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Electropolymerization of 4-aminobenzoic acid containing nano-Au deposited on carbon paste electrode for determination of acetaminophen

Xiao Liu, Liqiang Luo, Ying Zhang, Yaping Ding *

Department of Chemistry, Shanghai University, Shanghai, P. R. China
* *E-mail: wdingyp@sina.com*

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The conducting polymer films have been widely applied in the field of electrochemistry owing to their good stability, permselectivity, unique physical and chemical properties. A novel nano-Au/poly (4-aminobenzoic acid) (PABA) film modified carbon paste electrode was fabricated for sensitive detection of acetaminophen in this paper. The PABA film and nano-Au/PABA film were characterized by scanning electron microscope. The nano-Au/PABA film exhibited obviously catalytic performance to the determination of acetaminophen with a high sensitivity and a wide linear range. Under optimum conditions (pH, the concentration of 4-aminobenzoic acid, electro-polymerization cycles), linearity between the oxidation peak current and the acetaminophen concentration was obtained in the range of $1.0 \times 10^{-7} - 8.0 \times 10^{-5}$ mol L^{-1} with a detection limit of 8.0×10^{-8} mol L^{-1} (S/N = 3). In addition, the nano-Au/PABA film modified electrode was successfully employed to determine the acetaminophen in commercial drugs, and this work has shed some doubt on the redox mechanism of acetaminophen at the sensor.

Keywords: Poly (4-aminobenzoic acid); Nano-Au; Electrocatalysis; Acetaminophen

1. INTRODUCTION

In recent years, a lot of conducting polymer film has been widely applied in the field of electrochemistry owing to their good stability, permselectivity, unique physical and chemical properties [1–3]. Studies have indicated that polymer film modified electrodes show an enhanced response for the detection of various important biological and clinical species [4]. 4-aminobenzoic acid (4-ABA) is a non-protein amino acid which can be easily electro-polymerized on electrode surface to form poly-ABA (PABA) film. As an attractive modifier, PABA film has been used in lots of oxidation and reduction catalytic processes [5–8]. For example, the conducting polymers obtained from aminobenzoic acid (ABA) isomers have been synthesized and investigated at platinum electrode by Benyoucef et al [6]. The electrochemical polymerization of 2-, 3- and 4-ABA mixtures of aniline in various ratios on GCE by cyclic potential sweep has been reported by Thiemann et al [8]. Nano-Au is commonly applied in electrode modification for improving the analytical sensitivity owing to high electron density, dielectric property, catalytic function and excellent biocompatibility [9].

Acetaminophen (paracetamol, 4-acetamidophenol or N-acetyl-p-aminophenol) has been used commonly as an analgesic and antipyretic home drug to reduce fever and relieve pain for over 30 years since it was firstly presented in the medical profession in 1893 [10–12]. Generally, acetaminophen with normal therapeutic doses doesn't exhibit any harmful side effects, but an overdose of acetaminophen can produce toxic metabolite accumulation that may cause severe kidney and liver damage even fatal hepatoxicity and nephrotoxicity [13–16]. Therefore, it is

necessary to develop a simple, accurate, sensitive and inexpensive sensor for the determination of acetaminophen in pharmaceutical preparations.

So far, a variety of methods including titrimetry [17], spectrophotometry [18], fluorimetry [19], chemiluminescence [20], high performance liquid chromatography [21], capillary electrophoresis [22], chemometric method [23], amperometric batch injection analysis [24] and mass spectrometry [25] have been used for detecting acetaminophen in pharmaceutical formulations and biological fluids. Most of the above methods are unsuitable for routine acetaminophen analysis in pharmaceutical industry because they have some disadvantages such as high costs, long experimental times and stringent requirements for sample pretreatment [26].

In contrast, electrochemical method possesses many merits of quick response, high sensitivity, selectivity and miniaturization. So recently, the development and application of electrochemical techniques for the determination of acetaminophen [27–33] has attracted considerable attention, such as nano-Au modified indium tin oxide electrodes [28], poly (acid yellow 9)-nano-TiO2 modified glassy carbon electrodes [30], tetraoctylammonium bromide capped gold nanoparticles immobilized on 1, 6-hexanedithiol modified Au electrodes [31] and metal-dispersed porous carbon films modified electrodes [32]. In addition, a carbon paste electrode modified with nano-Au and glutamic acid has been explored as an electrochemical sensor for sensitive detection of acetaminophen by Y. Zhang et al. in our group [33].

