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Original Article

Voltammetric determination of chlorogenic acid in pharmaceutical products using poly(aminosulfonic acid) modified glassy carbon electrode

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ABSTRACT

In this work, a poly(aminosulfonic acid) modified glassy carbon electrode was fabricated and the electrochemical behavior of chlorogenic acid (CGA) was studied by cyclic voltammetry. Compared with a bare glassy carbon electrode, the modified electrode exhibits excellent catalytic effect on the electrochemical redox of CGA. Utilizing this catalytic effect, a sensitive and selective electrochemical method for the determination of CGA was developed. The analytical parameters were optimized. Under the optimized conditions, the oxidation peak current is linearly proportional to the concentration of CGA in the range from 4.00×10^{-7} to 1.20×10^{-5} mol/L and the detection limit is 4.00×10^{-8} mol/L. Further, the performance of the proposed method has been validated in terms of linearity ($r = 0.9995$), recovery (96.3–102.8%), reproducibility (RSD < 4.0%, $n = 6$) and robustness. The developed method has been successfully applied for the determination of CGA in a variety of pharmaceutical products.

1. Introduction

Cholorogenic acid (CGA), an ester of caffeic acid and quinic acid, is a naturally occurring phenolic compound with a structure as shown in Fig. 1 [1]. CGA is a potent antioxidant found in many foods and drinks, most notably in coffee. Clinical investigations have implied that consumption of CGA can have anti-hypertension and anti-obesity effects [2,3]. CGA also can serve as anti-inflammatory, antitumor, antimutagenic and anticarcinogenic agent [4,5]. As a bioactive compound that has many therapeutic effects, CGA can be found in more than 170 kinds of traditional Chinese pharmaceutical products such as tablets, capsules, and herbal injections. Therefore, fast and convenient determination of CGA in pharmaceutical products is of great importance.

Various methods for the determination of CGA have been developed, namely, near-infrared spectroscopy [6–8], capillary electrophoresis [9,10], nano-liquid chromatography-electrospray ionization mass spectrometry [11], high-performance liquid chromatography [12–18], ultra-performance liquid chromatography [19], liquid chromatography-mass spectrometry [20,21], chemiluminescence [22,23], and electrochemical

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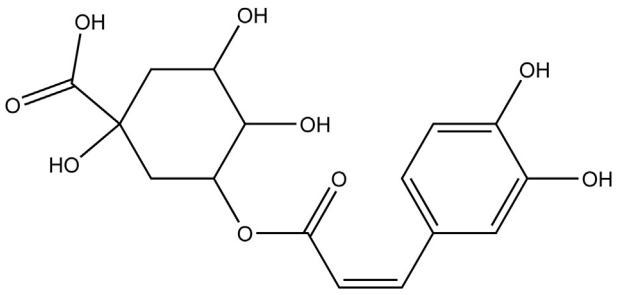


Fig. 1 – Molecular structure of chlorogenic acid.

methods [24–27]. Among these reported methods, near-infrared spectroscopy requires expensive equipment and resource-intensive calibrations. Capillary electrophoretic analyses are not very sensitive. Chromatographic methods require expensive equipment, large amounts of organic solvents, and are time-consuming. Electrochemical methods are obviously better due to their convenience, speed, higher sensitivity, and reproducibility [28–34]. For electrochemical determination of CGA, modified electrodes with modifiers such as ionic liquid containing iridium nanoparticles and polyphenol oxidase [25], horseradish peroxidase, DNA and silica–titanium composite [26], bean sprout homogenate and chitosan microspheres composite [27], and tetranuclear copper(II) complex [24] have been reported.

In recent years, electrically conductive polymers have been used as electrode modifiers due to their unique properties such as strong adherence to the electrode surface, more active sites and good chemical stability [35,36]. When fabricating polymer modified electrodes, polymer films are often electropolymerized to the surface of an electrode [37,38].

In the present work, a poly(aminosulfonic acid) modified glassy carbon electrode (PASA/GCE) for the determination of CGA was fabricated through electropolymerization and the fabricated electrode was characterized by scanning electron microscopy (SEM) and voltammetry. The electrochemical behavior of CGA at the PASA/GCE was investigated in detail by cyclic voltammetry and the polymer film showed an excellent electrocatalytical effect on the redox of CGA. Further, a novel voltammetric method for the determination of CGA in pharmaceutical products was established and validated. The fabrication conditions of the PASA/GCE and the analytical parameters were optimized. The results of CGA determination obtained using the PASA/GCE were compared with those obtained by other electrochemical methods reported in the literature.

