrode surface, which plays an important role in the enhancement of CAP electrochemical signals.

2. MATERIALS AND METHODS

2.1. Reagents and apparatus

Graphite (specific gravity of 1.82 g/cm³) was purchased from Tokai Carbon Co., Ltd, Japan. L-glutamic acid (99 %), potassium ferricyanide (99.9 %), potassium dihydrogen phosphate (99.9 %), di-potassium hydrogen phosphate (99.9 %), was purchased from Merck, Sigma-Aldrich. Chloramphenicol (98 %) was provided by Fisher. All reagents were of analytical grade and were commercially available.

Voltammetric measurements were carried out using an AUTO LAB PGSTAT320N. A three-electrode system consisting of an Ag/AgCl electrode, a platinum wire as the reference and auxiliary electrode, and a working graphite electrode was used.

The surface morphology of the electrodes was examined using field emission scanning electron microscopy (FESEM) (Hitachi S-4800, Japan).

2.2. Preparation of modified electrode

Graphite electrodes (from Japan) were initially polished with a 5000 grit sandpaper to get a flat, smooth surface; then, the electrode was soaked in 90 % ethanol and rinsed with double-distilled water to eliminate contaminants. A 5.0 g L⁻¹ glutamic acid (GA) solution was prepared by dissolving GA powder in 0.1 M PBS with pH 7 and sonicated for 300 s. The process of pGA polymerization on the graphite electrode was performed by cyclic voltammetry (CV) at a scan rate of 0.05 V s⁻¹ in a potential window between -1.2 and +2.0 V in the prepared GA solution.

2.3. Characterization of pGA/GrE

To characterize the electrochemical property of the modified electrode, cyclic voltammetry (CV) measurements were carried out in 5.0 × 10⁻³ M K₃Fe(CN)₆ in 0.1 M PBS with pH 7 at different scan rates ranging from 10 mV to 100 mV. In this examination, electrode surface areas of bare GrE and pGA/GrE were also evaluated.

2.4. Electrochemical measurements

To investigate the electrochemical determination of CAP, cyclic voltammetry (CV) and linear sweep adsorptive stripping voltammetry (LS-AdSV) methods were carried out in the potential range from -0.8 to 0.3 V. CV was performed to study electrochemical reaction of CAP on the electrode surface. The electrochemical detection of CAP was operated by LS-AdSV with a preconcentration step under open circuit condition. The detection conditions such as pH of buffer solution, preconcentration time were optimized.

All experiments were performed at a temperature of 25 ± 2 °C.

3. RESULTS AND DISCUSSION

3.1. Electrochemical polymerization of PGA onto graphite electrode

Figure 1a describes the cyclic voltammograms of electropolymerization in the glutamic acid solution. This electrochemical polymerization refers to a polymerization initiated by free radicals. Cathode and anode peaks appearing at -0.58 V and 1.65 V correspond to the deprotonation of carboxylic group to form -COO⁻ anion and the oxidation of amino group to form amino group radical cation (-NH₂^{•+}), respectively. Consequently, these ions react with each other to develop poly glutamic acid. Figure 1a shows the possible structure of the prepared

pGA. The gradual increase in the cathodic current and the anodic current with cycle number (Figure 1a) represents the growth of the electroactive pGA film onto the graphite electrode [14].

