Research Article

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The electrochemical redox mechanism and antioxidant activity of polyphenolic compounds based on inlaid multi-walled carbon nanotubes-modified graphite electrode

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Abstract: The electrochemical redox mechanism of polyphenolic compounds (gallic acid [GA], caffeic acid [CA], ferulic acid [FA], and vanillic acid [VA]) were investigated by electrochemical methods at the inlaid multi-walled carbon nanotubes-modified graphite electrode (MWCNTs/GCE). The obtained micro-information such as the number of electrons and protons were used to deduce the electrochemical oxidation mechanism of four polyphenolic compounds. The antioxidation activities of these compounds were also studied by two methods. The radical scavenging activity followed the order: GA > CA > FA > VA, which was in agreement with the result from the spectrophotometry method. The result indicated that compounds with lower oxidation peak potential (E_{pa}) showed stronger antioxidation activity. At the same time, compounds with high E_{pa} showed lower antioxidant activity. The greater the number of hydroxyls linked to the aromatic ring, the greater the antioxidation activities of four compounds. Structural analysis of these phenolic-based compounds suggested that multiple OH substitutions and conjugations determine their free radical scavenging activity and electrochemical behavior.

Keywords: antioxidant activity, polyphenolic compounds, cyclic voltammetry, differential pulse voltammetry

1 Introduction

Phenolic compounds attract a lot of attention due to their potent antioxidant activity and, as a result, health hazards [1–4]. However, these compounds are also used as preventive and therapeutic drugs for some oxidative stress illnesses, such as atherosclerosis, inflammation, cancer, etc. [5,6]. Their main benefits are related to their strong antioxidation activity [7,8]. For example, the antioxidation efficacy of phenolic acids depends on their structures and the OH group presence [9–11].

Recently, multi-walled carbon nanotubes (MWCNTs) attracted attention to fabricate MWCNT-modified electrodes because they possess high surface area, excellent mechanical and chemical properties, and superior electrocatalytic and charge transfer features [12–14]. However, the MWCNTs/GE was usually prepared by the method of dispensing or doping, and the process was comparatively complicated, which could take about 70–80 min, but stable carbon nanotube films could not be obtained. Therefore, MWCNTs' applications still demonstrate drawbacks such as poor dispersion and film-forming. For this reason, we inlaid MWCNTs into the electrodes directly by mechanically abrading in order to form a compact film on the electrode surfaces. It is found that the method has the advantage of simple, time-saving, and stable chemical properties.

Electrochemical techniques are versatile and powerful analytical tools, which have the benefit of high sensitivity, low detection limits, low cost, and enable rapid analysis of sample [15,16]. In addition to their fundamental science applications to study oxidation and reduction reactions and their mechanism, they are also used for kinetic and thermodynamic studies as well as to understand electron and ion transfer [17,18]. Phenolic compounds are often called “chain-breaking antioxidants” because of the strong radical scavenging ability of their H from the OH group or electron, both of which donate their charge capacity and

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delocalize/stabilize the phenoxyl radical [19,20]. This scavenging ability could be predicted by assessing the standard one-electron potentials of the corresponding compounds. For this purpose, voltammetry is frequently applied. Voltammetry is used for analyses of natural and synthetic antioxidants to study their reaction mechanisms. All this knowledge is then applied for the design of new efficient antioxidants [21–23]. The structure-property-activity relationship established for these compounds suggested that redox potentials reflect the antioxidant activity, which is useful as guidelines for drug discovery and development [24,25]. Cyclic voltammetry (CV) is also applied for the reduction ability characterization of natural phenols. Similarly, CV demonstrated an excellent correlation between its data on redox potential and phenols' antioxidation properties [26–29]. In general, strong scavengers oxidize at lower potential values [30], which was also observed by Firuzi *et al.* [31] during CV to obtain oxidation potentials and ferric reducing antioxidation power to determine their antioxidation properties. Typically, the higher the antioxidation activity and lower E_{pa} , the higher the OH group contents in the aromatic rings, and *vice versa*.

In the present work, the graphite electrode modified with an MWCNTs coating and CV were used to study the electrochemical redox mechanism of four polyphenolic compounds. Their antioxidation properties were assessed based on their redox potentials. The results coincide with that of spectrophotometry, which indicated that redox potentials reflect the antioxidation activity of the phenolic compounds, which, in turn, could be used for pharmaceutical applications.

