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Sensitive Determination of (−)-Epigallocatechin Gallate in Tea Infusion Using a Novel Ionic Liquid Carbon Paste Electrode

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ABSTRACT: This paper investigates the electrocatalytic oxidation of (−)-epigallocatechin gallate (EGCG), the main monomer flavanol found in green tea, with a novel ionic liquid, *n*-octylpyridinium hexafluorophosphate (OPFP) carbon paste electrode (CPE). Due to the natural viscosity and high conductivity of OPFP, this novel OPFP-CPE exhibited very attractive properties, such as high stability and electrochemical reactivity, low background current, and wide electrochemical window. Therefore, this electrode is a very good alternative to traditional chemically modified electrodes because the electrocatalytic effect can achieved without any further electrode modification. Comparative experiments were carried out using CPE and a glassy carbon electrode (GCE). With OPFP-CPE, highly reproducible and well-defined cyclic voltammograms were obtained for EGCG. Under optimal experimental conditions, the peak current of differential pulse voltammetry (DPV) response increased linearly with EGCG concentration over the range of 5.0×10^{-7}−1.25×10^{-5} M. The limit of detection (LOD) and the limit of quantification (LOQ) were 1.32×10^{-7} and 4.35×10^{-7} M, respectively. The method was applied to the determination of EGCG in green tea infusion samples, and the recovery of the spiked EGCG to the diluted (10-fold) tea extract was from 87.62 to 99.51%.

KEYWORDS: *(−)-epigallocatechin gallate, ionic liquid, carbon paste electrode, differential pulse voltammetry*

INTRODUCTION

Flavanols (also known as catechins) belong to a group of naturally occurring plant compounds called flavonoids and possess a characteristic tricyclic C6−C3−C6′ flavan skeleton.[1] Green tea, brewed from the leaves of the *Camellia sinensis* L. shrub, is the main nutritional source of monomer flavanols. These phenolic compounds account for 30−42% of the dry weight of the solids in green tea.[2,3] The most abundant flavanol found in green tea is (−)-epigallocatechin gallate (EGCG), followed by (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), and (+)-catechin (C) (Figure 1). These compounds are thought to be the major compounds responsible for the potential beneficial effects of green tea.[4] Among the polyphenols present in tea, EGCG, which comprises approximately 50% of the catechins in green tea,[5] is assumed to be the source of most of the biological activity of tea. EGCG has been found to possess antioxidant, antimutagenic, and anticancer properties.[6] In addition, EGCG is antibacterial,[7] and it may inhibit human immunodeficiency virus,[8] reduce platelet aggregation,[9] and prevent the development of atherosclerosis.[10] It has also been reported that EGCG may be closely associated with the prevention of inflammation caused by leukocyte elastase inhibition.[11]

For total or particular phenol analysis, numerous methods have been reported based, for example, on spectroscopy,[12,13] chemiluminescence,[14] spectrophotometry,[15,16] chromatography,[17,18] and so forth. Inherent limitations of the analysis differ from method to method (low sensitivity, interference by reducing substance, slow detection, and high price of equipment) have stimulated the study of simple, fast, and sensitive methods for the characterization of antioxidants. Recently, electrochemical methods have been exploited for the determination of polyphenolic compounds in natural samples.[19−21] Electrochemical studies provide useful information on the physicochemical properties of compounds. Therefore, electrochemistry has been used for the estimation of the antioxidant capacity of polyphenols. The electrochemical oxidation of (+)-catechin at a glassy carbon electrode was investigated over a wide range of conditions using cyclic,

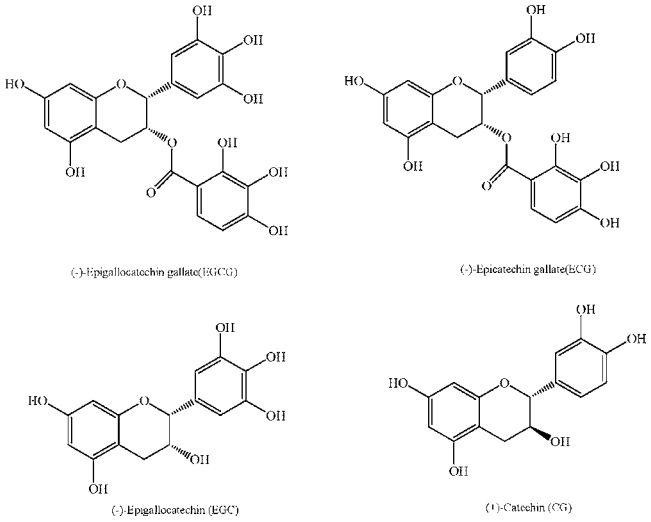

Figure 1. Molecular structures of (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (+)-catechin.

