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Studies on the Electrochemical Behavior of Chlorogenic Acid and Its Interaction with DNA at a Graphene Modified Electrode

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Graphene was used to modify the *glassy carbon electrode surface* using drop-casting method and the graphene modified electrode (Gr-GME) was prepared. The electrochemical behavior of chlorogenic acid (CGA) was studied. A new method to provide a rapid measure of CGA was established. A sensitive quasi-reversible redox peak was obtained from -0.4V to1.4V in pH=3.0 phosphate buffered saline (PBS) by cyclic voltammetry (CV). Compared with the unmodified glassy carbon electrode (GCE), the Gr-GME was observed to *greatly increase* the *reaction rate* of CGA, showing strong signal enhancement effects. The peak current of oxidation was proportional to the concentration of CGA and the linear range was from 3.20×10^{-7} to 4.50×10^{-5}mol L^{-1}. The detection limit was 8.00×10^{-9} mol L^{-1}. The relative standard deviation (RSD) for six replicate measurements was 1.5%. The interaction of CGA with DNA at a Gr-GME was also studied by CV. As a result, the peak current decreased and the peak potential shifted to the positive side after adding DNA into the solution containing CGA. We deduced that the interaction of CGA with DNA mainly is intercalation. *Hyperchromicity* and small red shifts were observed in its *Ultraviolet-Visible* (UV-Vis) *spectra* upon addition of *DNA. It* can be concluded that there existed intercalation and electrostatic interactions.

Keywords: Graphene, Chlorogenic acid, DNA, Interaction, UV-VIS spectroscopy

1. INTRODUCTION

Chlorogenic acid, [1, 3, 4, 5-tetrahydroxycyclohexane car-boxylic acid 3-(3,4-dihydroxycinamate)] (CGA), is natural chemical compound which is the ester of caffeic acid and quinic acid. CGA is widely distributed in different materials including many foods and drinks. It has been shown that CGA has a broad range of physiological and pharmacological activities. *For example*, it can help reduce blood pressure and weight [1-4]. CGA, which is a food additive, is used in coffee

products, chewing gum, and mints. Another study showed that CGA has a protective effect in neuroinflammatory conditions on dopaminergic neurons [5].

Many analytical techniques have been applied in the determination of CGA, such as high-performance liquid chromatography (HPLC) [6-10], capillary zone electrophoresis [11], near-infrared spectroscopy [12-14], liquid chromatography-mass spectrometry [15, 16], chemiluminescence [17, 18]. Among these reported methods, some methods require an expensive apparatus, large mounts of the organic solvents, and much time. Electrochemical methods are convenient, rapid, sensitive, and adequately reproducible [19-25]. Many modified electrodes with modifiers such as bean sprout homogenate and chitosan microspheres composite [26], horseradish peroxidase, DNA and silicaetitanium composite [27], tetranuclear copper(II) complex [28]，ionic liquid containing iridium nanoparticles and polyphenol oxidase [29], poly aminosulfonic acid[30], platinum nanoparticles, reduced graphene oxide and laccase[31], Vinyltrimethoxysilane, multi-walled carbon[32], have been reported. However, most of these electrodes are not so convenient due to the requirement of large time. Most methods are narrow of the linear range and not high enough of the sensitivity.

Graphene is a new form of two-dimensional carbon nano materials with high hardness and strong toughness. Its unique nanostructures, good physical and chemical properties, make graphene use as electronics, electromagnetics, optics, medicine and energy storage materials and so on. It is considered as the "revolutionary material". Because of the remarkable electronic, graphene has been used as a special material to fabricate sensor [33, 34].

As is known to all, *DNA* clearly *plays important roles* in the human body and is one of the most significant discoveries of the twentieth century. DNA is also a major target for drugs and some harmful chemicals. It has become an active research area at the interface between chemistry, molecular biology and *medicine*. The interaction of DNA with small molecule medicines was studied. It can provide instructive information for understanding how *drug molecules interact* with *DNA*. Therefore, the interaction of DNA with small molecule medicines has become an active research over the past few years. However, the behavior of CGA and its interaction with DNA at a graphene modified electrode have not been reported so far.