In this paper, nano-Au/PABA film was modified onto the surface of CPE to fabricate an electrochemical sensor for acetaminophen measurement. The CPE modified with nano-Au/PABA film (nano-Au/PABA/CPE) could remarkably enhance the electrochemical responses of acetaminophen and improve the sensitivity and selectivity of acetaminophen detection. Furthermore, the electrochemical sensor was successfully employed to determine the acetaminophen concentration in commercial drugs, and this work has shed some doubt on the redox mechanism of acetaminophen at the nano-Au/PABA/CPE.

2. EXPERIMENTAL

2.1 Chemicals

Acetaminophen, ascorbic acid and uric acid were purchased from Shanghai Aladdin Chemical Reagent Co., Ltd. $HAuCl_4$•4H2O, 4-ABA, glucose, graphite powder (spectral reagent), paraffin oil, Na_2HPO_4, NaH_2PO_4, NaOH and HNO_3 were obtained from Sinopharm Chemical Reagent Co., Ltd. All chemicals employed in this study were analytical grade. The phosphate buffer solution (PB solution, 0.1 mol L^{-1}) was chosen as the supporting electrolyte.

2.2 Apparatus

All electrochemical experiments were performed with a CHI 660C electrochemical workstation (Shanghai Chenhua Co., Ltd., China) with a three-electrode system. The system is consisted of a nano-Au/PABA/CPE working electrode, a saturated calomel reference electrode (SCE) and a platinum counter electrode, all potentials were given against the SCE. Scanning electron micrographs (SEM) were obtained by a scanning electron microscope (JSM – 6700F, 15.0 kV).

2.3 Preparation of the nano-Au/PABA/CPE

Firstly, the CPE was prepared by mixing carbon powder and paraffin oil with the proportion of 3:1 (w/w) in an agate mortar until perfect homogenization was received. Subsequently, a part of the mixture was packed into the glass tube (i.d. 3 mm) which had been ultrasonicated in HNO_3, NaOH solution and double distilled water in turns, and then the copper wire was inserted from another end

of the tube. Prior to use, the CPE was polished on a piece of weighing paper and rinsed with double distilled water.

Afterwards, the CPE was scanned in 0.1 mol L^{-1} PB solution (pH 7.0) containing 0.5 mmol L^{-1} 4-ABA within the scan potential ranges of –1.5 to 2.0 V at 100 mV s^{-1} for 20 cycles (Fig. 1) and the mechanism of the PABA polymerization was shown in scheme 1. Finally, the PABA/CPE was modified with nano-Au in 0.5 g L^{-1} $HAuCl_4$ + 0.1 mol L^{-1} KNO_3 aqueous solution by cyclic voltammetry (CV) in the potential range between 0 and 0.5 V for 30 continuous cycles at 100 mV s^{-1}. The modified electrode was carefully washed with double distilled water for use.