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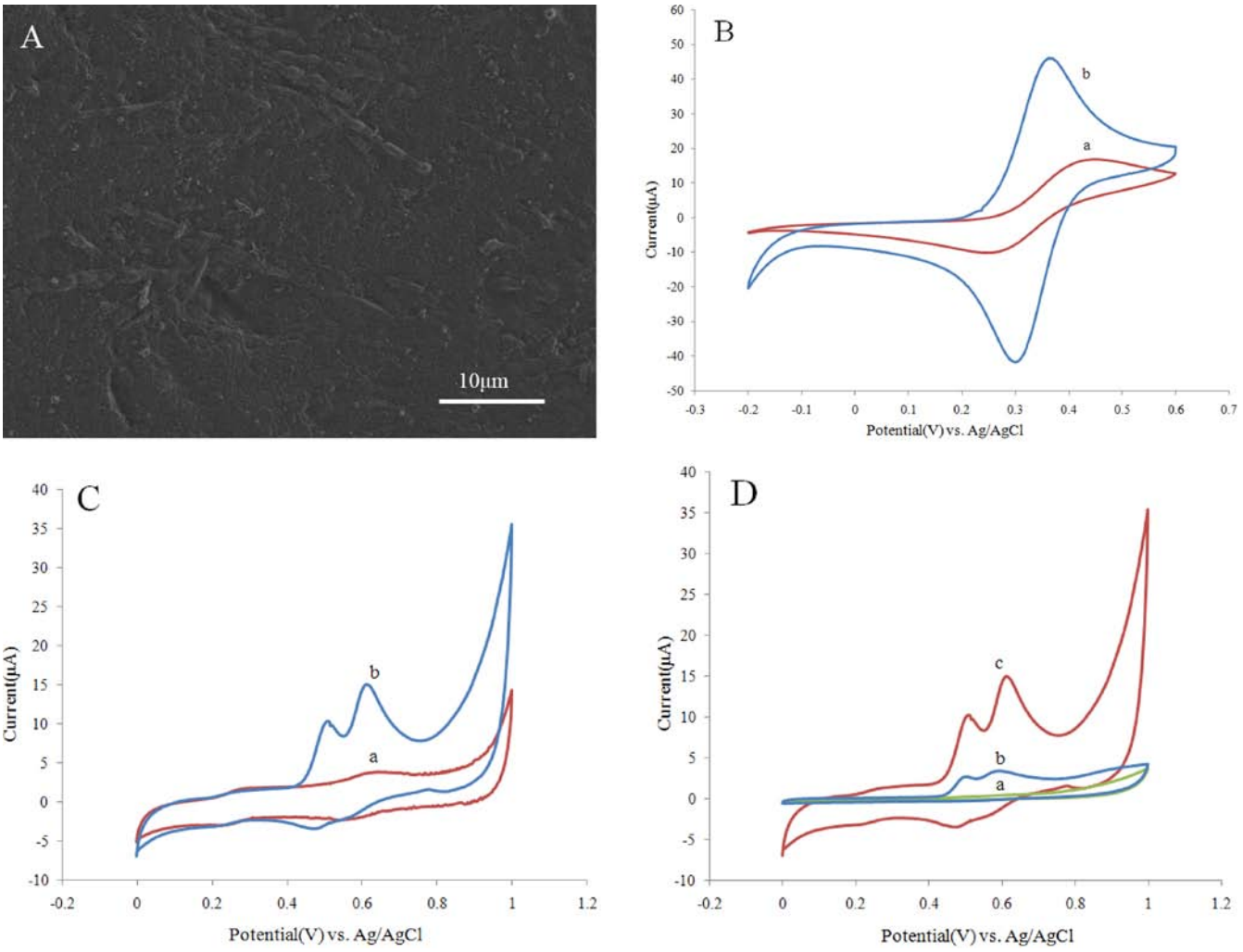


Figure 2. (A) SEM image of OPFP−CPE and (B) cyclic voltammograms for 1.0×10^{-3} mol L^{-1} $K_4[Fe(CN)_6]$ in 0.1 mol L^{-1} KCl, OPFP−CPE (a) and CPE (b). Scan rate = 100 mV s^{-1}. (C) Cyclic voltammograms of the OPFP−CPE in the absence (a) and presence (b) of 1×10^{-4} mol L^{-1} EGCG. (D) Comparison for cyclic voltammograms of 1×10^{-4} mol L^{-1} EGCG at the CPE (a), GCE (b), and OPFP−CPE (c). Scan rate = 50 mV s^{-1}.

differential pulse, and square-wave voltammery.[22] The investigation of the amperometric behavior of antioxidants was performed by using a functionalized gold wire electrode.[23] The mechanism of the electrochemical oxidation of EC and ECG on a glassy carbon electrode was investigated over a wide range of conditions, using cyclic and square-wave voltammetry.[24] In these papers, the authors mainly used traditional glassy carbon electrodes or metal electrodes, and these electrodes also need to be chemically modified to improve the performance of electrodes for the detection of polyphenols. That is, the performance of the electrode is very important for the determination of polyphenols.

Carbon electrodes are widely used in electroanalytical investigations because of their chemical inertness, relatively wide potential window, low background current, and suitability for different types of analysis. Carbon materials that have been widely used in the preparation of solid electrodes include glassy carbon, carbon fiber, carbon black, several forms of graphite from graphite powder to highly oriented pyrolytic graphite, carbon nanotubes, and highly ordered mesoporous carbons.[25−35] Among these, glassy carbon electrodes (GCEs) and carbon paste electrodes (CPEs) are the most popular, but the performance of common CPEs employing paraffin oils as nonelectroanlytical binder is not very satisfactory. Thus, many researchers want to find one compound to replace the paraffin as the binder of CPEs to improve the performance of electrodes.