In this work, the Gr-GME was prepared. The electrochemical behavior of CGA was studied. A new method of electrochemical detection of CGA was established. The interaction of CGA and DNA was studied at a Gr-GME by CV and its results were verified by UV-Vis spectroscopy. Based on the results of this study, we concluded that there exited intercalation and electrostatic interactions.

2. EXPERIMENTAL

2.1 Apparatus and materials

Electrochemical Workstation (CHI 660D)((Shanghai CH Instrument Company, Shanghai, China)was used for electrochemical analysis, with a three-electrode system consisting of a Gr-GME as working electrode, an SCE as reference electrode and platinum as counter electrode. A Model-78HW-*1* digital constant temperature magnetic stirrer was used for stirring *(*Jintan *Medical* Instrument

Factory, Jiangsu, China); A TU-1810 UV-Vis spectrophotometers （Beijing Purkinje General Instrument Co., Ltd, China）and MT infrared lamp (Guangzhou, China) were used and a KQ-100 ultrasonic cleaner (Kunshan, China).

CGA was purchased from Shanghai Fortune bio-tech Co., Ltd. (Shanghai, China), A 8.00×10^{-3} mol/L stock standard solution of CGA was stored in the dark at a temperature below 10°C; Buffer solution (PBS) was obtained by mixing the stock solutions of 0.20 mol L^{-1} sodium phosphate dibasic (Na_2HPO_4) (Guangdong *Xilong* Chemical Co., Ltd., Guangdong China) and 0.10 mol L^{-1} citric acid (Tianjin Kemiou Chemical Reagent Co., Ltd., Tianjin ,China)(pH 2.2~8.0), calf thymus DNA (*Shanghai Yuanye* Bio-Technology Co., Ltd, *Shanghai*, China). Synthesis of graphene from graphite powder was reported in our previously work [34]. 5 mg graphene powder was dispersed in 10 mL water after sonication to prepare a suspension solution of 0.5 mg mL^{-1}. All chemicals were of analytical reagent grade. All solutions were prepared from distilled water.

2.2 Preparation of Gr-GCE

First, a glassy carbon electrode, with diameter area of 3.8mm, was polished in wet suede, and then polished with alumina paste, and sonicated in nitric acid (1:1,V/V), ethanol and water for 10 min, respectively. Next, it was dried in air. The Gr-GCE was prepared by casting 5 μL of 0.5mg mL^{-1} graphene suspension on the electrode surface and dried under an infrared lamp.

2.3 Experimental method

2.3.1 Cyclic Voltammetry

A certain amount of PBS (pH=3.0) containing a specific amount of CGA solution or mixture of CGA with DNA solution was added into a cell. After stirring for 40s, the voltammograms were recorded at a Cr-GME modified electrode in the potential range from -0.4 V to 1.4 V by using CV. Scan rate was 120 mV s^{-1}. The modified electrode was placed in PBS (pH=3.0) and scanned until no peak for reuse.

2.3.2 UV-VIS method

5.00 mL of PBS (pH=3.0) , 5.00 mL of 1.00×10^{-4} mol L^{-1} solution of CGA and 0.10 mL 0.20 mg mL^{-1} of calf thymus DNA solution were added into a 25 mL volumetric flask, and than diluted to the scale with distilled water. The mixed solution was stored in the refrigerator at a temperature below 10 °C for one night and the UV -*VIS* spectra were recorded using a UV Spectrophotometer with 1cm path length quartz cuvettes in the wavelength range of 200-800 nm.