2 Materials and methods

2.1 Reagents and chemicals

Gallic acid, caffeic acid, ferulic acid, and vanillic acid reference substance (purity >99.0%) were acquired from the National Institute of China for the Control of Pharmaceutical and Biological Products (NICPBP). The 1.0 mg/mL stock solutions of gallic acid and caffeic acid were prepared in CH_3OH . The 50 mg/mL solutions of vanillic acid and ferulic acid were also prepared in CH_3OH . All the stock solutions were stored at 4°C. 0.10 mol/L of $\text{C}_6\text{H}_8\text{O}_7\text{--Na}_3\text{C}_6\text{H}_5\text{O}_7$ buffer solution, 0.10 mol/L of HAc--NaAc buffer solution, and KCl solution were used as supporting electrolytes. All other reagents were of analytical and reagent grades. Twice-distilled water was used throughout all experiments.

The TNMH_3 carboxyl-functionalized MWCNTs (>95% pure) were bought from the Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences. Spectrum pure graphite rod (0.6 cm in diameter) was from Qingdao Tengrui Carbon Co., Ltd.

2.2 Experimental setups and instruments

ZAHNER Zennium IM6 Electrochemical Workstation (Germany) was used for all tests. A typical setup contained a three-electrode system with MWCNTs/GE as a working electrode, platinum wire as a counter electrode, and a saturated calomel electrode as a reference electrode. UV-vis spectra were collected using an 8453 UV-Vis spectrophotometer (Agilent Technologies, USA).

2.3 Experimental methods

2.3.1 Preparation of the modified electrode

First, 6 cm graphite rod was taken and sealed in the polyethylene pipe using epoxy resin binder. One of the ends was used as electrode connection and the other was polished to a mirror-like surface by sandpaper. The mirror side was carefully polished for about 15 min using functionalized MWCNTs on parchment paper to embed MWCNTs uniformly on the electrode surface and the inlaid MWCNTs/GE were obtained. Finally, the prepared electrode was electrochemically cleaned in based solution with cycling potentials between -0.4 and $+2.0$ V at 0.1 V/s until a steady CV curve was observed.

2.3.2 Electrochemical analysis

One milliliter of sample solution at a specific concentration was added to 9.0 mL of appropriate buffer solution. CV and differential pulse voltammetry (DPV) analyses of the mixed solutions were performed using the three-electrode system under the optimization of the pulse amplitude, pulse interval, and pulse width conditions.

2.3.3 Determination of the antioxidant activity of four polyphenolic compounds

The antioxidation activity of four polyphenolic compounds was investigated by the electrochemical analysis method and spectrophotometry [32].

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Electrochemical behavior of the polyphenols under the MWCNTs/GE presence

3.1.1 CV analysis

The CV curves of four polyphenolic compounds under the optimum condition is shown in Figure 1. It is shown that four polyphenolic compounds have the obvious electrochemical response signal on the electrode, GA appeared as an irreversible oxidation peak at $E_{pa} = 0.46$ V; CA appeared as a pair of prominent redox peak at $E_{pa} = 0.42$ V, $E_{pc} = 0.27$ V, and $\Delta E = 0.15$ V; FA appeared as a

pair of obvious redox peak at $E_{pa} = 0.37$ V, $E_{pc} = 0.26$ V, and $\Delta E = 0.11$ V; VA appeared as a pair of prominent redox peak at $E_{pa} = 0.63$ V, and $E_{pc} = 0.55$ V, and $\Delta E = 0.08$ V, which indicated that redox reaction of four polyphenols occurred on the electrode surface (Table 1).

3.1.2 The DPV analysis

The DPV curve of four polyphenolic compounds under optimum conditions are shown in Figure 2 and Table 2. The DPV oxidation peak currents (i_{pa}) were detected. The linear relationships between i_{pa} and concentrations are shown in Table 3.