Nowadays, room temperature ionic liquids (RTILs) have attracted great attention in the field of chemistry due to their excellent properties such as high chemical and thermal stability, negligible vapor pressure, and good conductivity.[36,37] The capability of RTILs combining with carbon materials to form conductive composites makes them very attractive for the preparation of various electrodes.[38,39] IL−carbon composites such as IL−graphite, IL−carbon nanotubes, IL−mesoporous carbon, IL−carbon nanofibers, and IL−graphene have been developed for detection and analysis.[39−42] These IL-modified CPEs show some advantages over traditional CPEs, such as high conductivity and sensitivity, fast electron transfer, and good antifouling ability for electroanalysis.

In our previous works,[43] we introduced a new CPE in which the nonconductive paraffin oil binder was completely replaced by the ionic liquid octylpyridinum hexafluorophosphate (OPFP). This novel ionic liquid carbon paste electrode (OPFP−CPE) showed high performance electrode properties with many good features such as resisitivity toward biomolecule

fouling and high rates of electron transfer. The electron transfer kinetics on OPFP−CPE is much better than that of carbon nanotube paste and GCEs.

In this paper, the electrochemical behavior of the major tea flavanols (EGCG) at the IL−CPE was investigated for a wide range of solution conditions using differential pulse voltammery (DPV). The information on the oxidation mechanism of the above-mentioned compound was obtained from results at different pH values. Experimental parameters of the analytical procedure were optimized, and the method was applied for the determination of EGCG content in green tea infusion samples. According to our knowledge, there is no such report in the literature to date.

EXPERIMENTAL PROCEDURES

Reagents. All chemicals were of analytical grade and used without any further purification. Ionic liquid OPFP (99%) was purchased from Shanghai Chengjie Co., Ltd. (Shanghai, China). Graphite powder (size < 30 μm, spectral pure) and paraffin oil were obtained from Sinopham Chemical Reagent Co., Ltd. (Shanghai, China). Phosphate buffer solutions, 0.1 mol L^{-1} of different pH values, were used as the supporting electrolyte in all of the experiments. Millipore-Q (18.2 MΩ·cm) water was used for all experiments.

Electrode Preparation. The preparation of IL-CPE was similar to that given in our previous paper.[43] First, 0.6 g of graphite powder and 0.4 g of OPFP was hand-mixed in a mortar using a pestle for 30 min. Then a portion of the resulting paste was packed firmly into the electrode cavity (1.8 mm diameter) of a glass sleeve. The electrode was then heated in an oven to a temperature higher than the melting point of OPFP (mp 65 °C) for 2 min. Electrode contact was established via a copper wire. For comparison, the traditional CPE was prepared by a 70:30 (w/w) graphite to paraffin oil. The new surface of these working electrodes was obtained by smoothing the electrodes with a weighting paper.

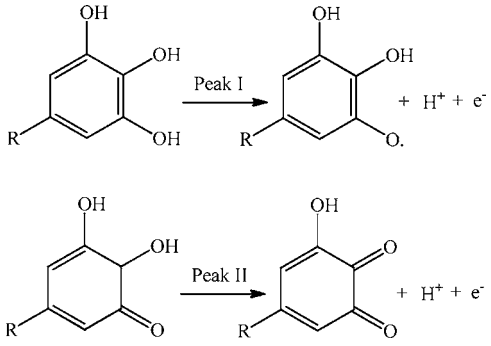
Apparatus. Cyclic and differential pulse voltammetric measurements were carried out on a CHI 440 electrochemical workstation (CH Instruments USA). A 10 mL capacity glass voltammetric cell was used. The electrochemical cell was assembled with a conventional three-electrode system: a saturated Ag/AgCl reference electrode, a Pt wire auxiliary electrode, and the prepared carbon ionic liquid working electrodes. All of the experiments were done at room temperature (25 ± 1 °C).

Sample Preparation and Determination of EGCG. Analyses of EGCG in the tea infusions by the proposed voltammetric method were carried out using samples of teas commercially available in China. For comparison reasons, all of the samples examined were produced recently, stored in the dark at 10 °C, and analyzed shortly after being opened. An aliquot of 1000 μL of tea infusions was added into the voltammetric cell containing 9 mL of phosphate buffer, pH 2.0, and homogenized with a magnetic stirrer. The differential pulse voltammograms were recorded in the potential range from 0.1 to 1.1 V, using a scan rate of 16 mV s^{-1}, a modulation time of 50 ms, and a pulse amplitude of 50 mV. The content of EGCG in these samples was determined according to the standard addition method.

RESULTS AND DISCUSSION

Figure 2A shows the scanning electron microscopy (SEM) images of the OPFP−CPE. The electrode made from the mixture powder of graphite and OPFP exhibited a uniform surface topography, suggesting the presence of OPPF filled the gaps well. The ohmic resistance of OPFP−CPE was 37 ± 1 Ω, which was much smaller than that of CPE (2.61 ± 0.06 kΩ). Meanwhile, the OPFP−CPE was quite stable because the natural viscosity of OPFP acts as a binder to provide a good cohesion between the graphite particles.