3. RESULTS AND DISCUSSION

3.1 Electrochemical properties of CGA at the Gr-GCE

The electrochemical propertiy of CGA at a bare GCE (Figure 1, a) and a Gr-GCE (Figure 1, c) were investigated by CV. The electrochemical behavior of a Gr-GCE was also investigated (Figure 1, b). The results showed that no redox peaks were observed in absent of CGA， but the value of the redox peak currents of CGA at the Gr-GCE (i_{pa}=-67.47 μA, i_{pc}=62.49 μA) were obviously increased in contrast to the poor response at the GCE (i_{pa}=-2.79 μA, i_{pc}=6.12 μA), which suggested that the Gr-GCE film on the electrode has a high electrocatalytic activity toward the redox of CGA and the electron transfer in the film is much faster duing to the unique structure of graphene. Graphene has stability because of its tightly packed carbon atoms and sp^2 orbital hybridization. The final p_z electron makes up the π-bond. The results, i_{pa}/i_{pc}=1.08≈1 and $\Delta E = E_{pa}-E_{pc}$ = 0.21V, suggested that the redox at the Gr-GCE performed a quasi-reversible process.

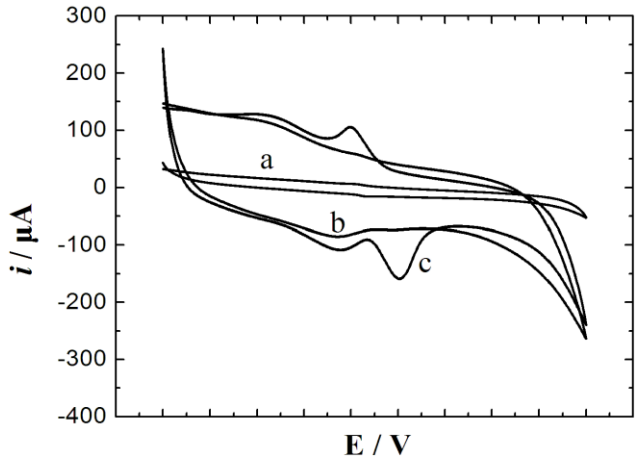

Figure 1. Cyclic voltammograms (CVs) of 5.00×10^{-5} mol L^{-1} CGA at a bare GCE (a) and a Gr-GCE(c); PBS at a CVs of Gr-GCE in pH 3.0 PBS (b).

3.2 Optimization of conditions for CGA detection

3.2.1 The Choice of Graphene Volume

The amount of the surface of graphene on a glassy carbon electrode has a larger effect on the peak current of the CGA, so we investigated the effect of the volume of graphene. We varied the volume of 0.5 mg mL^{-1} graphene from 1 to 5 μL, the oxidation current peak of CGA increased, and then decreased while the amount of graphene suspension exceeded 5 μL. However, the thickness of graphene on the GCE cannot be utilized effectively and also catalytic substrates have been hampered to spread to the electrode surface. Thus, 5 μL of a 0.5 mg mL^{-1} graphene suspension was used to in our experiments.

3.2.2 Effect of medium's pH

The medium's pH has a larger effect on the peak current of the CGA. We studied the effect of the medium's pH on the electrochemical signal in our work. A variety of pH supporting electrolyte (pH2.2~8.0) were investigated, respectively (Fig. 2). The oxidation peak current of CGA reached its maximum peak value in the pH=3.0 PBS, and then decreased while medium's pH exceeded 3.0. Thus, pH=3.0 PBS was chosen as the supporting electrolyte.