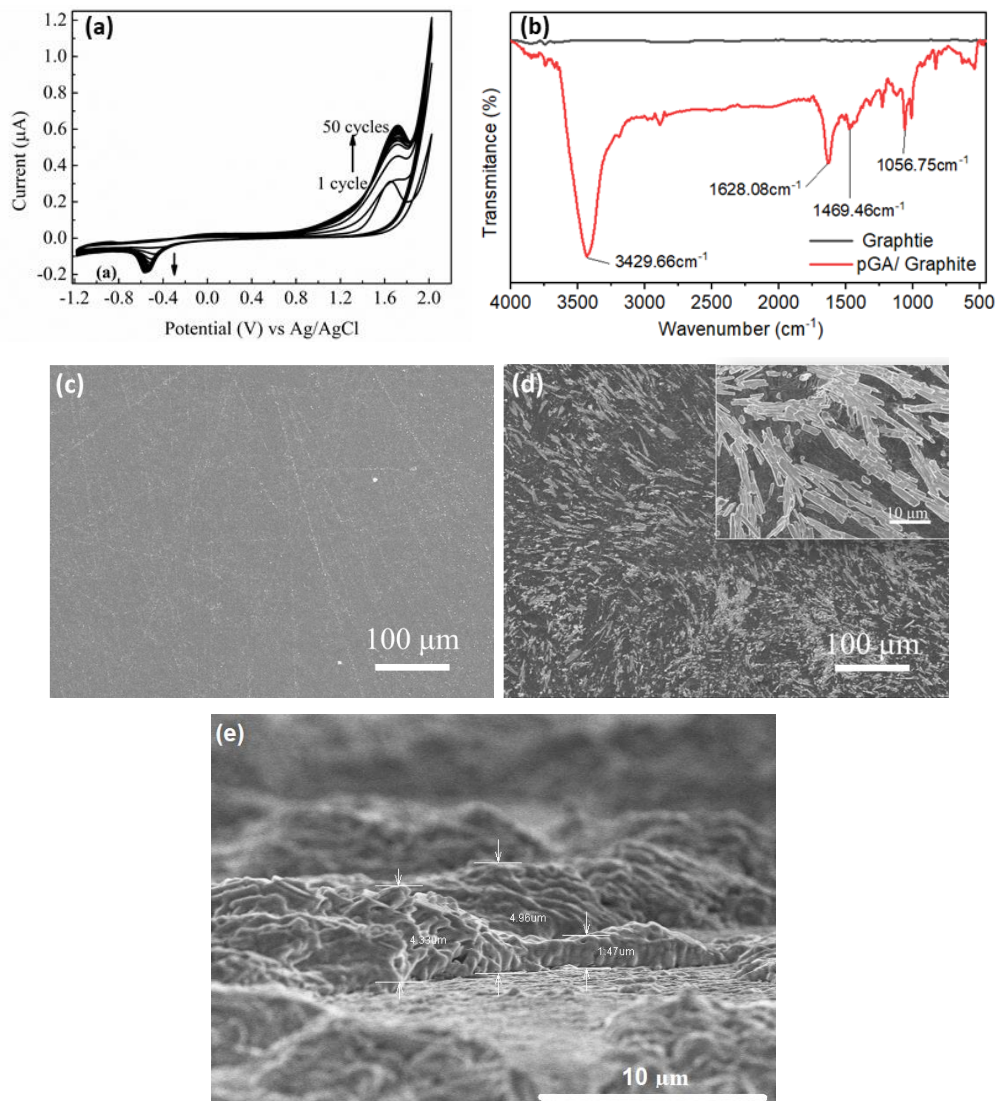


Figure 1. (a) Cyclic voltammograms of the electropolymerization of glutamic acid on graphite electrode surface from -1.2 V to 2.0 V for 50 cycles. (b) FTIR spectrum of graphite substrate, and electrochemically synthesized pGA film. SEM images of (c) graphite electrode, (d) pGA/GrE at different magnifications. (e) cross-section SEM image of pGA/GrE.

FTIR spectrum (Figure 1b) indicates the presence of poly-glutamic acid on the electrode surface with some characteristic peaks of glutamic acid. As can be seen that there are no sharp peaks observed in the IR spectrum of graphite substrate. Meanwhile, in the IR spectrum of the modified electrode with a poly-glutamic acid layer on the surface, a large peak is clearly observed at 3429 cm^{-1} corresponding to the N-H stretching vibration that was overlapped with O-H stretch. A sharp peak appearing at 1628.08 cm^{-1} assigns to the amide I combined with C=O (in carboxylic group). A peak of 1469.46 cm^{-1} is attributed to the C-C bending. The obtained