Scheme 1. Pathway Proposed for EGCG Oxidation



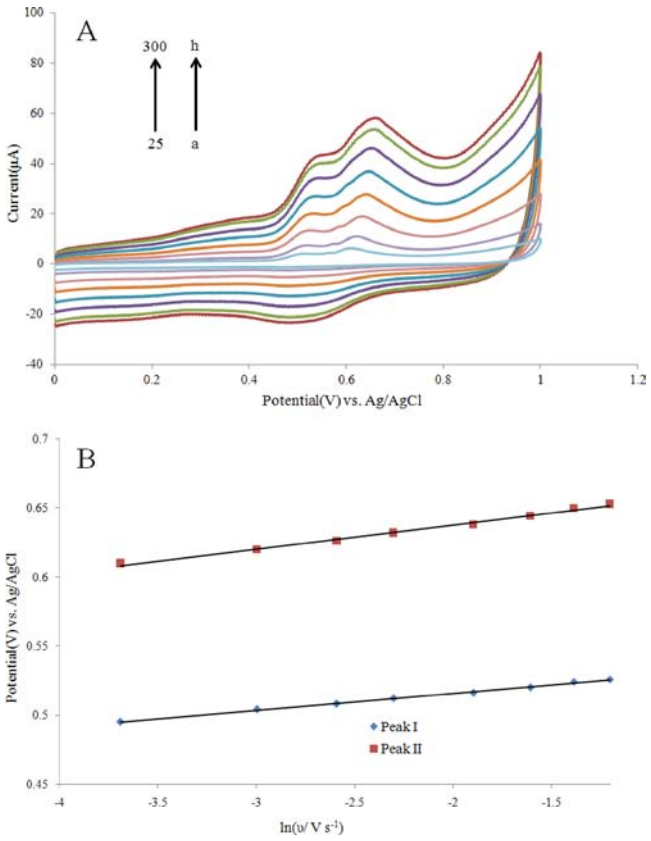


Figure 3. (A) Effect of scan rate on cyclic voltammetric responses for 1×10^{-4} mol L^{-1} EGCG on the OPFP-CPE at 25 (a), 50 (b), 75 (c), 100 (d), 150 (e), 200 (f), 250 (g), 300 (h) mV s^{-1}. (B) Plot of the oxidation peak potentials versus the logarithm of scan rates.

Figure 2B shows the typical cyclic voltammograms for $[Fe(CN)_6]^{3-/4-}$ at the OPFP−CPE and CPE. It is clear that the electron transfer rate was sluggish, with a peak-to-peak separation of 190 mV at the CPE. On the contrary, the OPFP−CPE exhibited a well-shaped cyclic response for the $[Fe(CN)_6]^{3-/4-}$ redox couple with a peak-to-peak separation of 62 mV, suggesting a dramatic increase in the electron transfer rate due to the high conductivity of OPFP. It is reported that the ionic liquid present in the carbon electrode not only acted as a binder to fill in the blanks of the carbon paste but also formed a layer of ionic liquid on the electrode surface.[43] Therefore, the OPFP in CPE could facilitate the electron transfer rate between the electron surface and electroactive species.

Figure 2C shows the typical cyclic voltammograms of the OPFP−CPE in the absence and presence of 1×10^{-4} mol L^{-1}

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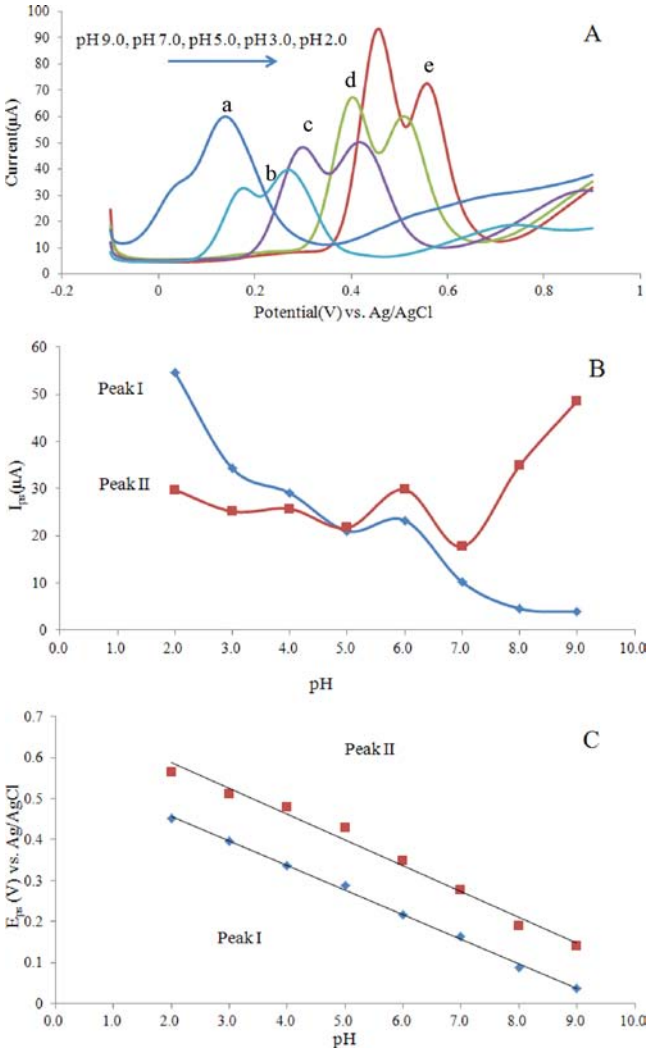