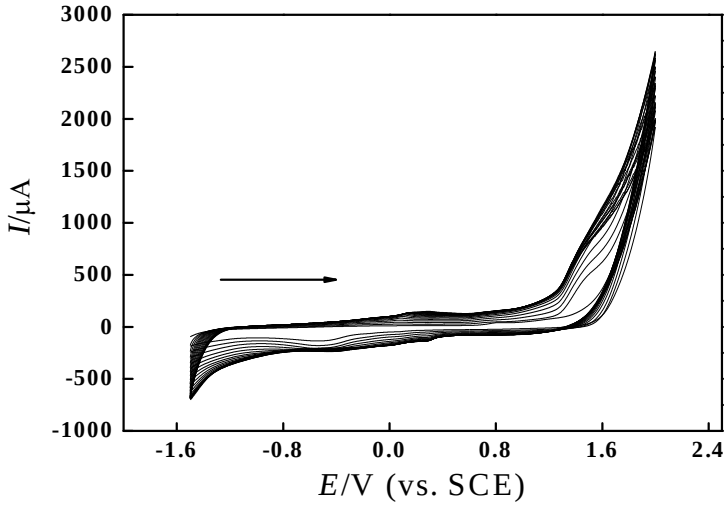
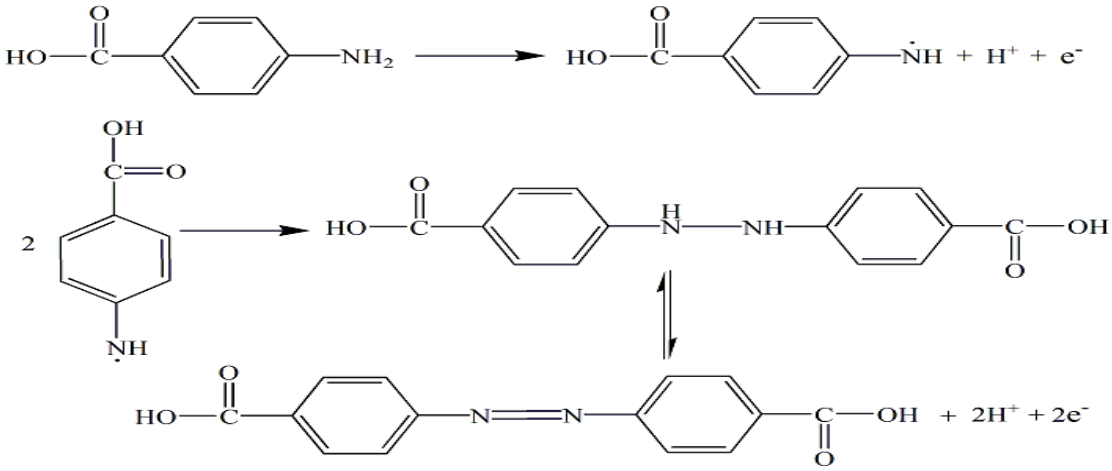


Fig. 1. The electro-polymerization of 4-ABA on CPE surface was carried out using cyclic sweeps between −1.5 V and 2.0 V for 20 cycles in 0.1 mol L^{-1} PB solution (pH 7.0) containing 0.5 mM 4-ABA.



Scheme 1. Mechanism of the PABA electrode reaction.

3. RESULTS AND DISCUSSION

3.1 The micrograph of nano-Au/PABA composite film

The morphology of PABA film and nano-Au/PABA film on the surface of CPE was characterized by scanning electron microscopy. Figure 2a shows the SEM of PABA film on the

surface of CPE. It is apparent that the CPE surface is coated with PABA film and the film is lamellae structure. Figure 2b exhibits clearly the surface state of nano-Au/PABA film. The nano-Au obviously exists on the modified electrode film and the mean nano-Au particle size is in the range of 80 – 100 nm.

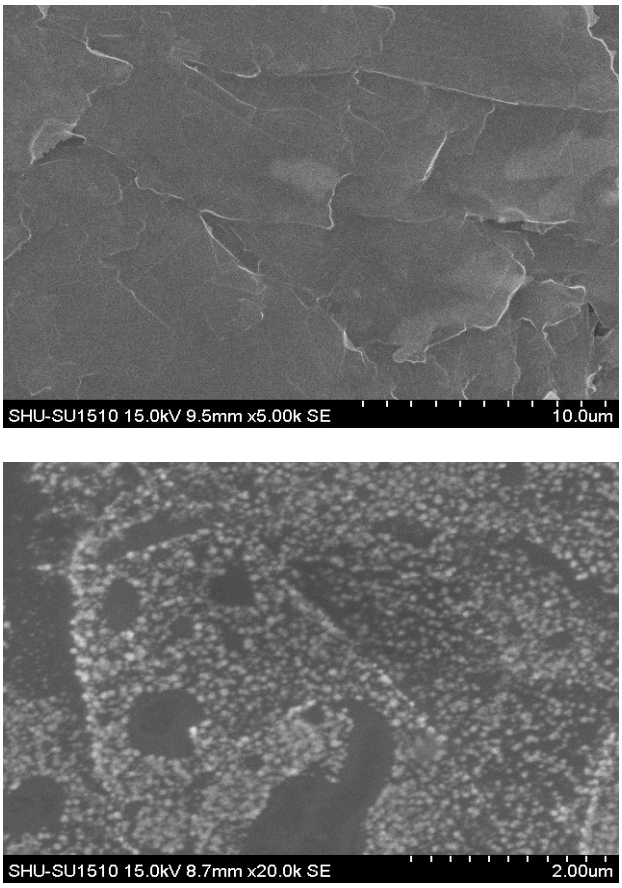


Fig. 2. SEM images of PABA film (a) and nano-Au/PABA film (b).