2. Experimental methods

2.1. Chemicals

All chemicals were of A.R. grade and were used as received without any further purification. CGA was purchased from Shanghai Fortune bio-tech Co., Ltd. (Shanghai, China). Aminosulfonic acid and all other chemical reagents were obtained from Beijing Chemical Reagent Company (Beijing, China). A 8.0×10^{-3} mol/L CGA stock solution was prepared by dissolving 0.147 g of CGA in 50.00 mL of absolute ethanol. Phosphate buffers (pH = 2.2 ~ 8.0) were prepared by mixing the stock solutions of 0.2 mol/L Na_2HPO_4 and 0.1 mol/L citric acid. All aqueous solutions were prepared using double-distilled water.

2.2. Apparatus

A CHI 660C Electrochemical Workstation (Chen-hua, Shanghai, China) was used for voltammetric measurements. A three-electrode system was employed with a bare GCE or PASA/GCE (3.8 mm in diameter) as the working electrode, a platinum wire electrode as the counter electrode, and an Ag/AgCl electrode as reference electrode. A field emission SEM Sirion 200 (FEI, Hillsboro, Oregon, USA) was used for recording the SEM image of the polymer film at the surface of the modified electrode. Acidity was measured using a PHS-3B Precision pH meter (Shanghai, China) and sonication was performed using a KQ-100 ultrasonic cleaner (Kunshan, China).

2.3. Fabrication of the PASA/GCE

The polymerization solution was prepared by mixing 10.00 mL of pH 8.0 phosphate buffer, 0.50 mL of 8.0×10^{-3} mol/L aminosulfonic acid solution and 9.50 mL of double-distilled water. The GCE was pretreated by polishing its surface successively with an abrasive paper (grit 1000) and an aqueous slurry of alumina powders (0.05 μm) on a polishing cloth, and then rinsed successively with 1:1 HNO_3, absolute ethanol, and distilled water to give a smooth and clean electrode surface. Then, the electrode was ultrasonicated in distilled water for about 30 seconds, and finally allowed to dry under an infrared lamp. Using the pretreated GCE as working electrode, an Ag/AgCl electrode as the reference electrode and a platinum electrode as the counter electrode, PASA/GCE was prepared by cycling the potential between −1.0 and 2.4 V for 12 cycles in the polymerization solution (prepared as described) at a scan rate of 120 mV/second. After polymerization, the modified electrode was rinsed with double-distilled water and dried in air at room temperature to give a PASA/GCE.

2.4. Sample preparation

2.4.1. Qingkailing injection and Mailuoning injection samples

Ten vials of each injection from different batches were mixed and analyzed directly without any pretreatment.

2.4.2. Honeysuckle samples

The traditional Chinese herbal medicine honeysuckle was carefully ground to a fine powder and sieved through a 600-mesh screen, then 5.0 g of the powder was extracted with 30 mL of ethanol for 30 minutes with ultrasonic agitation. The resulting mixture was filtered and the residue was similarly extracted twice. All filtrates were transferred into a 100 mL volumetric flask and diluted to scale with ethanol.

2.5. Electrochemical measurement

Cyclic voltammetry measurements were made in an unstirred, nondeaerated pH 4.0 phosphate buffer and all potentials

were measured and reported versus Ag/AgCl. In a typical run, 10 mL of pH 4.0 phosphate buffer, 10 mL of ethanol/water (v:v = 9:10) and 0.025 mL of CGA sample solution were transferred into the electrolytic cell. Accumulation was firstly performed under open-circuit with stirring for 30 seconds. Then, cyclic voltammograms (CVs) were recorded between 0.0 and 1.0 V at 120 mV/second. Upon completion of each scan, the PASA/GCE was placed in a blank supporting electrolyte and the cyclic scan was continued until there were no further peaks, then the electrode was washed with double-distilled water and dried in air for re-use.

3. Results and discussion

3.1. *Fabrication of PASA/GCE*

Cyclic voltammetry was used to fabricate the PASA/GCE. To fabricate an electrode that gives maximum response to CGA, polymerization conditions, such as pH, potential window, monomer concentration, number of scan cycles and scan rate were optimized.