result is in agreement with previous studies that proves the successfully preparation of polyglutamic based sensor in our study [15]. The formation of pGA film is also observed through the scanning electron microscopy images (Fig. 1c, d). These images display the morphologies of the electrode before and after the pGA polymerization. It can be seen that the graphite electrode has a flat surface (Figure 1c). Figure 1d shows that the pGA was homogeneously deposited on the graphite electrode after polymerization in glutamic acid/PBS solution. The deposited pGA had a string shape with cluster dimensions of approximately 20.0 μm in length and 1.0 μm in width, indicating the polymer layer's gradual growth. The thickness of pGA was studied through cross-section SEM image (Figure 1e). It is indicated that the polymer thickness ranges from around 1 μm to 5 μm .

3.2. Electrochemical properties of pGA/GrE

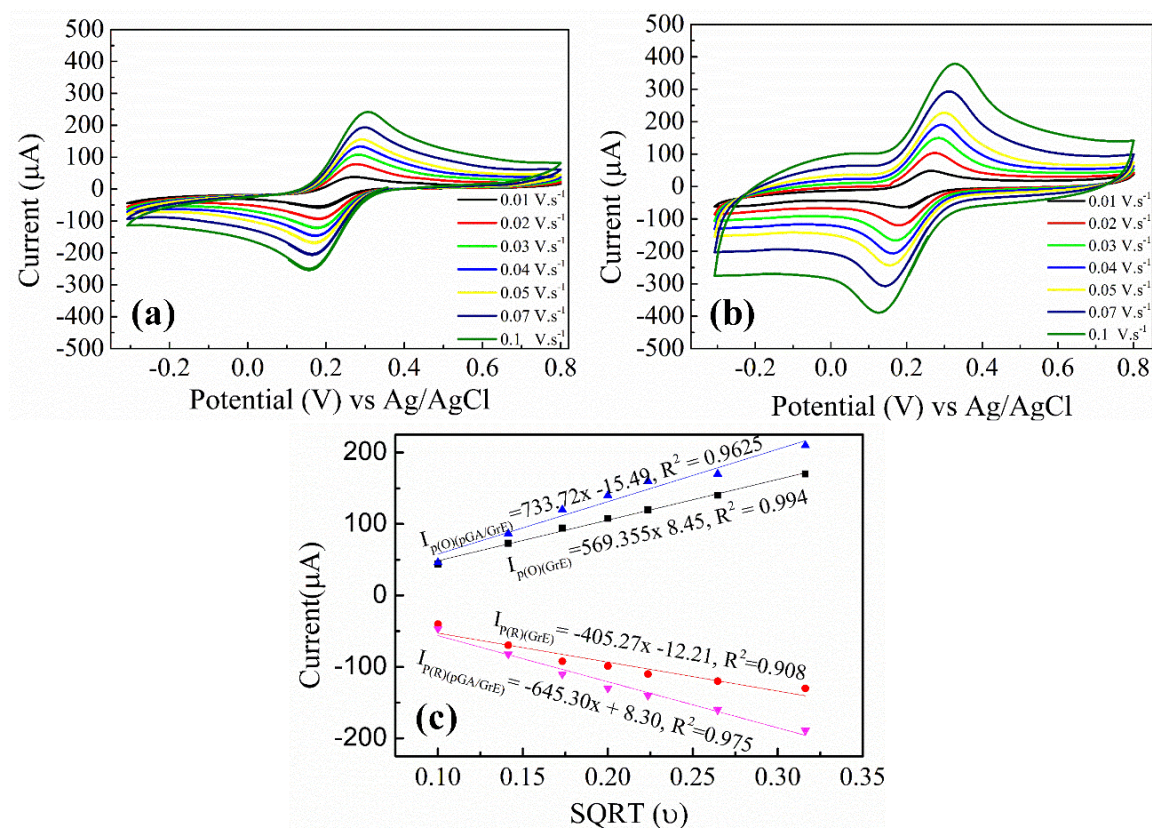


Figure 2. Cyclic voltammograms of 5 mM $\text{K}_3\text{Fe}(\text{CN})_6/0.1$ M PBS (pH 7.0) with different scan rates from 0.01 to 0.1 $\text{V}\cdot\text{s}^{-1}$ at bare GrE (a) and pGA/GrE (b).