3.2 Electrochemical behaviors of acetaminophen at nano-Au/PABA/CPE

The CPE modified with nano-Au/PABA film has good catalytic activity towards acetaminophen. CVs on the CPE (a), nano-Au/CPE (b), PABA/CPE (c), nano-Au/PABA/CPE (d) in 0.1 mol L^{-1} PB solution (pH 7.0) in the presence of 0.1 mM acetaminophen are shown in Fig. 3. The irreversible behavior of acetaminophen with the weakest oxidative peak current and an oxidative peak potential of 0.482 V is observed on CPE, while the potential of acetaminophen at nano-Au/CPE negatively shifts 45 mV and the current increases comparatively, which suggests that nano-Au has the catalytic effect to the acetaminophen oxidation. Furthermore, the oxidative peak potentials of the well-defined redox peaks negatively shift 73 and 63 mV respectively at PABA/CPE and nano-Au/PABA/CPE compared with nano-Au/CPE, indicating that the electrochemical reversibility of acetaminophen was ameliorated markedly at the two modified electrodes. We speculate that the benzene ring of PABA can provide recognition sites through a "π – π stacking" interaction with the aromatic structure of acetaminophen [34]. Therefore, PABA may play the role of enhancing the interaction between the modified electrode and the acetaminophen, and further improving the sensitivity and stability of the nano-Au/PABA/CPE.

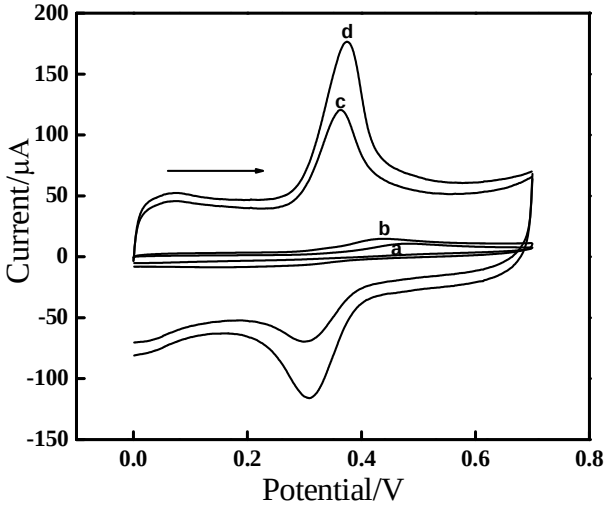


Fig. 3. CVs recorded at the bare CPE (a), nano-Au/CPE (b), PABA/CPE (c) and nano-Au/PABA/CPE (d) in 0.1 M PB solution (pH 7.0) with 0.1 mM acetaminophen. Scan rate: 100 $mV \cdot s^{-1}$.

Aim to understand the redox mechanism of acetaminophen at the modified electrode, the effect of scan rate on the redox of 0.1 mM acetaminophen in 0.1 mol L^{-1} PB solution (pH 7.0) was studied by CV (Fig. 4). The redox peak current in acetaminophen solution increases linearly with the scan rate in the range from 10 to 1000 $mV \cdot s^{-1}$ (inset, Fig. 4; linear regression equations: Ipa = 0.4148υ + 50.142, R = 0.996; Ipc = − 0.2495υ − 24.707, R = 0.998). This indicates that the redox reaction of acetaminophen at the nano-Au/PABA/CPE is an adsorption – controlled process.