Figure 4. (A) Effect of pH on the differential pulse voltammograms recorded for a 1×10^{-4} mol L^{-1} concentration solution of EGCG at a scan rate of 50 mV s^{-1}. pH values: 2.0, 3.0, 5.0, 7.0, 9.0. Reference electrode = Ag/AgCl. (B) Plot of Ipa (peak I, peak II) versus pH. (C) Plot of Epc (peak I, peak II) versus pH.

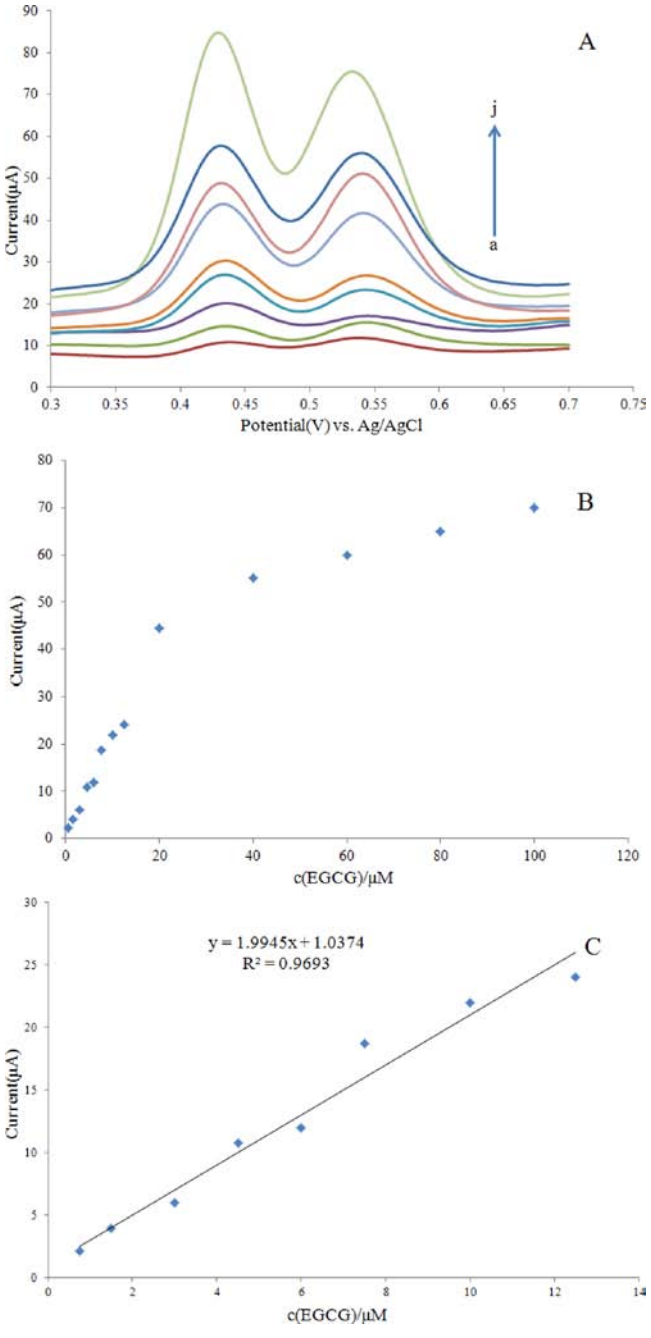

Figure 5. (A) Differential pulse voltammograms obtained under optimized conditions in 0.1 mol L^{-1} phosphate buffer (pH 2.0) solution containing (a) 0.0, (b) 0.5×10^{-6}, (c) 1.5×10^{-6}, (d) 3.0×10^{-6}, (e) 4.5×10^{-6}, (f) 6.0×10^{-6}, (g) 7.5×10^{-6}, (h) 10×10^{-6}, or (j) 12.5×10^{-6} mol L^{-1} EGCG. (B) Dependence of peak I currents on the concentration of EGCG. (C) Linear dependence of peak I currents on concentration of EGCG (concentration below 12.5 μM).

EGCG in phosphate-buffered saline (PBS) (0.1 M, pH 2.0). In the absence of EGCG, no obvious peak (Figure 2C(a)) was observed during the potential scan. After the addition of EGCG, two irreversible oxidation peaks (Figure 2C(b)) appeared at about +0.51 V (peak I) and +0.61 V (peak II). In general, the cyclic voltammograms indicated the electrochemical oxidation of EGCG by two anodic peaks for all of the electrodes tested. Peak I was attributed to the formation of the semiquinone radical, followed by its oxidation to the quinine form (peak II) as shown in Scheme 1.