3.2 Supporting electrolyte effect

For these tests, we used 0.01 mol/L of citric acid–sodium citrate buffer solution ($C_6H_8O_7$ – $Na_3C_6H_5O_7$), 0.01 mol/L of

Figure 1: The CV curve of four polyphenolic compounds on the MWCNTs/GE. (a)((a) Na_2HPO_4 – $C_6H_8O_7$, (b) Na_2HPO_4 – $C_6H_8O_7$ + 3 mg/L of GA, (c) Na_2HPO_4 – $C_6H_8O_7$ + 5 mg/L of GA)); (b)((a) HAc–NaAc, (b) HAc–NaAc + 0.5 mg/L of CA, (c) HAc–NaAc + 1 mg/L of CA)); (c)((a) $C_6H_8O_7$ – $Na_3C_6H_5O_7$, (b) $C_6H_8O_7$ – $Na_3C_6H_5O_7$ + 6 mg/L of FA, (c) $C_6H_8O_7$ – $Na_3C_6H_5O_7$ + 10 mg/L of FA)); and (d)((a) KCl, (b) KCl + 10 mg/L of VA, (c) KCl + 15 mg/L of VA)).

Table 1: The optimum condition of the CV curve of four polyphenolic compounds

Matter	Range of potential (V)	Based solution	Based solution pH	Scan rate (V/s)
GA	0.1–0.8	$\text{Na}_2\text{HPO}_4\text{--C}_6\text{H}_8\text{O}_7$	pH = 4.40	0.07
CA	0–0.8	HAc–NaAc	pH = 4.40	0.05
FA	–0.4 to 1.0	$\text{C}_6\text{H}_8\text{O}_7\text{--Na}_3\text{C}_6\text{H}_5\text{O}_7$	pH = 5.40	0.10
VA	–0.2 to 1.3	KCl	100 mmol/L	0.05

disodium hydrogen phosphate–potassium dihydrogen phosphate buffer solution ($\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$), 0.01 mol/L of phosphate buffer solutions (PBS), 0.01 mol/L of citric acid–disodium hydrogen phosphate buffer solution ($\text{C}_6\text{H}_8\text{O}_7\text{--Na}_2\text{HPO}_4$), 0.01 mol/L of acetic acid–sodium acetate buffer (HAc–NaAc), 0.01 mol/L of Britton–Robinson buffer (B–R), and 0.01 mol/L of potassium chloride buffer solutions (KCl) as supporting electrolytes for the MWNTs/GE system. Two CV redox peaks were observed for only five electrolytes (Figure 3). At the same time, well-defined CV responses with high redox peak currents for all four polyphenols were obtained in $\text{Na}_2\text{HPO}_4\text{--C}_6\text{H}_8\text{O}_7$ buffer solution (GA), HAc–NaAc buffer solution (CA), $\text{Na}_2\text{HPO}_4\text{--C}_6\text{H}_8\text{O}_7$ buffer solution (FA), and KCl buffer solution (VA).

The supporting electrolytes defined the electrochemical reactions discussed above; their chemistry affected the reaction mechanism, similar to how a solvent affects a chemical reaction [33]. A strong role of a supporting electrolyte is also related to its ability to tune the ionic conductivity of the system.

3.3 Influence of pH of supporting electrolyte

The influence of pH on the CV behavior of four polyphenolic compounds was investigated using the optimized buffer solutions. The oxidation peak potentials (E_{pa}) shifted negatively as pH increased (Figure 4). This dependency was linear (Table 4).

3.4 Scan rate effect on the electrodes

The influence of scan rate (ν) on the CV response of four polyphenolic compounds is shown in Figure 5. The oxidation peak potential (E_{pa}) shifted positively, while the reduction potential (E_{pc}) shifted towards more negative values as the scan rate increased. The oxidation and the

Figure 2: The DPV curve of four polyphenolic compounds on the MWNTs/GE.

Table 2: The optimum condition of the DPV curve of four polyphenolic compounds

Matter	Range of potential (V)	Based solution	Pulsewidth (ms)	Pulse amplitude (mV)	Pulse interval (ms)
GA	0.1–0.8	Na ₂ HPO ₄ –C ₆ H ₈ O ₇ (pH 4.40)	80	30	130
CA	0–0.8	HAc–NaAc (pH 4.40)	200	30	200
FA	–0.4 to 1.0	C ₆ H ₈ O ₇ –Na ₃ C ₆ H ₅ O ₇ (pH 5.40)	100	30	130
VA	–0.2 to 1.3	100 mmol/L KCl	20	30	220