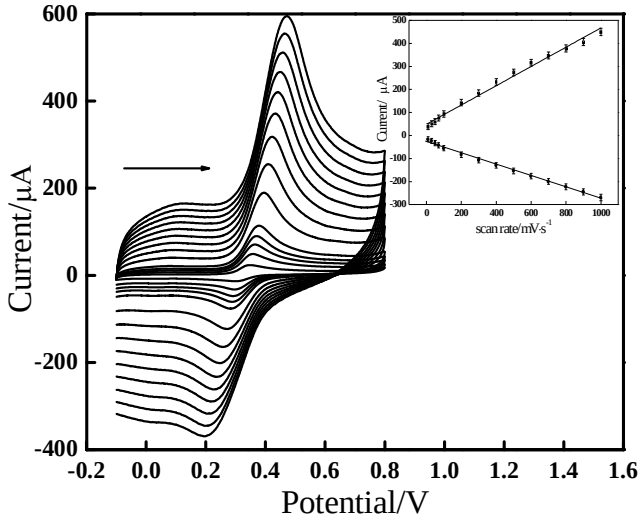
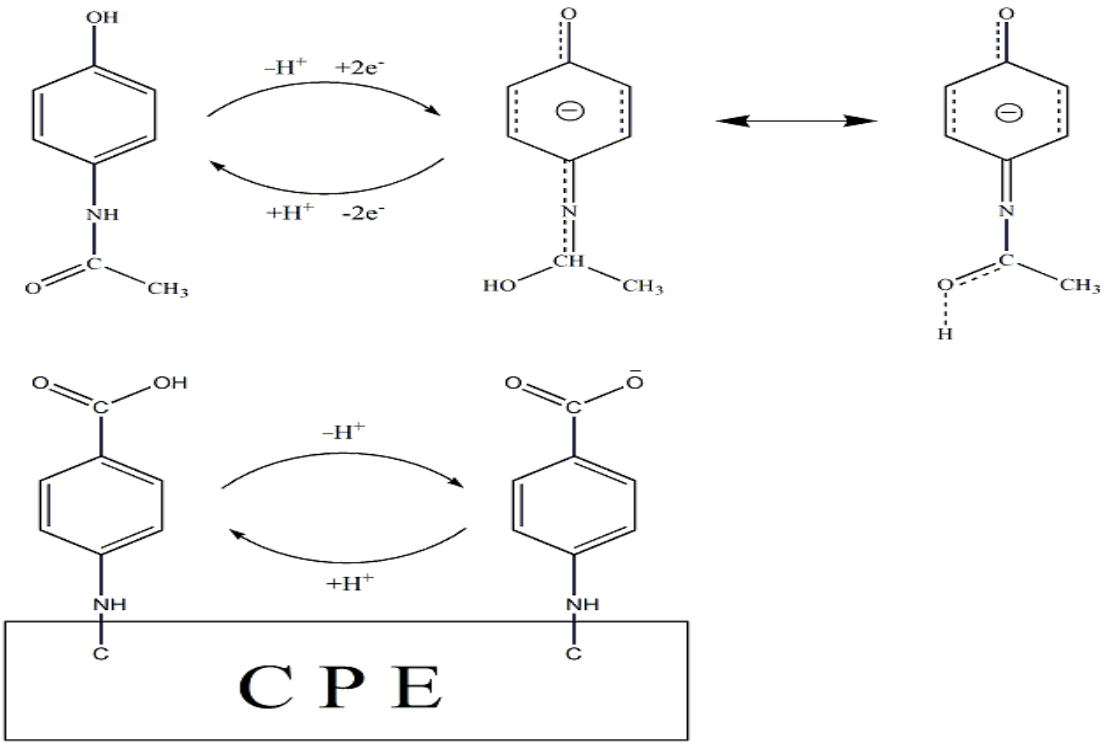


Fig. 4. CVs of nano-Au/PABA/CPE at the scan rate of 10 – 1000 $mV \cdot s^{-1}$ in PB solution (pH 7.0) with 0.1 mM acetaminophen. The inset shows the linear relationship between the peak current and the scan rate. Error bars represent the standard deviation for three independent measurements.

With the scan rate (υ) increased from 70 to 600 $mV \cdot s^{-1}$, the relationship between the Epa (Epc) and the logarithm of the scan rate are expressed as Epa = − 0.0252 lnυ + 0.4421 with R = 0.997 and Epc = 0.0382 lnυ + 0.1719 with R = 0.996. According to the slopes of the lines RT/(1−α)nF and

−(RT/αnF), the value of the electron transfer coefficient (α) and the electron transfer number (n) are calculated as 0.60 and 1.82, meaning that the electrochemical reaction between acetaminophen and the nano-Au/PABA/CPE is a double electron transfer process.