When optimizing polymerization pH, a series of PASA/GCEs were fabricated in phosphate buffers of pH from 2.2 to 8.0 and cyclic voltammograms of 2.0×10^{-5} mol/L CGA were recorded at these electrodes. It was found that the PASA/GCE fabricated in phosphate buffer of pH 8.0 gave the highest oxidation peak current. Thereby, a phosphate buffer of pH 8.0 was chosen as the supporting electrolyte for the fabrication. During the experiments, we found that it is difficult for aminosulfonic acid to form a polymer film at the surface of the GCE in acidic buffer, while in neutral or weak basic buffer, the polymer film can be easily formed. This may be because that aminosulfonic acid mainly exists in its deprotonated state (SO_3^-) in neutral or weak basic buffer, which is conducive to its interaction with amino group radical cation to make polymerization occur.

The influence of the potential window used in electrode fabrication on its electrocatalytic activity to CGA was also studied. Modified electrodes were fabricated in different potential ranges and cyclic voltammograms of 2.0×10^{-5} mol/L CGA were recorded using these fabricated electrodes. Firstly, the upper potentials were varied from 1.2 to 2.6 V during electropolymerization while fixing the lower potential at −1.0 V. Oxidation peak current of CGA at the fabricated PASA/GCE first increased with the increase of polymerization terminal potential and reached maximum at 2.4 V, then decreased when the polymerization terminal potential continued to increase. Therefore, 2.4 V was chosen as the optimum terminal potential. Similarly, −1.0 V was obtained as the optimum lower potential. As a result, a potential range of −1.0 to 2.4 V was chosen for the polymerization. During the experiments, it is found that the PASA film cannot be formed when the positive potential is lower than +1.4 V, whereas a positive potential beyond +2.4 V leads to a deterioration of PASA film; the reason for this may be that free radicals cannot be formed at a lower potential, where a potential that is too high can cause over-oxidation of CGA.

The monomer concentration, number of scan cycles and scan rate were also optimized and the best response was obtained in the presence of 2.0×10^{-4} mol/L aminosulfonic acid for 12 cyclic scans at a scan rate of 120 mV/second.

Fig. 2 shows the repetitive cyclic voltammograms of 2.0×10^{-4} mol/L aminosulfonic acid at a GCE under the above optimized polymerization conditions. Two anodic peaks and one cathodic peak appeared. Peak currents for all three peaks became larger with the increase in scan numbers, reflecting the continuous growth of the film. However, the increase in peak current amplitude became smaller, indicating that the film growth rate became slower as the film tends to become complete. When the modification is completed, a uniform adherent blue polymer film was formed on the GCE surface. Fig. 3 shows the SEM image of the PASA/GCE. From this figure we can see that PASA immobilized on the GCE surface was assembled into a fiber-like structure, indicating that aminosulfonic acid has been successfully polymerized onto the GCE. A possible mechanism for the polymerization may be as follows, aminosulfonic acid monomer is firstly oxidized to its corresponding free radical, which is then covalently linked to the electrode surface. Aminosulfonic acid has a pK_a of 0.99 and exists in its deprotonated state (SO_3^-) in pH 7.0 phosphate buffer. The SO_3^- group interacts with oxidized amino group radical and polymerization occurs, resulting in a PASA film at the electrode surface. A possible structure of the immobilized polymer film is shown in Scheme 1.

3.2. *Electrochemical behavior of CGA at the PASA/GCE*

The electrochemical behavior of CGA at a bare GCE and a PASA/GCE were investigated by cyclic voltammetry. From Fig. 4 we can see that the intensity of the oxidation peak current at the PASA/GCE (Fig. 4, curve 4) was significantly increased in contrast to the response at the bare GCE (Fig. 4, curve 3). The oxidation peak potential negatively shifted from 430 mV to 360 mV and the oxidation peak current increased from −17.25 μA to −30.97 μA; The reduction peak potential positively shifted from 190 mV to 290 mV and the reduction peak current increased from 6.68 μA to 21.20 μA,