To characterize the electrochemical property of the modified electrode, cyclic voltammetry (CV) experiments were conducted in 5.0×10^{-3} M $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M PBS with pH 7.0 at the bare GrE and pGA/GrE (Figure 2). It can be clearly seen that both electrodes show a good electrochemical property through well-defined redox peaks. However, the higher redox peaks observed in the CV curves of the pGA/GrE indicate that the pGA/GrE has higher electroactive surface area and better electrochemical properties. This suggests that the surface property of the

modified electrode has been significantly changed. The total active surface area of the electrodes can be estimated by the Randles–Sevcik equation [16]:

$$I_p = 2.69 \times 10^5 n^3/2 A D^{1/2} C_0 \nu^{1/2}$$

where, I_p is the peak current (1.27×10^{-4} A for GrE, 1.90×10^{-4} A for pGA/GrE), C_0 is the concentration of the electroactive species (5.0×10^{-6} mol cm^{-3}), n is the number of electrons exchanged ($n = 1$), D is the diffusion coefficient (7.5×10^{-6} $\text{cm}^2 \text{s}^{-1}$), ν is the scan rate (0.1 V s^{-1}) and A is the electroactive surface area (cm^2).

For pGA/GrE, the calculated electroactive surface area is 0.163 cm^2 that is nearly 1.5 times higher than that of GrE (0.109 cm^2).

3.3. The response signal of CAP on the pGA/GrE

3.3.1. The response signal of CAP

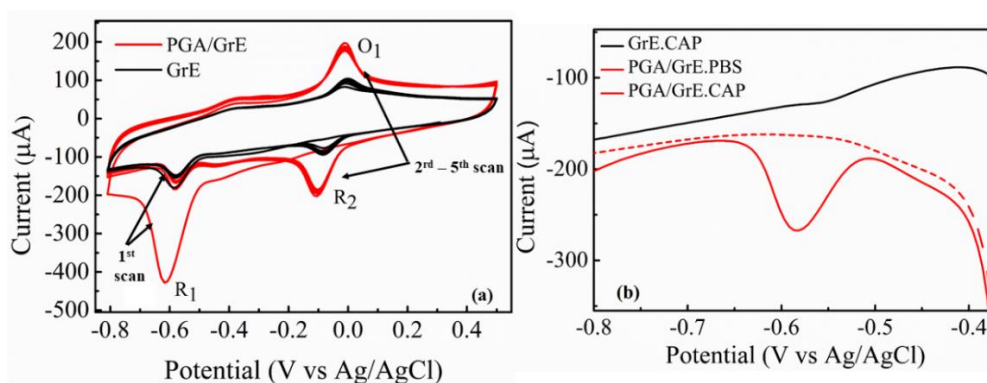
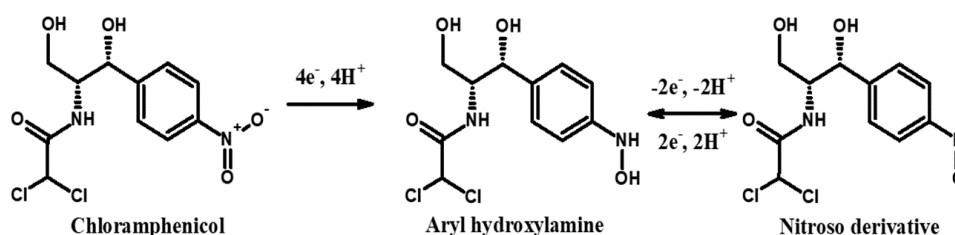


Figure 3. (a) Cyclic voltammograms of bare GrE and pGA/GrE in 0.1 M PBS (pH 7.0) containing 500 μM CAP. (b) Linear sweep voltammograms of bare GrE and pGA/GrE in 0.1 M PBS (pH 7.0) containing 20 μM CAP.



Scheme 1. Schematic illustration of proposed mechanism of CAP electrochemical redox reaction [17].