To show the superior electrochemical activity of the OPFP–CPE for the oxidation of EGCG, other carbon electrodes such as commercial GCE and homemade CPE were used to direct the electrochemical oxidation of EGCG. Figure 2D compared the cyclic voltammetric responses of EGCG at these carbon electrodes. For the homemade CPE (Figure 2D(a)), the response current (0.43 V, 0.18 μA; 0.61 V, 0.53 μA) was very low and the peak potential was high, probably due to the poor electrochemical activity of the paraffin oil between the graphite particles in CPE. On the contrary, OPFP–CPE exhibited a higher peak current and lower overpotential for the electrochemical oxidation of EGCG (Figure 2D(c)), indicating a dramatic increase in the electron transfer rate due to the high conductivity of OPFP. The ionic liquid OPFP in the modified electrode not only acted as a conductive binder but also formed a layer of IL film on the electrode surface. Ping et al.[39] used the OPFP to fabricate a CPE and found an enhanced electron transfer kinetics for the ascorbic acid, which could be ascribed to the electrostatic interaction between the negatively charged ascorbic acid and the cationic OPFP film present on the electrode surface. Therefore, the negatively charged EGCG

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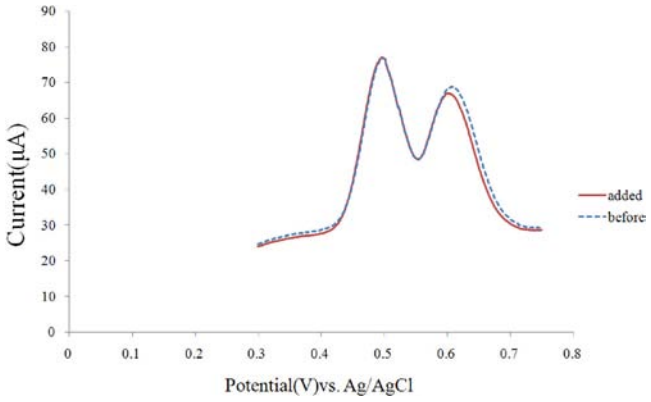

Figure 6. DPV voltammograms of the OPFP−CPE in a 0.1 mol L^{-1} phosphate buffer solution, pH 2.0, containing 1×10^{-5} mol L^{-1} EGCG in the absence (dotted line) and presence (solid line) of caffeic acid, ascorbic acid, ECG, and EGC (1.0 and 10 mg mL^{-1}; 1×10^{-4} and 1×10^{-4} mol L^{-1}).

Table 1. Results of the Recovery of the Spiked EGCG to the Diluted (10-fold) Tea Extract Sample

no.	added EGCG (μM)	found[a] EGCG (μM)	recovery (%)
1	–	9.27	–
2	10	19.18	99.51
3	30	34.41	87.62
4	40	47.18	95.75

[a]Relative standard deviation (RSD) for six repetitive measurements was <4.5%.

(zeta potential = −10 ± 1 mV) was also easily adsorbed onto the surface of OPFP−CPE. The electrochemical response of EGCG at the OPFP−CPE was also superior to that obtained at the GCE (Figure 2D(b)).

The effect of scan rate on the cyclic rate on the cyclic voltammetric response of EGCG at the OPFP−CPE was also investigated and is shown in Figure 3A. It can be observed that with the increase of scan rates, the oxidation peak currents of both peaks I and II increased gradually. The linear relationship was established between the oxidation peak currents and the scan rate in the range of 25−300 mV s^{-1}, indicating the partitioning of the negatively charged EGCG into an organic layer, which is positively charged. Moreover, both of the oxidation peak potentials shifted to positive direction with the increase in the scan rate. As shown in Figure 3B, the oxidation peak potentials show a good linear relationship with the natural logarithm of the scan rate (ln υ, V s^{-1}). The linear regression equations were calculated as E_p (V) = 0.54 + 0.012 ln υ for peak I and E_p (V) = 0.67 + 0.017 ln υ for peak II.

The effect of pH on the oxidation of EGCG (1×10^{-4} mol L^{-1}) was investigated over a range between 2.0 and 9.0 using phosphate buffer solutions. It was observed that EGCG presented well-defined peaks at pH values of <7.0 (Figure 4A). In addition, pH values of >7.0 were avoided because peak I disappeared in this condition. The obtained results indicated that the current values of the anodic peaks reached the maximum value at pH 2.0 for peak I and at pH 9.0 for peak II, as shown in Figure 4B. The anodic peak potentials (peaks I and II) obtained for EGCG oxidation at pH values ranging from 2.0 to 9.0 presented shifts of 60 mV (peak I) and 63 mV (peak II), respectively, by pH unit to more negative values (Figure 4C), thereby indicating that the electrode process is influenced by the protonation reactions. For further studies, a phosphate buffer solution at pH 2.0 was selected.

The amount of ionic liquid in the carbon paste had a significant influence on the DPV response. The peak currents increased with an increasing amount of OPFP up to 50% (w/w). For amounts >50% (w/w), the anodic peak currents became almost constant, probably due to a saturation of the conductive area at the electrode surface. In this way, the best carbon paste composition was achieved with 50% (w/w) OPFP and 50% (w/w) graphite powder. The effect of the scan rate on the OPFP−CPE (solution containing 5.0×10^{-4} mol L^{-1} EGCG) using DPV was investigated in the range of 1−50 mV s^{-1}. For scan rates >16 mV s^{-1}, the increase in the values of the anodic peak currents was accompanied with a broadening and distortion of the peaks. As a result, the optimum scan rate of 16 mV s^{-1} was chosen, and this value was adopted throughout the subsequent studies. Differential pulse voltammograms recorded at several potential pulse amplitude values (10−100 mV) indicated that the values of the anodic peak currents increased with an increase in the potential pulse amplitude. However, the use of a potential pulse amplitude >50 mV led to an increase in the background currents. From the obtained results, a potential pulse amplitude of 50 mV was chosen due to the best voltammetric profile and higher sensitivity.