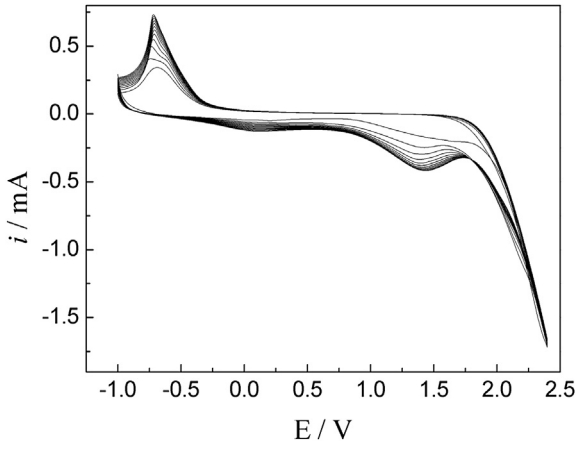


Fig. 2 – Cyclic voltammograms for electropolymerization of aminosulfonic acid on a glassy carbon electrode surface in pH 8.0 phosphate buffer containing 2.0×10^{-4} mol/L aminosulfonic acid. Scan rate: 120 mV/second.

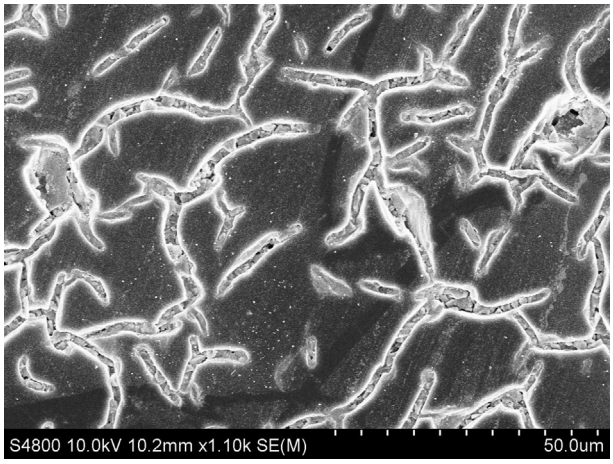


Fig. 3 – Scanning electron microscopy image of the poly(aminosulfonic acid) film on the glassy carbon electrode.

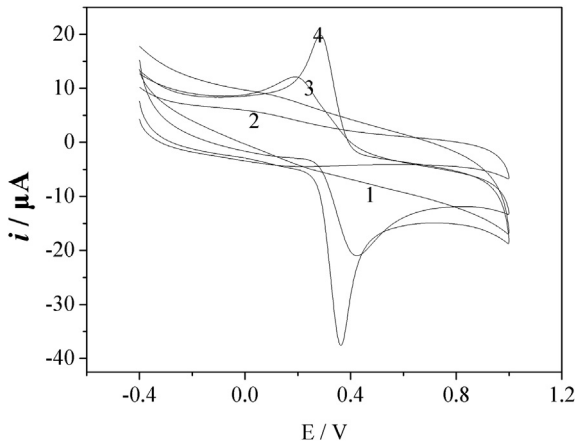


Fig. 4 – Cyclic voltammograms of bare glassy carbon electrode (GCE; curve 1) and the poly(aminosulfonic acid) modified glassy carbon electrode (PASA/GCE; curve 2) in pH 8.0 phosphate buffer and cyclic voltammograms of 1.00×10^{-5} mol/L CGA at the bare GCE (curve 3) and the PASA/GCE (curve 4) in pH 8.0 phosphate buffer. Scan rate: 120 mV/second.

suggesting that the poly(aminosulfonic acid) film imobilized on the electrode has a high electrocatalytic activity toward the redox of CGA and the electron transfer rate in the film is much faster. The electrocatalytic activity of the poly(-aminosulfonic acid) film towards CGA may be attributed to the formation of hydrogen bond between the hydroxyl group in CGA and the nitrogen atom in poly(aminosulfonic acid) film. The formation of a hydrogen bond can weaken the bond energy between hydrogen and oxygen and the electron transfer is liable to occur *via* the bond of N–H–O. At the PASA/GCE, $\Delta E = 70$ mV, $i_{pa}/i_{pc} > 1$, suggesting that the reaction process at the modified electrode is a quasi-reversible process.

Fig. 5 shows the cyclic voltammograms of 2.00×10^{-5} mol/L CGA at the PASA/GCE at various scan rates. The figure inset shows that the both the oxidation and the reduction peak currents are proportional to the square root of the scan rate and their linear regression equations can be expressed as: $i_{pa}(\mu A) = 20.82 - 11.57v^{1/2}$, $r = -0.9981$ (a) and $i_{pc}(\mu A) = -12.89 + 9.37v^{1/2}$, $r = 0.9974$ (b), respectively, indicating that the electrode process of CGA at the PASA/GCE is a diffusion controlled process.