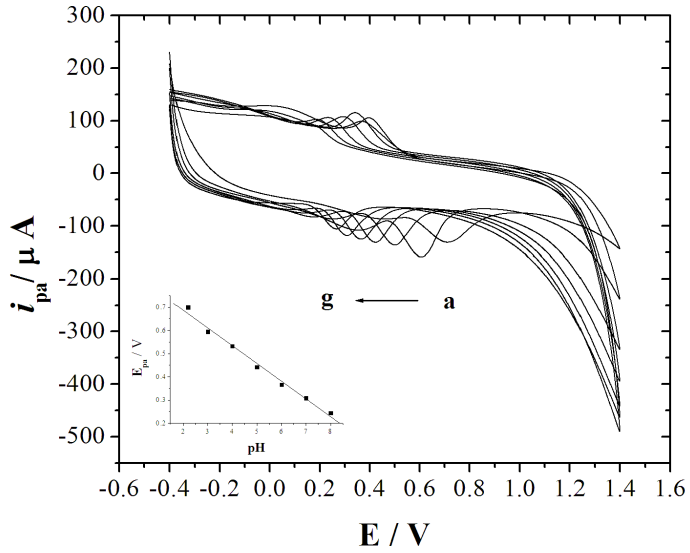

Figure 2. CVs of 5.00×10^{-5}mol L^{-1} CGA at a Gr-GCE at different pH (from a to g): 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0, respectively. Inset is the plot of oxidation peak currents versus pH.

It was found that peak potential shifted negatively with the increase of solution pH (see Figure 2), indicating that the oxidation of CGA at the Cr-GCE is a pH-dependent reaction, which was similar to that reported in the literature [30, 35]. We can described the relationship between the oxidation peak potential (E_{pa}) and the solution pH (over a pH range between 2.2 and 8.0) by the following equation (Fig. 2, Insert) of *Epa* = 0.76 - 0.065 pH, with a correlation coefficient of R = 0.9972. The slope of -0.065 was obtained, which was close to the theoretical value of -0.059, demonstrating that the numbers of electron and proton taking part in the electrode reaction is equal [36].

3.2.3 Effect of electrode potential

We study the effect of *high* or low electrode potential for CGA under the experimental conditions within the potential range -0.8 V to +1.8V. It was found that the oxidation peak current of CGA reached its maximum peak value at -0.4V and +1.4v respectively. Hence, the potential range -0.4 to 1.4V was used throughout the study.

3.2.4 Effect of scan rate

We investigated the effects of scan rates on the oxidation peak current of CGA. The redox peak currents (i_{pa} and i_{pc}) of CGA increased linearly with the scan rate (v) at scan rate values: i_{pa}=-1.12×10^{-5}-6.22×10^{-7}ν (mV s^{-1}), R=0.9929; i_{pc}=1.10×10^{-5}+5.91×10^{-7}ν (mV s^{-1}), R=0.9916, respectively, which suggested that the electrode reaction was controlled by the adsorption process. This was different from the electrode process of CGA on poly(aminosulfonic acid) modified glassy carbon electrode [30]. It was also found that the limit of detection became lower when the scan rate is higher than120 mV s^{-1} than other peaks. Therefore, 120 mV s^{-1} was used as the scan rate in our work.

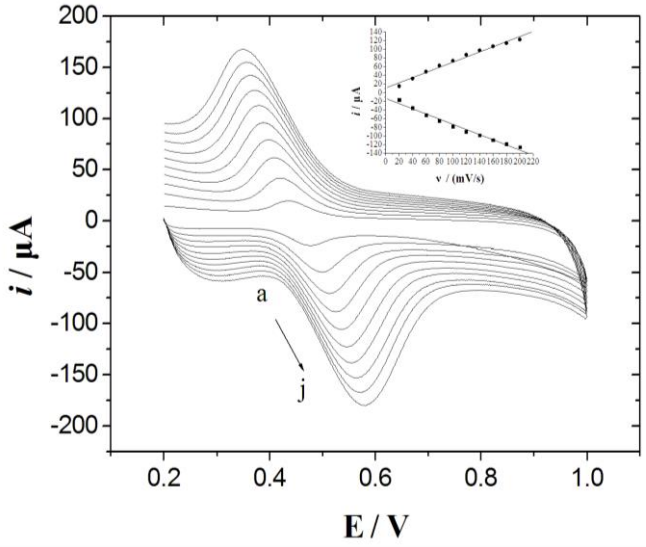

Figure 3. CVs of 5.00×10^{-5}mol L^{-1} CGA at a Gr-GCE at different scan rate. Each of the numbers from a to j corresponds to a scan rate of 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mV s^{-1}, respectively. Inset is the plot of redox peak currents of CGA versus scan rates.