After optimization of the operating conditions for the OPFP−CPE (scan rate = 16 mV s^{-1}, pulse amplitude = 50 mV, pH 2.0, electrode composition of 50% (w/w) graphite powder), the DPV measurements were carried out in solutions containing different EGCG concentrations. Figure 5 shows the voltammograms obtained and the respective analytical curve. Because the relationship between peak currents of peak II and concentration of EGCG is not linear in any range, the data of peak I were selected. Figure 5A shows the relationship between peak currents of the first oxidation peak and concentrations of EGCG. In the wider concentration range ($c > 1 \times 10^{-5}$ M) this relationship is not linear (Figure 5B), indicating the saturation of the electrode surface, which could be caused by the

Table 2. General Characteristics of the Electrochemical Methods Used for Antioxidant Estimation in Several Samples

electrode	technique	linear dynamic range	sample	ref
glassy carbon electrode modified with polyaspartic acid	differential pulse voltammetry	2.5×10^{-7}–3.0×10^{-5} mol L^{-1} (catechin)	tea	21
glassy carbon electrode modified with multiwalled carbon nanotubes	cyclic voltammetry	100−1000 mg L^{-1} (polyphenols)	tea	19
glassy carbon electrode	square wave voltammetry	1.0×10^{-7}–1.0×10^{-6} mol L^{-1} (EGCG)	tea	24
glassy carbon electrode modified with single carbon nanotubes	differential pulse voltammetry	3.7×10^{-10}–2.4×10^{-9} mol L^{-1} (catechin)	aqueous media	20
ionic liquid carbon paste electrode	differential pulse voltammetry	5.0×10^{-7}–1.25×10^{-5} mol L^{-1} (EGCG)	tea	this work