Fig. 6 shows the correlation between the oxidation and reduction peak potentials and the logarithms of scan rates, log (v/mV/second). According to Laviron's theory describing quasi-reversible electrochemical reactions [39], variations in the peak potential values are a function of logarithms of scan rates and the following equations can be used to determine the electron transfer number and coefficient:

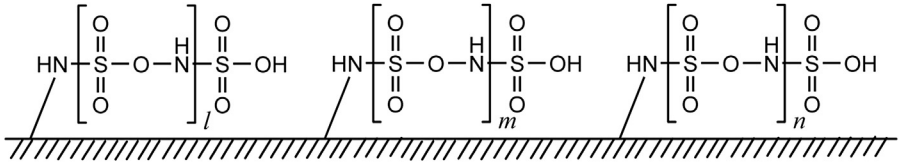
$$E_{pa} = a + (2.303RT/(1-\alpha)n_{\alpha}F)\lg\ v \quad (1)$$

$$E_{pc} = b - (2.303RT/\alpha n_{\alpha}F)\lg\ v \quad (2)$$

where a and b are constants.

From Fig. 5 we can see that with a pH 4.0 phosphate buffer and a scan rate range of 80–160 mV/second, $E_{pa} = 0.284 + 0.119\lg v$, $R = 0.9983$; $E_{pa} = 0.518 - 0.0998\lg v$, $R = 0.9985$. Based upon Equations (1) and (2), the calculated electron transfer number (n_{α}) and the electron transfer coefficient (α) are 1.1 and 0.54, respectively. The electron transfer coefficient is close to theoretical value 0.5, which further proved that the electrode process is quasi-reversible.

Fig. 7 shows the effect of the pH value of supporting electrolyte on CGA redox peak potential and peak current. In the pH range from 2.2 to 8.0, both the oxidation and reduction peak potentials shifted negatively with the increase of the pH of supporting electrolyte and E_{pa} shows a linear relationship with pH and the linear regression equation can be expressed as $E_{pa} = 0.87 - 0.083$ pH, $r = 0.9933$ (inset A of Fig. 7), which indicates the redox reaction of CGA at the PASA/CGA involves a proton. According to the following formula: [40] $dE_p/dpH = 2.303mRT/nF$, in which m is the proton transfer number and n is the electron transfer number, the proton transfer number is calculated to be 1.54.



Scheme 1 – A possible structure of the poly(aminosulfonic acid) film at the glassy carbon electrode.

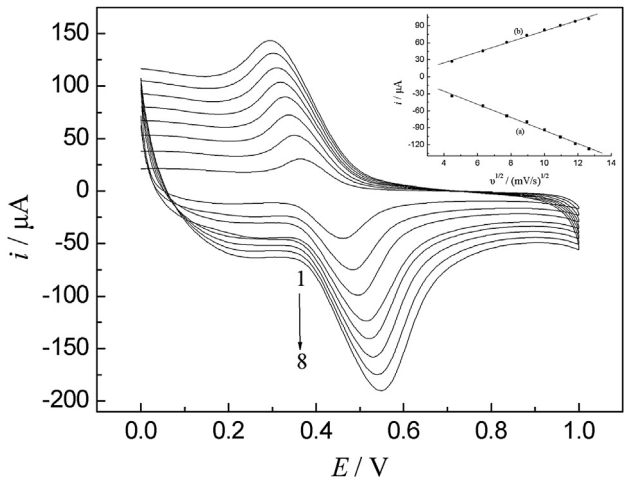


Fig. 5 – Cyclic voltammograms of 2.00 × 10^{-5} mol/L chlorogenic acid (CGA) at a poly(aminosulfonic acid) modified glassy carbon electrode. Each of the numbers from 1 to 8 correspond to a scan rate of 20, 40, 60, 80, 100, 120, 140 and 160 mV/second, respectively. The inset shows the plot of redox peak currents of CGA versus the square roots of scan rates.