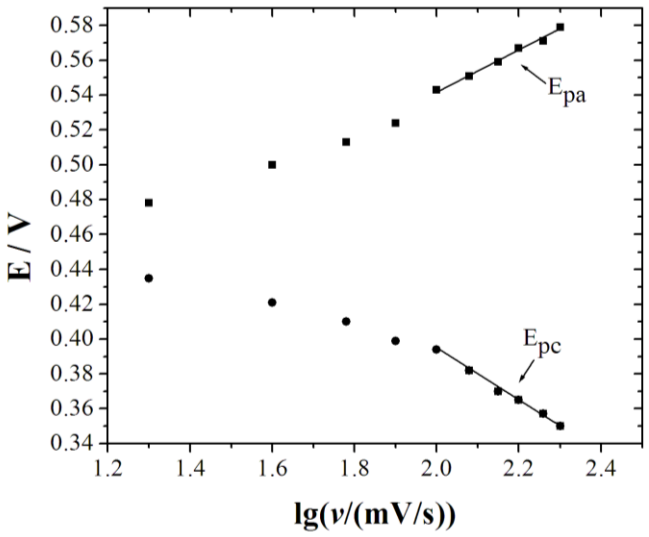

Figure 4. Plots of the peak potentials of 5.00×10^{-5} mol L^{-1} CGA at a Gr-GCE versus the logarithms of the scan rates.

Figure 4 showed the plots of the peak potentials versus the logarithms of the scan rates. The linear regression equations of the E_{pa} and E_{pc} versus the scan rates were expressed as: E_{pa}=0.30+0.12lg$v$ (mV s^{-1}), R=0.9922 (1); E_{pc}=0.67-0.14 lg$v$ (mV s^{-1}), R=-0.9966 (2). Laviron's equation [37] can express by the following equation: $E_{pa}=a + (2.303RT/(1-\alpha)n_{\alpha}F)$ lg$v$ (mV s^{-1}); $E_{pc}=b - (2.303RT/\alpha n_{\alpha}F)$ lg$v$ (mV s^{-1}), where a and b are all constant, F the Faraday constant (96,487 C), R the universal gas constant (8.314 J K^{-1} mol^{-1}), T the Kelvin temperature (K), n the number of electron transferred and α the electron transfer coefficient.

By combining equations 1 and 2, we obtained n_{α} = 0.92 and α=0.46. Thus the electron transfer number was 1. It can be seen that the value of α was quite close to its theoretical value, indicating that the redox at the the Gr-GCE performed a quasi-reversible process. According to the literature [36], dEp/dpH =2.303mRT/nF, the number of proton transferred (m) can be calculated was 1, which was consistent with literature [38, 39].

3.2.5 Influence of accumulation time

We studied the effect of accumulation time on the analytical signal. The accumulation time was examined in the range 20 to100 s. Experimental results showed that the current increased with accumulation time till 40 s and it remained constant beyond 40 s. Therefore, this accumulation time was selected in our experiment.

3.3 Linear range and the detection limit

Under optimum conditions, the CV technique was performed to investigate the determination of CGA by the Gr-GCE. Figure 5 shows the CVs of different concentrations of CGA on the Gr-GCE in pH 3.0 PBS. The peak current of oxidation was proportional to the concentration of CGA and the linear range was from 3.20×10^{-7} to 4.50×10^{-5} mol L^{-1}. The regression equation is i_{pa}=9.62×10^{-6}+1.45c （mol L^{-1}） with R=0.9987. Detection limit is estimated by gradually decreasing the concentration levels of CGA and the detection limit was estimated to be about 8.00×10^{-9} mol L^{-1}.