The electrochemical response of CAP at pGA/GrE was estimated and compared to that at bare GrE using the CV and LSV. Figure 3a shows multi-cycle CV curves from 0.3 V to -0.8 V of bare GrE and PGA/GrE with $500 \mu\text{M}$ CAP in phosphate buffer solution (pH 7). At the first forward scan, the CV curve of bare GrE presents only one reduction peak (R_1) at -0.58 V (black line), while a higher reduction peak of CAP was observed at around -0.61 V on the curve of pGA/GrE (red line). The peak current decreases remarkably from the second scan. This peak corresponds to the irreversible reduction of the nitro group ($-\text{NO}_2$) in CAP structure to

phenylhydroxylamine. At the reverse scan and next cycles, an oxidation peak (O_1) and a reduction R_2 are also observed at around 0.0 V and -0.1 V, respectively, ascribed to the reversible reduction-oxidation reaction of the redox pair of hydroxylamine/nitroso derivative (Scheme 1) [17,18]. The signal of the R_1 peak was chosen for CAP determination by LS-AdSV technique.

As can be seen in the LS-AdSV voltammograms (Figure 3b), the R_1 peak signal of CAP received on the pGA/GrE is significantly greater (nine times) than that on the bare GrE. This result could be assigned to the increase of the electrochemical active surface area of pGA/GrE (calculated in Section 3.2) as well as the enhancing of its adsorption capacity towards CAP. The negative shift of the peak potential on the electrode signifies the capacity in CAP adsorption since the electrode was coated by poly glutamic acid film [18].

3.3.2. The influence of polymerization cycle number on the CAP signal

The influence of cycle number on the detection of CAP at pGA/GrE was examined by LSV in 0.1 M PBS (pH 7.0) with 20 μ M CAP. As shown in Figure 4a, on the pGA/GrE fabricated in various polymerization cycles (from 10 to 60 cycles), the CAP peak current increased gradually with the increase in cycle number. The peak current reached the highest when the number of cycles was 50, then significantly decreased at a higher number of cycles. The smaller peak current at 60 cycles is assigned to a thicker pGA layer that interferes with the charge transfer on the electrode surface. Thus, the number of polymerization cycle of 50 was chosen for the electrode fabrication in the following experiments.

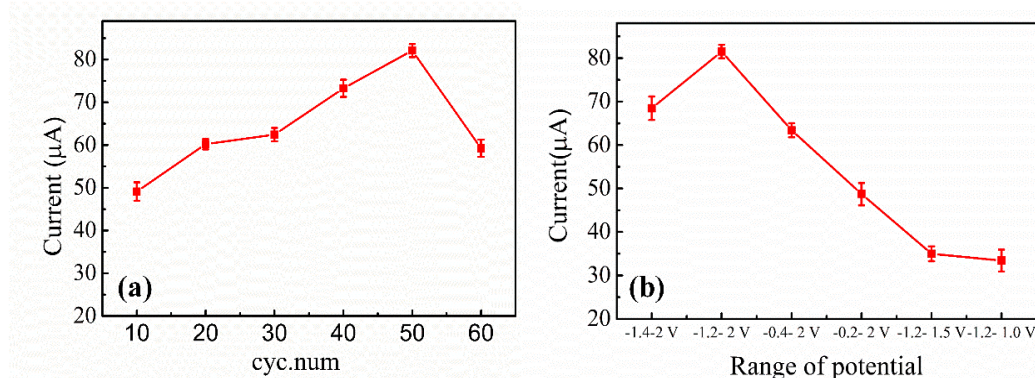


Figure 4. Effect of electropolymerization cycle number (a) and potential range (b) for pGA electropolymerization on the reductive current response of 20.0 μ M CAP at pGA/GrE.