In addition, the potential of redox peak varies linearly with pH, suggesting that H^+ transfer is involved in the electrode reaction. In the pH ranging from 4 to 9, the equation can be expressed by the relation: $E^{\theta'} = -0.0483\ pH + 0.7008$, (R = 0.997). According to the dEp/dpH = 0.059X/αn, the proton number (X) is calculated as 1. So, the electrochemical reaction of acetaminophen belongs to a two electron and one proton transfer process, and this is in accordance with the mechanism of acetaminophen as shown in Scheme 2 [4].



Scheme 2. Possible redox mechanism of acetaminophen on the nano-Au/PABA/CPE.

3.3 Optimization of the experimental conditions

Figure 5 illustrates the influence of pH value on the oxidative peak current of 0.1 mM acetaminophen at the nano-Au/PABA/CPE. The oxidative peak current increased sharply with the increase of pH from 4.0 to 7.0 and decreased with the increase of pH from 7.0 to 10.0. So 7.0 was selected as the optimum pH value.

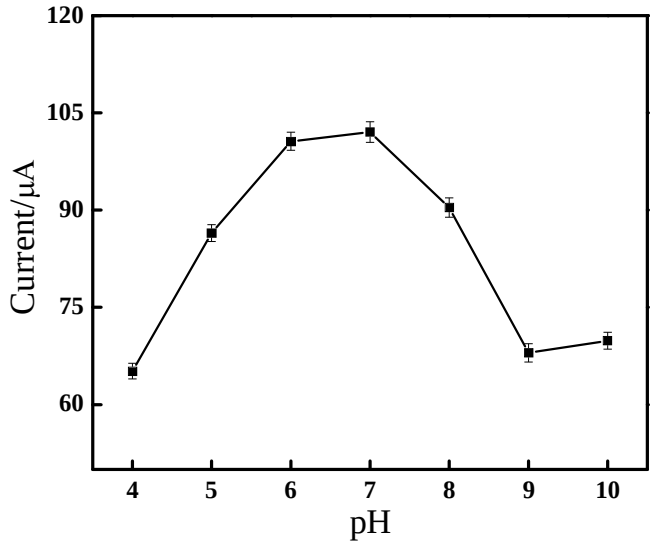


Fig. 5. The effect of pH value on the oxidative peak current of 0.1 mM acetaminophen at nano-Au/PABA/CPE. Error bars represent the standard deviation for three independent measurements.

The relationships between the oxidative peak current of acetaminophen and the 4-ABA concentration were investigated in Fig. 6. The concentration of 4-ABA was changed in the range of 0.1 mM – 5.0 mM and both the oxidative peak current and the reductive current reached the maximum value at 0.5 mM. So 0.5 mM was selected as the optimum 4-ABA concentration.

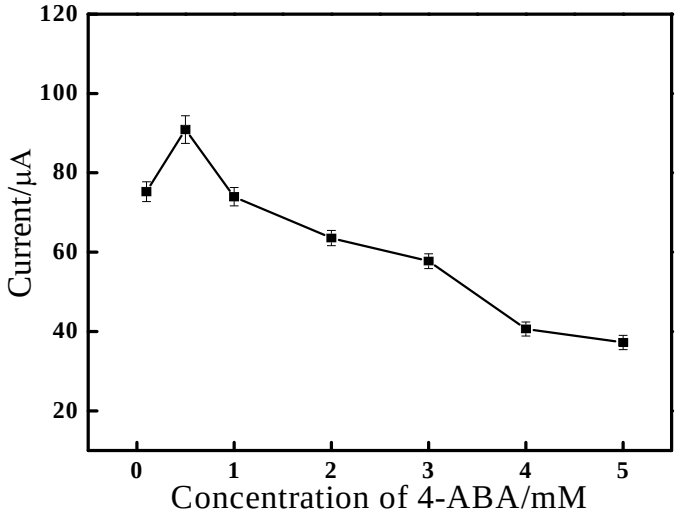


Fig. 6. The relationships between 4-ABA concentration and the oxidative peak current of 0.1 mM acetaminophen at nano-Au/PABA/CPE. Error bars represent the standard deviation for three independent measurements.