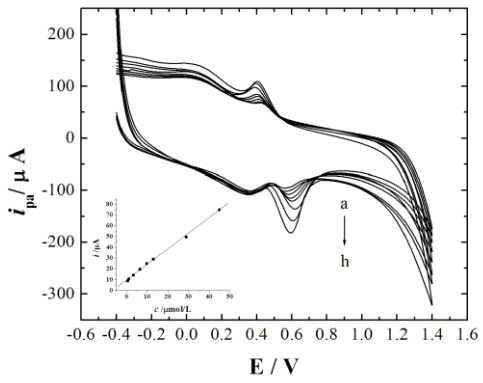

Figure 5. CVs of CGA at the Gr-GCE at different concentrations. Each of the letters from a to h corresponds to a concentration of: 0.32, 0.96, 3.20, 6.40, 9.60, 13.00, 29.00, and 45.00 respectively (in μmol L^{-1}). Inset is the plot of the oxidation peak current versus concentration of CGA Scan rate: 120 mV s^{-1}.

3.4. Comparison of the proposed method with those reported in the literature

The comparison of this developed method and those of previously reported electrochemical methods is shown in Table 1. These results indicated that Gr-GCE was an appropriate platform for the determination of CGA. This method has a fairly large linear range and high sensitivity.

Table 1. Comparison of different electrodes for chlorogenic acid determination.

Modifiers	Linear range (μmol/L)	Detection limit (μmol/L)	References
Tetranuclear copper(II) complex	5.0~145	0.8	26
Bean sprout homogenate and silica composite	4.89~48.5	0.852	27
An ionic liquid containing iridium nanoparticles and polyphenol oxidase	3.48~49.5	0.915	28
HRP, DNA and silicaetitanium composite	1.0~50	0.7	29
Poly aminosulfonic acid	0.4~12	0.08	30
Platinum nanoparticles, reduced graphene oxide and laccase	2.91~26.47	2.67	31
Vinyltrimethoxysilane, multi-walled carbon nanotubes and a molecularly imprinted siloxane film	0.08~500	0.032	32
Glasscarbon electrode	5~50	1.2	35
Graphene	0.32~45	0.008	This work

3.5. Reproducibility of Gr-GCE and interference studies

Under the optimal conditions, the reproducibility of the Gr-GCE was tested by detecting the CV response of 5.00×10^{-5}mol L^{-1} CGA in PBS (pH = 3.0) with the same electrode six times. The result shows that the RSD of the detection was 1.5%, which indicated that the modified electrode had excellent reproducibility.

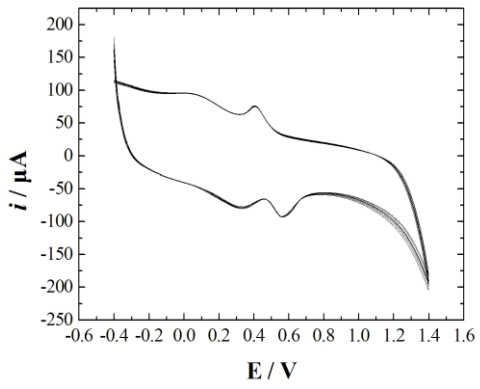

Figure 6. CVs of 5.00×10^{-5} mol L^{-1} CGA with the same electrode six times

The influence of some possible interference was investigated in PBS (pH = 3.0) and the results showed that about 300-fold sucrose, KCl and NaCl; about 50-fold Cu^{2+}, Mg^{2+}, thymol, rutin, quercetin did not significantly influence the peak currents at the Gr-GCE under the selected experiment conditions.

3.6. Recovery experiment

3.6.1 Sample preparation

Two products of different samples including *honeysuckle* and Qingkailing Injection were chosen. They were purchased from the local drug store.

Sample solution of *honeysuckle*: The *honeysuckle* product *was* crushed into powder by a mill. Five grams of the *honeysuckle* powder was weighted accurately to a flask, and then 40 mL absolute alcohol was added. The contents in the flask were heated to boiling and adjusted the pH value of solution with hydrochloric acid. The extraction solution was filtered. *Filter residue* was putted into a flask, and then 40 mL absolute alcohol was added with the same procedure. All the extraction solution was collected and then was diluted to 100 mL with absolute alcohol and stored at 5°C to be used.