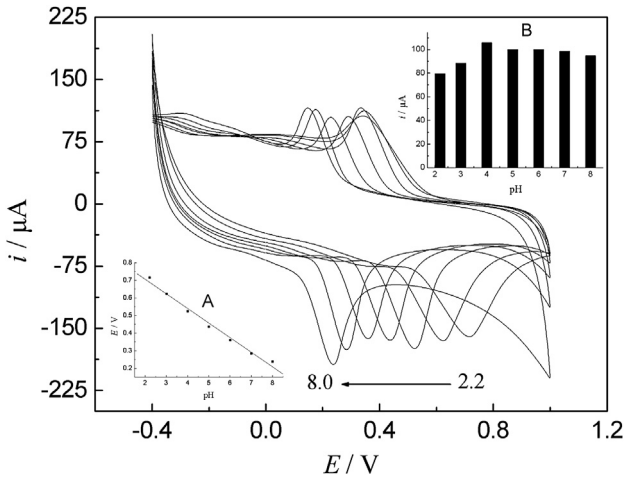


Fig. 7 – Cyclic voltammograms of 2.00 × 10^{-5} mol/L chlorogenic acid (CGA) at a poly(aminosulfonic acid) modified glassy carbon electrode in phosphate buffers of various pH. Scan rate: 120 mV/second. Each of the numbers from 1 to 7 corresponds to a pH of 2.2, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0, respectively. Inset A shows the plot of peak potential of CGA versus pH of supporting electrolyte. Inset B shows the variation of the oxidation peak response of CGA versus the pH of supporting electrolyte.

3.3. *Optimization of CGA determination parameters*

CGA determination parameters were optimized by investigating the effects of the scan rate, the pH of the supporting electrolyte, and the sample accumulation time. The peak current of CGA linearly increases with the scan rate and the scan rate of 120 mV/second gave the best peak shape (Fig. 5). In a pH range of 2.2–8.0, oxidation peak current firstly increases with increasing pH and reaches a maximum at pH 4.0, then decreases as pH continues to increase (inset B of Fig. 7). Due to the adsorption process of CGA at the PASA/GCE, the accumulation time was investigated to study its effect on the peak current. Lower, middle, and higher concentrations of CGA in the linear range was investigated by varying the accumulation time between 0 and 120 seconds. Results showed that the peak current increases as stirring time increases, and reaches its maximum value at 30 seconds and then stabilizes. Thereby, a scan rate of 120 mV/second, a phosphate buffer solution of pH 4.0 and an accumulation time of 30 seconds were chosen as the determination parameters in this study.

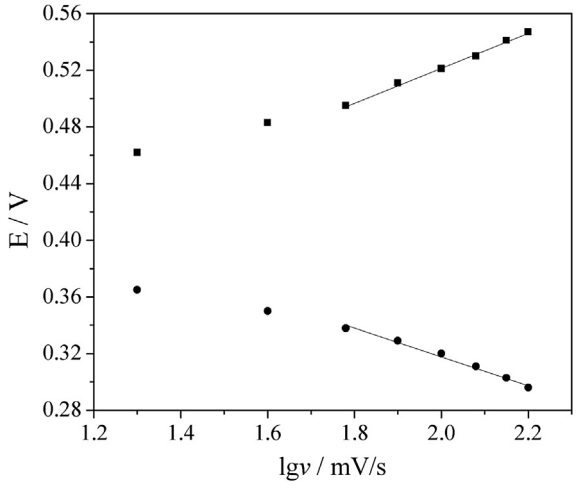


Fig. 6 – Plots of the peak potentials versus the logarithms of the scan rates.

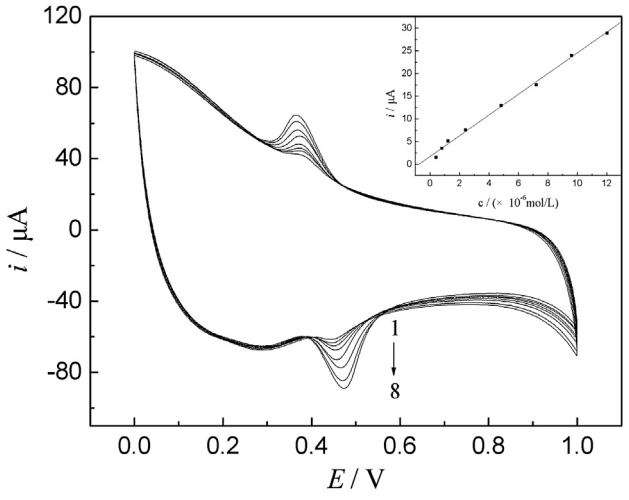


Fig. 8 – Cyclic voltammograms of chlorogenic acid (CGA) at various concentrations at a poly(aminosulfonic acid) modified glassy carbon electrode in pH 4.0 phosphate buffer. Each of the numbers from 1 to 8 corresponds to a concentration of 4.00 × 10^{-7}, 8.00 × 10^{-7}, 1.20 × 10^{-6}, 2.40 × 10^{-6}, 4.80 × 10^{-6}, 7.20 × 10^{-6}, 9.60 × 10^{-6}, and 1.20 × 10^{-5} mol/L, respectively. The inset shows the plot of oxidation peak current of CGA versus its concentration. Scan rate: 120 mV/second.