Table 3: The linear relationship between *i*_{pa} and concentration of four polyphenolic compounds

Matter	Range of concentration (g/L)	Linear regression equation	<i>r</i>	RSD (<i>n</i> = 5)
GA	2.0×10^{-6} – 8.0×10^{-4}	$i_{pa} (\mu A) = 0.3187c + 0.3329$	0.9996	1.015
CA	6.0×10^{-7} – 1.0×10^{-4}	$i_{pa} (\mu A) = 1.8564c + 0.9736$	0.9988	4.038
FA	2.0×10^{-6} – 1.2×10^{-4}	$i_{pa} (\mu A) = 2.0850c - 0.5263$	0.9912	1.575
VA	6.0×10^{-6} – 5.0×10^{-4}	$i_{pa} (\mu A) = 0.3411c + 0.8118$	0.9996	2.743

reduction peak currents (*i*_{pa} and *i*_{pc}, respectively) increased linearly with the scan rate for all four polyphenols tested in this work (Table 5). Thus, the electrode reactions of these

polyphenols at the MWCNTs/GE were controlled by the adsorption [34]. A high and stable peak current for four polyphenolic compounds was obtained at the scan rate of

Figure 3: The influence of supporting electrolyte on the redox peak current of four polyphenolic compounds. (a((a) Na₂HPO₄–KH₂PO₄, (b) PBS, (c) C₆H₈O₇–Na₃C₆H₅O₇, (d) Na₂HPO₄–C₆H₈O₇, (e) HAc–NaAc, *c* = 0.01 mol/L and *v* = 0.05 V/s)); (b((a) C₆H₈O₇–Na₃C₆H₅O₇, (b) PBS, (c) HAc–NaAc, (d) B–R e: KCl, *c* = 0.01 mol/L and *v* = 0.05 V/s)); (c((a) Na₂HPO₄–KH₂PO₄, (b) C₆H₈O₇–Na₃C₆H₅O₇, (c) Na₂HPO₄–C₆H₈O₇, (d) PBS, (e) HAc–NaAc, *c* = 0.01 mol/L and *v* = 0.10 V/s)); and (d((a) HAc–NaAc, (b) KCl, (c) PBS, (d) B–R, *c* = 0.01 mol/L and *v* = 0.05 V/s)).

Figure 4: pH effect on the redox peak current of four polyphenolic compounds. (a)(a) pH = 7.00, (b) pH = 6.00, (c) pH = 5.00, (d) pH = 4.40, (e) pH = 4.00, (f) pH = 3.00, and (g) pH = 2.20); (b)(a) pH = 3.60, (b) pH = 4.00, (c) pH = 4.40, (d) pH = 4.80, (e) pH = 5.40, and (f) pH = 5.80); (c)(a) pH = 3.00, (b) pH = 4.00, (c) pH = 5.00, (d) pH = 5.40, (e) pH = 6.00, (f) pH = 6.60, and (g) pH = 7.00); and (d)((a) 400 mmol/L, (b) 200 mmol/L, (c) 40 mmol/L, (d) 80 mmol/L, and (e) 100 mmol/L).

Table 4: The linear relationship between pH and peak potential E_p of three polyphenolic compounds

Matter	Range of pH	Linear regression equation	r	m
GA	2.20–7.00	$E_{pa} (V) = -0.0599 \text{ pH} + 0.8057$	0.9984	3.04
CA	4.00–5.80	$E_{pa} (V) = -0.6190 \text{ pH} + 0.9786$	0.9879	2.10
	3.60–5.80	$E_{pc} (V) = -0.0586 \text{ pH} + 0.5824$	0.9983	1.99
FA	3.00–7.00	$E_{pa} (V) = -0.0848 \text{ pH} + 0.9680$	0.9908	1.44
	3.00–7.00	$E_{pc} (V) = -0.0731 \text{ pH} + 0.6791$	0.9902	1.24

70 mV/s (GA), 50 mV/s (CA), 100 mV/s (FA), and 50 mV/s (VA).