Qingkailing Injection： Ten kind of *Qingkailing injection* samples from *different batches* were mixed together without treatment and stored at 5°C to be used.

3.6.2 Application

In order to check whether the given condition is appropriate, the sensor was applied to analyze the amount of CGA in *honeysuckle* and Qingkailing injection samples respectively. These analyses were performed by the standard addition method and the results obtained were shown in table 2. The experimental results show that the proposed method(a) and high performance liquid chromatography method(b) for the determination of real samples with a high degree of consistency, proved the accuracy of the method.

Table 2. Determination results of CGA in pharmaceutical products (n=6).

Sample	Added ($\times10^{-6}$mol/L)	Found ($\times10^{-6}$mol/L)	Recovery (%)	RSD (%)
Honeysuckle	-	3.78^{a},3.84^{b}	-	-
	1.00	4.76^{a},4.81^{b}	98.0^{a}, 97.0^{b}	2.3^{a}, 3.5^{b}
	2.00	5.79^{a},5.83^{b}	100.5^{a},99.5^{b}	2.6^{a},2.4^{b}
	4.00	7.89^{a},7.77^{b}	102.8^{a},98.2^{b}	3.1^{a},3.3^{b}
Qingkailing injection	-	2.42^{a},2.47^{b}	-	-
	1.50	3.95^{a} ,4.01^{b}	102.0^{a},102.6^{b}	2.5^{a},2.9^{b}
	2.50	4.89^{a},4.90^{b}	98.8^{a}, 97.2^{b}	3.3^{a},3.1^{b}
	3.50	5.83^{a},5.90^{b}	97.4^{a}, 98.0^{b}	2.6^{a} ,3.0^{b}

a: results obtained by the proposed method; b: results obtained by HPLC method.

4. CGA INTERACTION WITH DNA ON THE GR-GCE

The CVs of 5.0×10^{-5} mol L^{-1} CGA in the presence of different concentrations of DNA were recorded on the Gr-GCE in pH 3.0 PBS. As can be seen from Fig. 7, We could find that there were a couple of reversible redox peaks for CGA (curve a)，both the cathodic and anodic peak currents of CGA decreased significantly and its peak potential positively shifted with increasing the concentration of DNA, indicating that the interaction of CGA with DNA led to form a new electrochemically active complex at the electrode. Among the three kinds of binding modes for small molecules to DNA, if the peak positively shifted to more negative value when small molecules interacted with DNA, the interaction mode was electronic binding. On the contrary, if the peak positively shifted to more positive value, the interaction mode was intercalative binding [40, 41]. According to above results, we deduced that the interaction of CGA with DNA is mainly via intercalation mode [42].

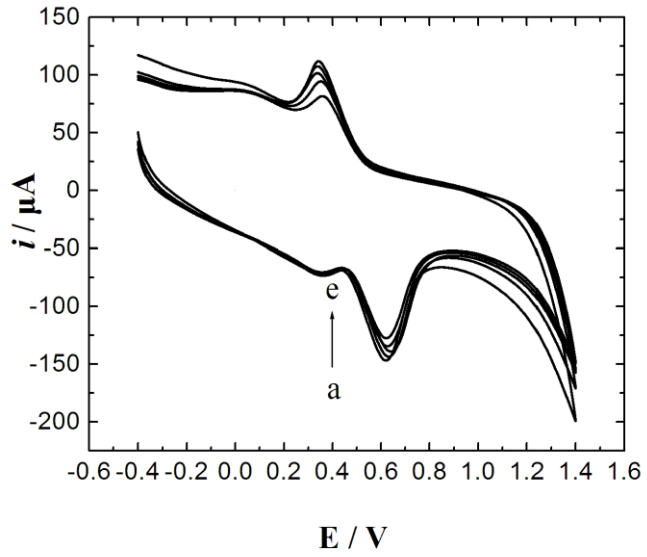

Figure 7. CVs of CGA containing *different concentrations of DNA* at the Gr-GCE. Each of the letters from a to e corresponds to a concentration of DNA: 0.00, 0.002, 0.004, 0.006 and 0.008, respectively (in mg mL^{-1}).