3.3.3. The influence of the polymerization potential range on the CAP signal

The polymerization potential was investigated in five different ranges, which were from -1.4 to 2.0 V, -1.2 to 2.0 V, -0.4 to 2.0 V, -0.2 to 2.0 V, -1.2 to 1.5 V and -1.2 to 1.0 V to optimize the electrode fabrication for the CAP detection. The result (Figure 4b) shows that the electrode fabricated in the potential range from -1.2 to 2.0 V provided the highest peak current of CAP. It could be explained by the complete polymerization of poly glutamic acid on the electrode surface in this polymerization range compared to the other smaller ranges. The decrease of the CAP peak current on the pGA electrode fabricated at a wider potential range (-1.4 - 2.0 V) could be due to the release of H_2 bubbles, which competes with the pGA

polymerization. Therefore, the potential range from -1.2 to 2.0 V was selected for further studies.

3.4. Optimization of analytical conditions

The conditions for detecting CAP were optimized by investigating the influences of pH of supporting electrolyte and accumulation time.

3.4.1. The influence of pH on pGA/GrE

The electrochemical behavior of CAP is dependent on the pH of the solution. The CAP signal recorded in PBS solutions containing $20.0 \mu\text{M}$ CAP with pH varying from 5 to 9 is shown in Figure 5a. Accordingly, the reduction peak potential of CAP gradually shifted to the negative direction as the pH of electrolyte environment changed from acidic to alkaline. This demonstrates the participation of protons in the electrochemical reduction of CAP [18].

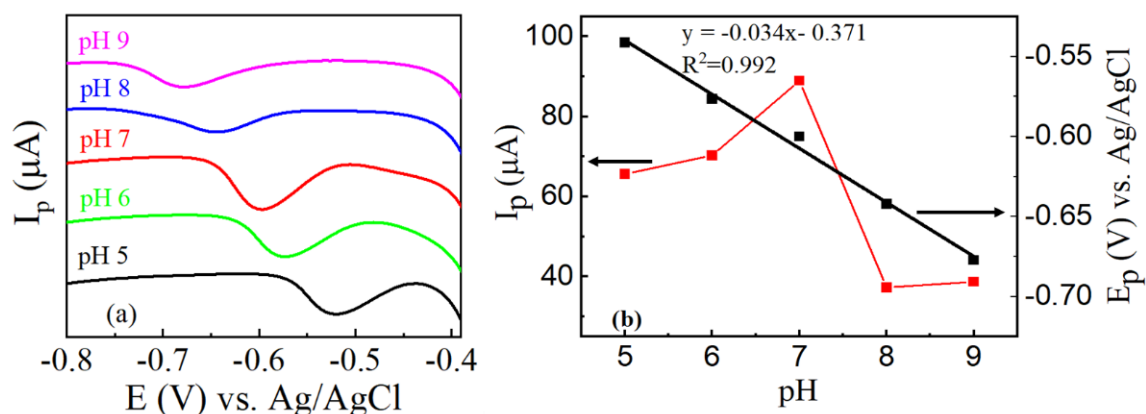


Figure 5. Effects of pH on the LSV of $20.0 \mu\text{M}$ CAP in 0.1 M PBS (pH 7.0) at pGA/GrE. (a) effects of a range of pH from 5.0 to 9.0, (b) the plot of I_{pc} vs pH (red) and linear plot of E_{pc} vs pH (black).

Besides, as illustrated in Figure 5b, the CAP reduction peak height reached a maximum value at pH 7. The gradual increase of peak current when pH increased from 5 to 7 could be attributed to the existing transformation of CAP ($\text{p}K_a = 5.52$), which is favorable for CAP adsorption on the electrode surface. However, in the alkaline medium (pH varies from 8 to 9), the CAP signal gradually decreased, which could be caused by the hydrolysis of CAP [18]. Therefore, the pH 7.0 medium was selected for further experiments.

3.4.2. Accumulation time

The accumulation time of CAP on the modified electrode surface has a crucial impact on the LSV signals of $20.0 \mu\text{M}$ CAP. To acquire an appropriate time for the analytical process, the accumulation time was varied from 0 s to 600 s for investigation. Figure 6 shows that when accumulation time increases, the peak current goes up and reaches a peak at 180 s. Then, the peak current slightly decreases at 240 s, and remains unchanged when the accumulation time varies from 240 s to 360 s. This is attributed to the desorption of CAP and the establishment of

CAP adsorption/desorption equilibrium on the electrode surface when the preconcentration time was extended. So, the optimum accumulation time of 180 s was chosen for the next steps.