3.5 Analysis of the kinetic parameters

Electron transfer number, n [35] is a key parameter of an electrochemical reaction at the electrode. Because the reactions of four polyphenols on the electrode were adsorption-controlled [36], they could be described by the Langmuir isotherm described as follows [37]:

$$i_p = \frac{n^2 F^2 A \Gamma_T v}{4RT} = \frac{nFQv}{4RT}, \quad (1)$$

$$Q = nFA\Gamma_T, \quad (2)$$

where Q is the charge value calculated from the oxidation or reduction CV peak areas, i_p is peak current (in μA), $F = 96,485 \text{ C/mol}$, $R = 8.1345 \text{ J/K/mol}$, $T = 298.15 \text{ K}$, Γ_T is the surface coverage of the electrode (in mol/cm^2); and A is the electrode surface area (equal to 0.2826 cm^2). Our calculations showed that the electron transfer numbers (n) of four polyphenolic compounds calculated from the

Figure 5: Electrochemical behavior of the polyphenols as a function of the CV scan rate (u).

data obtained at 0.05 V/s of scan rate were equal to 3.01 (GA), 1.98 (CA), 1.31 (VA), and 1.29 (FA).

The proton number m is also one of the most essential electrochemical parameters. We used the Nernst equation and data from Table 4 to calculate m as shown below:

$$E = E^\theta + \frac{RT}{nF} \ln \frac{[O][H^+]}{R} = E^\theta + \frac{RT}{nF} \ln \frac{[O]}{R} + \frac{mRT}{nF} \ln [H^+], \quad (3)$$

$$E = k - \frac{mRT}{nF} \text{pH} = k - \frac{m}{n} \times 0.059(25^\circ\text{C}), \quad (4)$$

where the slope k is equal to $0.059 m/n$. Thus, m of the oxidation peak can be obtained from the corresponding n values.

The electron transfer coefficient α was calculated from the equation below:

$$E_p = k + \frac{RT}{\alpha nF} \lg v, \quad (5)$$

According to the equation, the slope (k) = $k = 2.303RT/(\alpha nF)$, and α could be calculated using corresponding n values.

The apparent constant of electron transfer rate (k_s) was calculated by the following equation:

Table 5: Linear relationship of four polyphenolic compounds between oxidation peak current and scan rate

Matter	Range of scan rate (V/s)	Linear regression equation	r	Control process
GA	0.02–0.07	$i_{pa} (\mu\text{A}) = 240.0v + 21.03$	0.9987	Adsorption control
	0.07–0.12	$i_{pa} (\mu\text{A}) = -182.9v + 51.22$	0.9984	
CA	0.01–0.12	$i_{pa} (\mu\text{A}) = 255.7v + 27.49$	0.9979	Adsorption control
	0.01–0.12	$i_{pa} (\mu\text{A}) = 246.3v + 27.04$	0.9980	
FA	0.02–0.10	$i_{pa} (\mu\text{A}) = 326.48v + 10.60$	0.9924	Adsorption control
	0.02–0.10	$i_{pa} (\mu\text{A}) = 481.80v + 1.526$	0.9972	
VA	0.02–0.08	$i_{pa} (\mu\text{A}) = 283.2v + 29.97$	0.9958	Adsorption control
	0.02–0.08	$i_{pa} (\mu\text{A}) = 385.4v + 14.54$	0.9942	

Table 6: The determination of kinetic parameters of four polyphenolic compounds

Sample	The linear relationship	r	n	Γ_T (mol/cm ²)	m	α	k_s (/s)
GA	$E_{pa} \text{ (V)} = 0.0477 \lg v + 0.5054$	0.9900	3	2.27×10^{-10}	3	0.41	0.27
CA	$E_{pa} \text{ (V)} = 0.0843 \lg v + 0.6150$	0.9967	2	6.18×10^{-10}	2	0.35	2.32
FA	$E_{pa} \text{ (V)} = 0.0683 \lg v + 0.4334$	0.9992	1	1.41×10^{-10}	1	0.87	0.86
VA	$E_{pa} \text{ (V)} = 0.0619 \lg v + 0.5512$	0.9983	1	2.08×10^{-9}		0.96	1.08

$$\lg k_s = \alpha \lg(1 - \alpha) + (1 - \alpha) \lg \alpha - \lg \frac{RT}{nFv} - (1 - \alpha) \alpha \frac{nF\Delta E_p}{2.303RT} \quad (6)$$

All kinetic parameters of our polyphenolic compounds were calculated using the formulas shown in Table 6.