The optimum number of CVs cycles employed to form the electro-conductive PABA film on the CPE was determined from the following experiment in which electrodes were fabricated with different numbers in the range of 5 – 25 cycles in 0.1 M PB solution (pH 7.0) with 0.5 mM 4-ABA.

As shown in Fig. 7, the response of acetaminophen at nano-Au/PABA/CPE was found to reach the largest peak current when the cycles were 20. 20 cycles were selected as the optimum polymerization cycles.

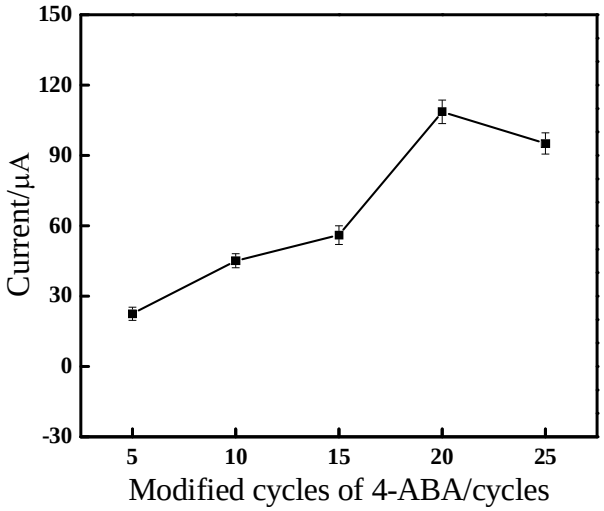


Fig. 7. The influence of modified cycles of 4-ABA on the oxidative peak current of 0.1 mM acetaminophen at nano-Au/PABA/CPE. Error bars represent the standard deviation for three independent measurements.

3.4 Calibration curve and interferences

Under optimum conditions, a calibration plot was obtained in 0.1 mol L^{-1} PB solution (pH 7.0) with different concentration of acetaminophen by CV. The linear response range of nano-Au/PABA/CPE to acetaminophen concentration is 1.0×10^{-7} – 8.0×10^{-5} mol L^{-1} (Fig. 8) and the linear regression equation is described as follows: Ip (μA) = 4.812 + 1.193 c (μM) (R = 0.9960). The detection limit is 8.0×10^{-8} mol L^{-1} at the S/N of 3.

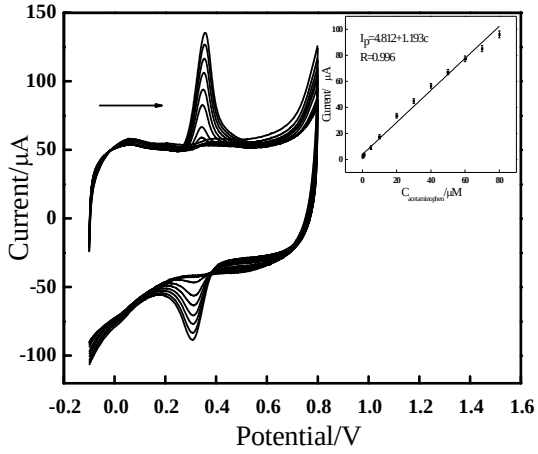


Fig. 8. CVs acquired on the nano-Au/PABA/CPE in a 0.1 mol L^{-1} PB solution (pH 7.0) at different concentrations of acetaminophen from 1.0×10^{-7} to 8.0×10^{-5} mol L^{-1}. Inset: Calibration curve of the oxidation currents vs. acetaminophen concentration. Error bars represent the standard deviation for three independent measurements.

Table 1 displays the analytical levels of the methods reported previously using for detecting acetaminophen. It can be seen that the dynamic range of nano-Au/PABA/CPE for acetaminophen is wider than that obtained at Polypyrrole/GCE [18] and Au/HDT/TOAB-AuNPs electrode [22]. The detection limit of nano-Au/PABA/CPE is lower than that of AuNPs/ITO [19], C–Ni/GCE [20] and PAY/nano-TiO_2/GCE [21]. Compared with these literatures, the electrochemical performance of nano-Au/PABA/CPE here is favorable.