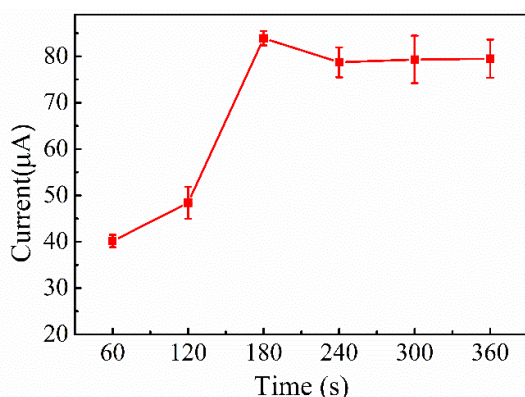


Figure 6. Effect of accumulation time on the reductive current response of 20.0 μM CAP at pGA/GrE in 0.1 M PBS (pH 7.0).

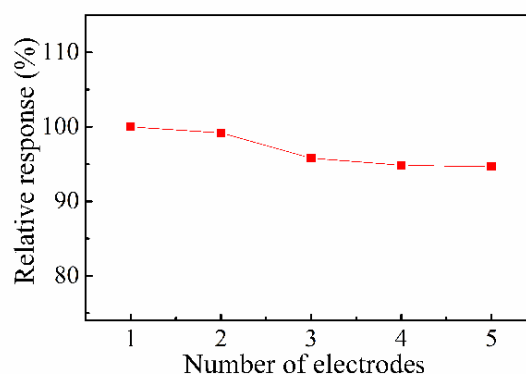


Figure 7. Peak signals of CAP at five measurements corresponding to five electrodes.

3.5. Reproducibility, selectivity and calibration curve for CAP detection

To study the reproducibility of the pGA/GrE, LSV measurements were carried out in the PBS solution containing 20 μM CAP using five electrodes (Figure 7). With the relative standard deviation of peak current of 2.18 %, the electrode exhibits satisfactory reproducibility.

The interferences of some inorganic ions and organic compounds on the CAP signal were investigated to evaluate the selectivity of the developed electrode. It is indicated that 25-fold concentration of ions (Cu^{2+} , Na^+ , K^+ , Pb^{2+} , Zn^{2+} , Fe^{2+} , SO_4^{2-} , Cl^- , NO_3^-) had a negligible influence on the CAP signal with a signal change below 2 %. Similarly, 10-fold concentration of amoxicillin and ascorbic acid caused a decrease by only 0.12 and 0.47 % in the CAP signal. Meanwhile, oxytetracycline, glucose, sulfamethoxazole, ciprofloxacin highly interfered the CAP detection when their concentrations were higher than CAP concentration in the analytical solution.

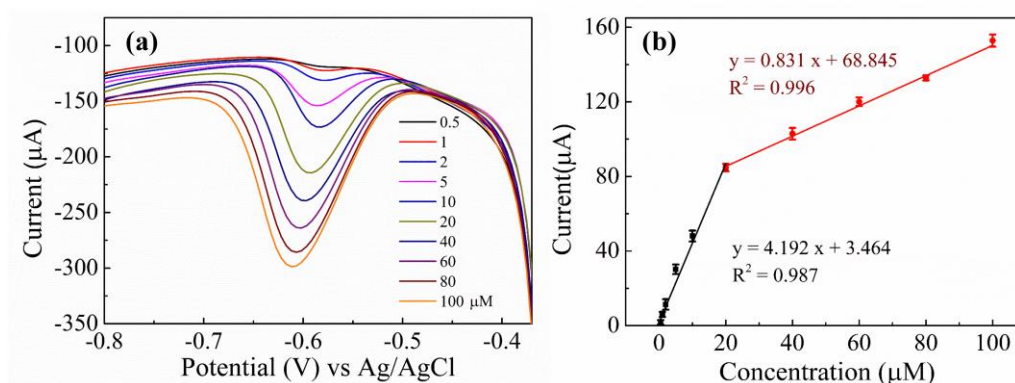


Figure 8. Linear sweep voltammograms for different concentrations of CAP in 0.1 M PBS (pH 7.0) versus Ag/AgCl electrode (a). Plot of concentration of CAP versus peak current of CAP (b).