To provide further evidence about the interaction between CGA and DNA, *UV-VIS* Spectra was employed to study the interaction in this work. We deduced there were some changes in absorbance (hypochromism) and the position of the band would red shift when a small molecule interacts with DNA. The possible reasons to explain why the spectral have some effects may be that [43, 44] it can cause an energy decrease, and a decrease of the π→π* transition energy when the empty π* -orbital of the small molecule couples with the π-orbital of the DNA base pairs. As a result, the absorption band of the small-molecule should exhibit red-shifted absorption band. Furthermore, electrons have partially filled the empty π* -orbital, reducing electron transition probability, and this leads to hypochromism.

Figure 8 showed the *UV-VIS* Spectra for DNA (a), CGA (b) and CGA–DNA complexes(c). It can be seen that CGA produced two absorption peaks at 240 and 324 nm respectively. The absorption

peak at 324 nm decreased with the addition of DNA, which was hypochromic effect, confirming interaction between CGA and DNA. The results indicated that the CGA molecule intercalated into the base-pairs of DNA, and the complex of CGA–DNA was formed. However, when DNA was added to CGA solution, the absorption peak at 240 nm increased and a small red shifted, that was hyperchromic effect, confirming electrostatic interaction between CGA and DNA [45, 46]. Our study showed that there exited intercalation and electrostatic interactions.

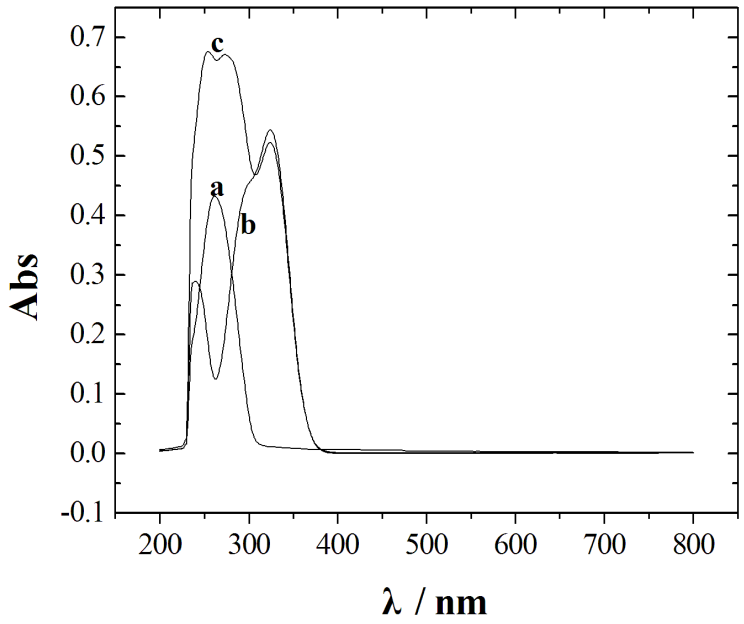

Figure 8. UV-Vis spectra of the mixture of (a) DNA; (b) CGA; and (c) the mixture of CGA and DNA

5. CONCLUSIONS

The Gr-GCE was prepared and the electrochemical behavior of CGA was studied. It was found that this sensor has high sensitivity and selectivity as well as a wide linear range. The electrochemical methods using Gr-GCE have been widely *applied to the* detection *of* samples, exhibiting *good reproducibility and* high yields.

The interaction of CGA with DNA had been studied in CV *method*. The peak current of CGA decreased significantly and the peak potential positively shifted, indicating the interaction of CGA with DNA is intercalation. Similar result was confirmed by *UV-VIS method*. At the same time, it was found that the interaction of the CGA and DNA were intercalation and electrostatic interactions.

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