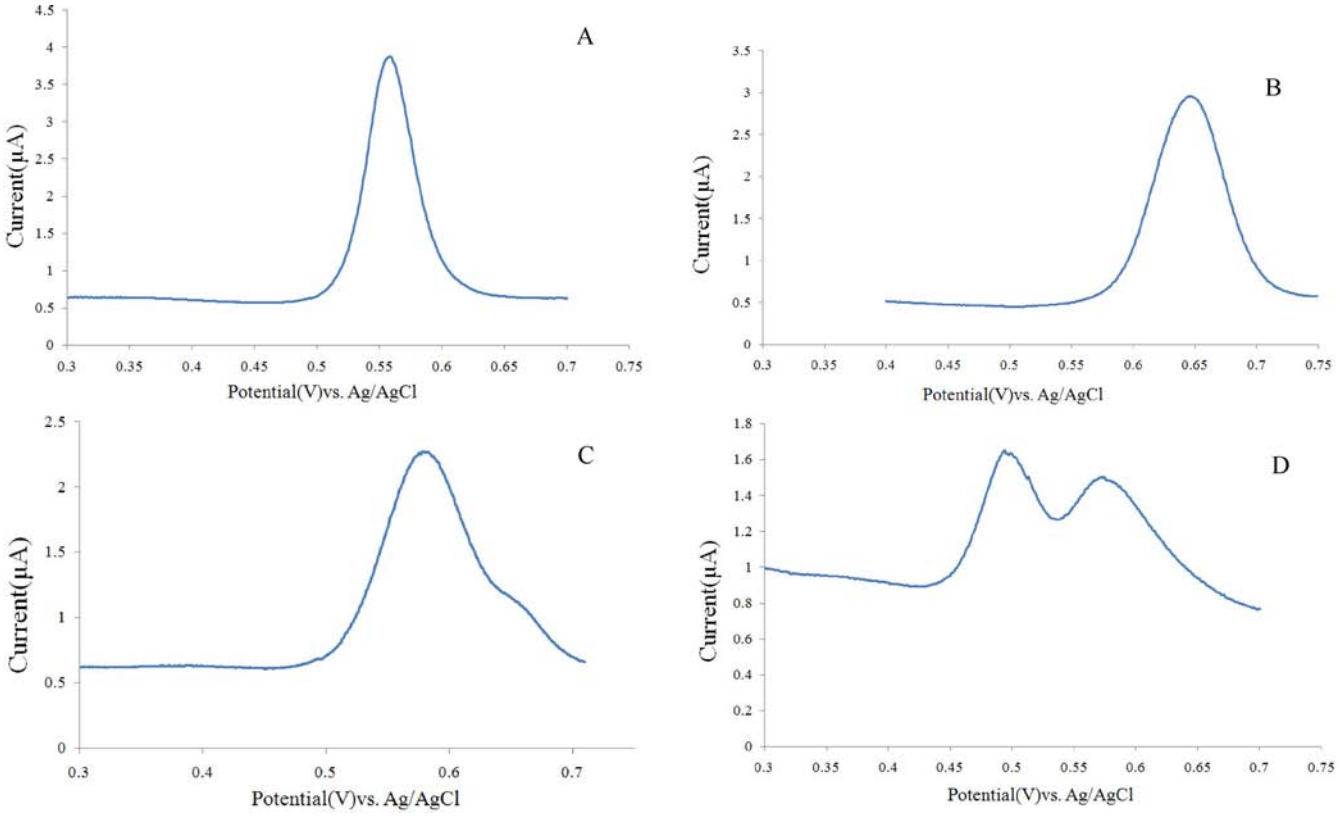

Figure 7. Differential pulse voltammograms obtained under optimized conditions in 0.1 mol L^{-1} phosphate buffer (pH 2.0) solution containing 1.0 × 10^{-6} mol L^{-1} ECG (A), EGC (B), C (C), or EGCG (D).

adsorption of the products of electrode reaction. However, good linearity was found in the concentration range from 5 × 10^{-7} to 1.25 × 10^{-5} M (Figure 5C), which is represented by the line equation

$$i_p\ (\mu A) = 1.0374 + 1.9945[\mathrm{EGCG}]\ (\mu M),$$

$$R^2 = 0.9693$$

Because the EGCG of the green tea was vulnerable to degradation caused by temperature,[44] the linearity of the equation is not good enough. In the future, we will control the temperature of the analysis process to improve the linearity of the equation.

The sensitivity of DPV was determined on the basis of the values obtained for detection and quantification limits. The limit of detection (LOD) is defined as the lowest concentration of analyte that can be detected with an acceptable accuracy, whereas the limit of quantification (LOQ) represents the lowest amount of analyte that can be reliably quantified. LOD and LOQ were calculated from the parameters obtained from the analytical curve, using LOD = $3S_b/s$ and LOQ = $10S_b/s$, where S_b is the standard deviation of the *y*-intercept and *s* is the slope. Under the given conditions, the calculated LOD and LOQ of EGCG were found to be 1.32 × 10^{-7} and 4.35 × 10^{-7} M, respectively. The relative standard deviation (RSD) of ten times repetitive measurements of 5 × 10^{-6} M EGCG with the same electrode was 2.8%, whereas the RSD was 3.5% for 10 different electrodes.

To investigate the concomitant effects of compounds usually present in tea, DPV voltammograms for the OPFP−CPE were carried out in a 0.1 mol L^{-1} phosphate buffer solution, pH 2.0, containing 1 × 10^{-5} mol L^{-1} EGCG in the absence and presence of caffeic acid, ascorbic acid, ECG, and EGC (1.0 and 10 mg mL^{-1}; 1 × 10^{-4} and 1 × 10^{-4} mol L^{-1}). The caffeic acid, ascorbic acid, ECG, and EGC were added to the solution together. In the present study, no significant influence on the voltammetric response for the EGCG was observed (Figure 6).

To illustrate the feasibility of the developed OPFP−CPE for routine analysis, the electrode was applied to determine EGCG concentration in the diluted real tea sample. The sample was diluted with PBS at a volume ratio of 1:9. The analytical results are summarized in Table 1. One can see that the developed OPFP−CPE exhibited exact recovery results, indicating the potential application of OPFP−CPE for the determination of EGCG in real green tea infusion samples. A standard addition plot of peak height versus added EGCG concentration is described by the linear equation

$$i_p\ (\mu A) = 4.066 + 1.7546[\mathrm{EGCG}]\ (\mu M), \quad R^2 = 0.9737$$

The analytical characteristics of the proposed and other electrochemical methods applied to the estimation of antioxidant properties are shown in Table 2.

We used our electrode to analyze the extract from tea and also four catechins (ECG, EGC, C, EGCG) (Figure 7). From the results, we conclude that the differential pulse voltammograms for (−)-epigallocatechin (EGC) (Figure 7A), (−)-epicatechin gallate (ECG) (Figure 7B), and (+)-catechin (C) (Figure 7C) have only one current peak; however, only the differential pulse voltammogram of (−)-epigallocatechin gallate (EGCG) (Figure 7D) has two current peaks at 0.49 and 0.60 V, respectively, which is similar to the differential pulse voltammogram of the extract from tea.

From the above, we can confirm that our experimental data are related to the content of EGCG, not the content of total phenols.

Perhaps the common disadvantage of OPFP−CPE is that it suffers from large capacitive charging currents. Therefore, in our future study, we may make great efforts to overcome this major disadvantage.

For the first time, the present study related the application of a novel ionic liquid carbon paste electrode for the antioxidation estimation in real green tea infusion samples by using the differential pulse voltamety technique. The proposed OPFP−CPE represents a good and easy method for monitoring EGCG in real green samples, exhibiting a good analytical performance due to its stability and reproducibility associated with an easy and rapid preparation, low cost, and longer lifetime when compared with biosensors. The practical usefulness of the OPFP−CPE was demonstrated by the estimation of the content of EGCG in real green tea samples, using an extremely simple procedure involving the direct addition of a sample aliquot in the electrochemical cell, dispensing with any sample pretreatment.

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Notes

The authors declare no competing financial interest.

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