Table 1 – Accuracy and precision (intra- and inter-day) under the optimized conditions ($n = 6$).

Concentrations (mol/L)	Intra-day			Inter-day		
	Found (mol/L)	Recovery (%)	RSD (%)	Found (mol/L)	Recovery (%)	RSD (%)
2.00×10^{-7}	1.98×10^{-7}	99.0	2.9	1.97×10^{-7}	98.5	3.6
1.00×10^{-6}	9.86×10^{-7}	98.6	3.6	9.83×10^{-7}	98.3	3.9
2.50×10^{-6}	2.45×10^{-6}	98.0	2.6	2.44×10^{-6}	97.6	3.3
5.00×10^{-5}	4.96×10^{-6}	99.2	2.7	4.95×10^{-6}	99.0	2.6
1.00×10^{-5}	9.81×10^{-6}	98.1	3.1	9.77×10^{-6}	97.7	3.4

3.4. *Interference studies*

Under optimal experimental conditions, the interferences of some metal ions and organic molecules have been evaluated individually by cyclic voltammetry. The oxidation peak current of 1.50×10^{-6} mol/L CGA in the absence and presence of various foreign species were measured. If the peak current change is no more than ±5% when a species is added, we assume no interference occurs. Experimental results showed that a 60-fold excess of Na^+, Zn^{2+}, Fe^{3+}, Ca^{2+}, K^+, Mg^{2+}, sucrose and glucose did not interfere with the analysis of CGA. This indicates that the determination of CGA at PASA/GCE is not affected by the common interfering species and the selectivity of the method is satisfactory. Further, to ascertain there is no interference coming from matrix components, negative control samples were prepared using same formulations of Qingkailing injection and Mailuoning injection, except that honeysuckle was not added. The negative control samples were similarly analyzed as directed in Section 2.5. No peaks were observed, indicating that matrix components contained in the injections do not interfere with the determination of CGA.

3.5. *Validation studies*

3.5.1. *The linearity range and limit of detection*

Under the above optimized conditions, the variation of peak current with concentration of CGA at the PASA/GCE was studied by cyclic voltammetry and the result is shown in Fig. 8. From the figure we can see that the oxidation peak current of CGA at the PASA/GCE was proportional to its concentration in pH 4.0 phosphate buffer in a range of 4.00×10^{-7} to 1.20×10^{-5} mol/L with a linear regression equation that can be expressed as i_{pa} (A) $= 1.88 \times 10^{-6} + 2.27c$ (mol/L), r = 0.9996. The limit of detection was estimated by gradually decreasing the concentration levels of CGA. When the concentration of CGA was decreased to 8.0×10^{-8} mol/L, the oxidation peak can still be observed, but the oxidation peak almost disappeared when the concentration was further decreased. Therefore, the limit of detection was evaluated to be 8.0×10^{-8} mol/L.

3.5.2. *Accuracy and precision*

The precision of the method was validated under the optimized conditions in terms of repeatability (intra-day) and intermediate precision (inter-day). Six replicate measurements for each of five samples containing lower, middle, and higher concentrations in the linear range were made over a single day (intra-day, $n = 6$) and for 5 days over a period of 1 week (inter-day, $n = 6$). Satisfactory recoveries and relative standard deviations (RSD) were obtained and are reported in Table 1. The recoveries obtained confirmed the high accuracy and the relative standard deviations obtained confirmed the good precision of the method.

3.5.3. *Robustness*

The robustness of the developed method has been evaluated by investigating the effect of small variations in pH value of supporting electrolyte, accumulation time and scan rate on the recovery of CGA. Recoveries for CGA under all variable conditions were in the range of 96.9–99.7%. No significant variations were observed when these parameters were slightly changed, which indicates a very good robustness of the proposed method.

3.6. *Comparison of this developed method with those reported in the literature*

The analytical performance of this developed method and those of previously reported electrochemical methods are shown in Table 2. From the comparison we can see that this

Table 2 – Comparison of various electroanalytical methods proposed for the determination of CGA.