Polyphenolic compounds are antioxidants with the ability to convey electrons and protons. Their oxidation

reaction removes free radicals, when phenolic hydroxyl was oxidized to the ketone group [38]. That is, the mechanism of phenolic acids scavenging free radicals is by supplying the hydrogen on phenolic hydroxyl groups to the free radicals and forming relatively stable phenolic hydroxyl radicals to prevent a free radical chain reaction. Due to the reaction mechanism of natural antioxidants scavenging free radicals is consistent with the electrochemical

Figure 6: The electrochemical oxidation mechanism of four polyphenolic compounds.

oxidation mechanism, which are both achieved through the electron transfer, the oxidation reaction of antioxidants on the electrode is similar to its antioxidative reactions *in vivo* [39]. The antioxidant reaction process *in vivo* of polyphenolic compounds can be learned by the determination of electrochemical behavior.

From the results of the research, for GA, the electrochemical oxidation reaction takes place involving three electrons and protons; For CA, quasi reversible adsorption reaction takes place involving two electrons and protons; For FA and VA, single electron adsorption reaction takes place, and a proton is involved in the reaction of FA. So, we concluded that electrochemical oxidation reaction involving electrons and protons take place in polyphenolic compounds. We can infer the possible reaction mechanism as follow:

Enhanced selectivity of the sensors could be achieved by the electrochemical formation of conducting polymeric layers. Phenolic polymerizable monomers are widely used to obtain electrochemically deposited coatings [40]. The essential feature of all polyphenols is the presence of one or more hydroxylated benzene rings. For example, GA contains both three hydroxyls (–OH) and carboxyl (–COOH) groups. Thus, it can be electrochemically polymerized because of its two electrochemically active groups, which could be oxidized differently by the benzoic acid and its derivatives. These hydroxyl and carboxyl groups could also act as reactive sites to selectively bond to metals or as biomolecules active as electrocatalysts and chemical and biological sensors [41].

Polyphenol oxidation occurs by an electron transfer through the phenoxyl radical (semiquinone) formation [42]. This radical is unstable and decays by dimerization or polycondensation reactions. Thus, these four compounds could also undergo dimerization or polycondensation. Therefore, the oxidation mechanism of GA is described from this point of view (Figure 6). Abdel-Hamid and Newair have suggested an irreversible transformation of GA to its semiquinone radical cation (GA^+) by transferring one electron. After this radical releases a proton, it forms a semiquinone radical ($GA^\cdot$) [43], which, in turn, undergoes a second irreversible electron transfer

forming quinone cation (GA^+) [44]. GA^+ deprotonation ends the two-electron process yielding quinone, which manifested as an irreversible peak.

Summarizing all comments and discussions presented above, it was proposed that the electrochemical oxidation of polyphenols induced electrochemical polymerization. Potential GA cycling in acidic buffer solutions could form polymeric layers, which will further grow during anodic electropolymerization, which involves deprotonation of the phenols. These protons first become chemisorbed at the electrode surface, which then gradually oxidizes at anodic potentials.

3.6 Comparison of antioxidant activity of four polyphenolic compounds

3.6.1 The structure of four polyphenolic compounds (Figure 7)

3.6.2 Comparison of antioxidation activity of the polyphenol

The electrochemical response of four polyphenolic compounds was investigated in 0.01 mol/L of HAc–NaAc buffer solution (pH = 4.00) on the MWCNTs/GE at 50 mV/s scan rate. The peak potential of four polyphenolic compounds (GA, CA, VA, and FA) is 0.48, 0.51, 0.60, and 0.65 V, respectively. And the CV of four polyphenolic compounds is showed in Figure 8.

Wellington et al. investigated the electrochemical oxidation behavior of caffeic acid, ferulic acid, chlorogenic acid, and *p*-coumaric acid in HAc–NaAc (pH = 5.6) by CV. They concluded that the antioxidation activity of these polyphenols is related to the oxidation potential (E_{pa}), and caffeic acid ($E_{pa} = 0.31$ V) > chlorogenic acid ($E_{pa} = 0.38$ V) > ferulic acids ($E_{pa} = 0.43$ V) > *p*-coumaric acid ($E_{pa} = 0.73$ V). The result indicated that compounds with lower oxidation potentials show stronger antioxidation activity, whereas compounds with high E_{pa} values show lower antioxidant activity. So, we can infer that the

Figure 7: The structure of four polyphenolic compounds.