A calibration curve was built to evaluate the CAP quantification by LS–AdSV method. Figure 8a shows the LSV curves obtained on pGA/GrE in the range of 0.5 - 100.0 μM CAP under optimal experimental conditions. Figure 8b illustrates that the height of peaks increases linearly with the increase in the concentration of CAP. The calibration curve shows two linear ranges. In the lower concentration range of 0.5 - 20.0 μM , the linear regression equation was

$$I_{pc} (\mu\text{A}) = 4.192 C (\mu\text{M}) + 3.464 (R^2 = 0.987),$$

while in the higher concentration range from 20.0 to 100.0 μM , it was

$$I_{pc} (\mu\text{A}) = 0.831 C (\mu\text{M}) + 68.845 (R^2 = 0.996).$$

Accordingly, the sensitivity of 4.192 $\mu\text{A}/\mu\text{M}$ was calculated from the first calibration curve. The detection limit was estimated from the first linear curve at a lower concentration range. LOD of 0.28 μM was obtained using the equation $\text{LOD} = 3 \sigma/b$, where, σ and b are the standard deviation and the slope of the linear line, respectively. This result is comparable with previous reports, as shown in Table 1.

Table 1. Comparison between different electrodes in previous reports with proposed electrode for determination of CAP.

Modified electrode	Method	Concentration Range (μM)	Detection limit (μM)	Ref.
Cu-MoS ₂ /SPE ^a	DPV	0.5 - 50	0.19	[19]
AuNPs/GO ^b	Amperometric	1.5 - 2.95	0.25	[8]
ZnWO ₄ /GCE ^c	CV	50 - 500	0.32	[20]
SPAN-MoS ₂ ^d	DPV	0.1-1000	0.065	[21]
N-G/AuE ^e	LSV	2 - 80	0.59	[22]
PGA/GrE	LSV	0.5 -100	0.28	This study

^aCu–MoS₂ nanocomposite;

^bGold nanoparticles decorated graphene oxide;

^cZinc tungstate nanowires;

^dMolybdenum disulfide nanosheets and self-doped polyaniline;

^eNitrogen-doped graphene nanosheets decorated with gold nanoparticles.

4. CONCLUSIONS

In this study, a voltammetric sensor for CAP detection based on polyglutamic acid film has been successfully fabricated by electropolymerization on a bare graphite electrode. The string shape of the pGA with lateral dimensions of 20.0 μm in length, 1.0 μm in width, and thickness ranging from 1.0 μm to 5.0 μm was characterized by SEM. The modified electrode by pGA provided a considerable increase in reduction current of CAP compared to the pristine electrode. The PGA/GrE electrodes exhibited the smallest detection limit of up to 0.28 μM in the wide range from 0.5 to 100 μM of CAP. The results come from the high active surface area and CAP adsorption capacity of the fabricated electrode. The CAP signal is affected by

electropolymerization conditions such as cycle number, scanning potential range, as well as by analytical conditions such as electrolyte pH and accumulation time. The prepared electrode shows a good performance in the chloramphenicol detection by linear sweep adsorptive voltammetry. The method has satisfactory repeatability, a wide linear range, and an acceptable limit of detection.

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CRedit authorship contribution statement. Nguyen Thi Kim Ngan: Methodology, Investigation, Experiment, Formal analysis. Vu Thi Thu Ha: Formal analysis, Manuscript writing, Supervision. Dang Van Thanh: Experiment, Data processing. Nguyen Tra My: Investigation, Manuscript writing. Pham Thi Hai Yen: Methodology, Experiment, Formal analysis, Funding acquisition, Supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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