Modifiers	Recoveries	Limit of detection (mol/L)	Linearity range (mol/L)	Sample analyzed	References
An ionic liquid containing iridium nanoparticles and polyphenol oxidase	93.2–105.7	9.15×10^{-7}	3.48×10^{-6}–4.95×10^{-5}	Coffee	[24]
HRP, DNA and silica–titanium composite	93–98	7.0×10^{-7}	1.0×10^{-6}–5.0×10^{-5}	Coffee, tea	[25]
Bean sprout homogenate and chitosan microspheres composite	96.5–102.6	8.02×10^{-7}	4.89×10^{-6}–3.20×10^{-4}	Coffee	[26]
Bean sprout homogenate and silica composite	91.3–115.5	8.52×10^{-7}	4.89×10^{-6}–4.85×10^{-5}	Coffee	[26]
Tetranuclear copper(II) complex	93.2–106.1	8.0×10^{-7}	5.0×10^{-6}–1.45×10^{-4}	Coffee	[27]
PLPA/GCE	96.3–102.8	8.0×10^{-8}	4.0×10^{-7}–1.2×10^{-5}	Injections, honeysuckle	This work

Table 3 – Determination results of CGA in pharmaceutical products ($n = 6$).

Sample	Added ($\times 10^{-6}$ mol/L)	Found ($\times 10^{-6}$ mol/L)	Recovery (%)	RSD (%)
Qingkailing injection	—	2.47[a], 2.39[b]	—	—
	1.50	3.99[a], 3.86[b]	101.3[a], 98.0[b]	3.4[a], 2.7[b]
	2.50	5.04[a], 4.86[b]	102.8[a], 98.8[b]	3.1[a], 4.1[b]
	3.50	5.91[a], 5.81[b]	98.3[a], 97.7[b]	2.7[a], 3.6[b]
Mailuoning injection	—	10.02[a], 9.98[b]		
	5.00	14.88[a], 14.73[b]	97.2[a], 95.0[b]	3.5[a], 3.9[b]
	10.00	20.16[a], 19.92[b]	101.4[a], 99.4[b]	2.4[a], 2.8[b]
	15.00	24.88[a], 24.73[b]	99.1[a], 98.3[b]	2.9[a], 3.7[b]
Honeysuckle		3.95[a], 3.88[b]		
	3.00	6.84[a], 6.81[b]	96.3[a], 97.7[b]	1.9[a], 2.5[b]
	4.00	8.02[a], 7.83[b]	101.8[a], 98.8[b]	3.6[a], 4.3[b]
	5.00	8.89[a], 8.76[b]	98.8[a], 97.6[b]	2.7[a], 3.6[b]

[a] Results obtained by the proposed method.
[b] Results obtained by HPLC method.

developed method has a large advantage over the other reported ones in terms of linearity range, limit of detection, recovery, and number of samples analyzed.

3.7. *Analytical application*

The analysis of CGA in honeysuckle, Qingkailing injection, and Mailuoning injection was performed using the PASA/GCE. First, real samples were analyzed. Then, the analyzed samples were spiked with CGA standard solutions and similarly analyzed. The concentration of CGA in the electrolytic cell and calculated recoveries are listed in Table 3. Good recoveries demonstrated the applicability of the modified electrode for determination of CGA. After calculation, the corresponding concentrations of CGA in the real samples of honeysuckle, Qingkailing injection and Mailuoning injection were 2.8%, 4.94×10^{-8} mol/L and 1.00×10^{-4} mol/L, respectively. For further evaluation of the validity of the proposed method, HPLC was used to compare the analysis results. Table 3 lists the analytical results of real samples obtained by HPLC and the proposed method. The results obtained are in high agreement, demonstrating the high accuracy of the proposed method.

4. Conclusions

A PASA/GCE for the voltammetric determination of CGA was fabricated. The fabricated electrode showed an excellent electrocatalytic effect toward the redox of CGA and the redox peak currents of CGA were remarkably increased at the PASA/GCE. Based on the electrocatalytic effect, a convenient method for the determination of CGA was developed and the proposed method showed good recovery, reproducibility, and sensitivity. This method can be used for the determination of CGA in a variety of traditional Chinese pharmaceutical products. Because CGA can be found in more than 170 types of traditional Chinese pharmaceutical products, it is expected that the proposed method will be useful in CGA determination of these products and thus would be of great help to pharmaceutical industries.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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