Table 1. Comparison of Dynamic Ranges and Detection Limits at the Various Modified Electrodes for the Electrochemical Determination of Acetaminophen.

Method	Dynamic ranges (μM)	Detection limits (μM)	References
Polypyrrole/GCE	0.33 – 1.65	0.298	[18]
AuNPs/ITO	0.2 – 1500	0.18	[19]
C–Ni/GCE	7.8 – 110	2.3	[20]
PAY/nano-TiO_2/GCE	12 – 120	2.0	[21]
Au/HDT/TOAB-AuNPs electrode	0.15 – 13.4	0.0026	[22]
nano-Au/PABA/CPE	0.1 – 80	0.08	Present work

GCE, glassy carbon electrode; AuNPs, gold nanoparticles; ITO, indium tin oxide electrode; C–Ni, carbon-coated nickel magnetic nanoparticles; PAY, poly(acid yellow 9); HDT, 1,6-hexanedithiol; TOAB, tetraoctylammonium bromide.

The selectivity of nano-Au/PABA/CPE was assessed by studying the effect of different interfering molecules on the measurement of acetaminophen. Voltammetric responses of acetaminophen were detected in the presence of some possible interfering substances like ascorbic acid, uric acid and glucose, which were existent in biological fluids and might interfere with the detection of acetaminophen by traditional methods. Determination of acetaminophen was not affected in presence of 100–fold glucose, 100–fold ascorbic acid and 5–fold uric acid.

3.5 Stability, repeatability and reproducibility of nano-Au/PABA/CPE

The nano-Au/PABA/CPE was very stable towards determination of acetaminophen. It was found that the oxidation current of acetaminophen only lost 4.8% after the nano-Au/PABA/CPE storing in the refrigerator for 12 days, indicating that this present sensor has a good stability.

The repeatability of the nano-Au/PABA/CPE was investigated for 0.1 mM acetaminophen by CV. The relative standard deviation (R.S.D.) of the electrode response to 0.1 mM acetaminophen for six successive measurements was 1.28%. Under the same and independent conditions, it was found that the oxidation current of acetaminophen almost remained the same with a R.S.D. of 3.84% by five electrodes.

3.6 Determination of acetaminophen in commercial drugs

The standard addition method was applied to determine acetaminophen commercial tablets such as “Acetaminophen, Aminophenazone, Caffeine and Chlorphenamine Maleate Tablets” (Shanghai Huashi Pharmaceutical Co., Ltd.) and “Vitamin C Yinqiao tablets” (Shanghai Lei Yun

Shang Pharmaceutical Co., Ltd.). Firstly, one of the tablets was ground to powder and dissolved in 250 mL volumetric flask using double distilled water. Subsequently, acetaminophen concentration was laid in the range of calibration plot by adding 20 μL above solution into 10 mL 0.1 M PB solution (pH 7.0) and gradually increased the acetaminophen concentration in the determination solution. The concentration of acetaminophen was detected using the proposed analytical procedures, which was in good agreement with the manufacturers' stated contents of acetaminophen (as shown in Table 2).

Table 2. Determination of Acetaminophen Concentration in two Commercial Tablets (Tablet 1: Acetaminophen, Aminophenazone, Caffeine and Chlorphenamine Maleate Tablets; Tablet 2: Vitamin C Yinqiao tablets) Using Nano-Au/PABA/CPE.

Commercial tablet	Reported content	Detected content	R.S.D. (%) (n=3)	Recovery (%) (n=3)
Tablet 1	150 mg	145 mg	3.33	98.6
Tablet 2	105 mg	102 mg	2.86	99.1

4. CONCLUSION

In this study, a novel nano-Au/PABA/CPE for the determination of acetaminophen was successfully fabricated. The nano-Au/PABA film enhanced the peak current of acetaminophen and decreased the overpotential obviously, indicating an excellent electrocatalytic effect on the oxidation of acetaminophen. Under the optimum conditions, the modified electrode showed the satisfying results of wide liner range, low detection limit, good selectivity and reproducibility. The voltammetric sensor has successfully employed to determine the concentration of acetaminophen in commercial drugs, and the redox mechanism of acetaminophen at the modified electrode has been discussed adequately.

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