Figure 8: The cyclic voltammogram of four polyphenolic compounds.

antioxidant activity of four polyphenolic compound: GA ($E_{pa} = 0.48$ V) > CA ($E_{pa} = 0.51$ V) > FA ($E_{pa} = 0.60$ V) > VA ($E_{pa} = 0.65$ V).

Based on the theoretical analysis, polyphenolic acids are suitable hydrogen donors, whose ability to remove free radicals is mainly associated with phenolic OH groups, and $-\text{CHCHCOOH}$ groups are not the necessary groups for phenolic acids scavenging free radicals [45]. Their anti-oxidation activities are related to the number and position of phenolic OH groups and the other factors such as dehydrogenation reaction and the stability of the generated free radicals. The more hydroxyl groups on the benzene ring, the stronger the antioxidant activity is. The

antioxidant activity decreases when the hydroxyl groups on the benzene ring are replaced by the electron-withdrawing groups, whereas the antioxidant activity will be increased.

From Figure 8, GA with three *ortho*-hydroxyls showed the strongest antioxidant capacity compared with the other four polyphenolic compounds. CA has less antioxidant capacity than GA, and CA turns into FA when the *ortho*-hydroxyl of CA is replaced by electron-withdrawing group (methoxyl group), and the antioxidant capacity decreased, which indicated the antioxidant capacity of CA stronger than FA. The results further confirmed the sequence of antioxidant activities of the four polyphenols.

Figure 9: DPPH free radical scavenging of polyphenols studied in this work.

Table 7: The linear relationship of four polyphenolic compounds on DPPH free radical clearance

Matter	Linear equation	R^2	IC ₅₀	Linear range
GA	$y = 49.34x + 4.5849$	0.9755	0.92 µg/mL	0–1.6 µg/mL
CA	$y = 14.518x + 0.1982$	0.9913	3.43 µg/mL	0–6.0 µg/mL
FA	$y = 74.736x + 9.116$	0.9282	0.55 mg/mL	0–1.0 mg/mL
VA	$y = 17.457x + 10.388$	0.9565	2.27 mg/mL	0–4.75 mg/mL

3.6.3 Comparison of antioxidant activity of four polyphenolic compounds by spectrophotometry

DPPH free radical scavenging of our polyphenols possessed an excellent linear relationship between concentration and clearance E before reaching the maximum clearance (Figure 9).

In addition, the 50% radical scavenging activity (IC₅₀) of our polyphenols was obtained using linear equations shown in Table 7. Lower IC₅₀ values imply higher radical scavenging. Thus, the radical scavenging ability was in the following order: GA > CA > FA > VA, which agreed with the electrochemical method's result. Thus, our electrochemical method to evaluate antioxidation activities of polyphenols is reliable, and the micro-information of the reaction mechanism is also obtained.

4 Conclusion

Electrochemical methods are powerful analytical tools capable of providing insights into the reaction mechanisms and electron transfer. The electrochemical behavior of four polyphenolic compounds was investigated by CV using the inlaid MWCNTs/GE. The obtained micro-information such as the number of electrons and protons were used to deduce the electrochemical oxidation mechanism of four polyphenolic compounds. The antioxidation properties of these polyphenols were analyzed electrochemically and by UV-vis spectrophotometry. The compounds with lower oxidation potentials demonstrated stronger antioxidation abilities. The compounds with high E_{pa} values showed lower antioxidant activity. The scavenging ability of our polyphenols could be described in the following order: GA > CA > FA > VA, which was corroborated by the spectrophotometry data. Thus, experimentally obtained oxidation potentials strongly correlate with the structures of the polyphenolic compounds. The polyphenol structures also influence their corresponding antioxidation activities. The compounds with several electron-donating groups typically possess lower anodic peak potentials and

higher antioxidant abilities than monosubstituted phenols. However, phenolic OH groups have stronger effects than OCH₃ ones.

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Data availability statement: Raw data were generated at the facility: ZAHNER Zennium IM6 Electrochemical Workstation using UV-Vis spectrophotometer, etc. Derived data supporting the findings of this study are available from the corresponding author on request.

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