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*Short Communication*

# Electrochemical Detection of Liquiritin in Liquorice Based on Carbon Materials

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To quantify liquiritin, an electrochemical sensor was synthesized with a glassy carbon electrode (GCE), which was modified by the carboxylic acid-based graphene (C-GO) and acidified MWCNT. Compared with the original GCE, the GCE after modification with MWCNT or C-GO exhibited a well-defined oxidation and reduction of liguiritin. Moreover, the current response was enhanced significantly by this process, where the current response of the oxidation peak of liquiritin showed a linear relationship with the concentration of liquiritin in the range of 0.05 to 100 μM when the limit of detection was 0.03 μM. The modified voltammetric performance of the electrochemical sensor demonstrated that it could be practically applied in the determination of liquiritin for the real licorice specimens.

**Keywords:** Electrochemistry, Sensor, Liquiritin, Liquorice, Carbon nanotube; Graphene

## 1. INTRODUCTION

Either in Eastern or Western medicine, licorice have been one of the most essential medicinal herbs. So far, around 200 chemical components have been identified through chemical and pharmaceutical studies [1-4], where triterpenes and flavonoids have been considered as the remarkable bioactive compositions. These ingredients possess various pharmacological activities, such as anti-ulcer, anti-oxidant, anti-viral, immunomodulatory and anti-inflammatory, which have widely been applied in diverse areas including tobacco, food, confectionery and medicinal industry.

To study the components of licorice comprehensively, analyzing the licorice extracts qualitatively and quantitatively is effective and necessary, which is further practical in isolation, purification, production and quality control of the initial materials. For the aqueous licorice extracts, in

general, most of them is liquiritin ((2S)-2-[4-[(2S,3R,4S,5S,6R)-3-[(2R,3S,4S)-3,4-dihydroxy-4-(hydroxymethyl)oxolan-2-yl]oxy-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-7-hydroxy-2,3-dihydrochromen-4-one), which could be employed to evaluate the quality of the licorice as an indicator [5-9]. By far, several analytical approaches have been reported by using various techniques, including gas chromatography (GC) [10], high performance liquid chromatography (HPLC) [11], spectrophotometry [12], polarography [13], micellar electrokinetic chromatography (MEKC) [14] and capillary zone electrophoresis (CZE) [15-17]. For instance, a quantitative analysis of liquiritin in *Glycyrrhiza uralensis* extract was reported by Shen and co-workers through employing HPLC combined with an evaporative light scattering detector [12]. Then a developed and valid HPLC, especially the photodiode array detection was demonstrated by Seo et al. [18] to analyze the liquiritin in *Galgeun-tang*.

HPLC with UV detection have been selected to determine the extracts of licorice among all the methods described above. Nevertheless, some problems have generally been observed in this proposed method. Some practical standard reference materials are difficult to be achieved although numerous chemical compositions of licorice have been recognized. Generally, the diverse compounds have different response factors. In addition, the unstable baselines might be induced by the mobile phase gradient elution, and some non-chromophoric compounds also could not be recognized. Thus, based on these features, it may not be easy or available to quantify some compositions.

Recently, developing electrochemical sensors for detecting molecules has attracted considerable attention, owing to the advantages of electrochemical sensors including high sensitivity and selectivity, low instrumental requirements, short analysis time and simple experimental procedure, which is available in various physiological specimens [19]. The liquiritin could be measured by the normal electrodes, but over-potential and electrode contaminants would be induced by the samples. Moreover, the biological molecules, which could undergo reduction or oxidation under a similar potential window as liquiritin, would also induce an interference. Consequently, the surface modification of the electrode can be employed to improve the analytical capacity for the liquiritin electrochemical determination.

Carbon could be extensively used in electro catalysis and analysis, owing to its high electric conductivity and electro-catalysis activity, the remarkable capacity to accumulate analyte and alleviate surface contamination, as well as the ability of surface modification. Moreover, it has been demonstrated to be stable in most solution and exhibit excellent behavior in a wide range of temperatures. Recently, it has been reported that the electrochemical reactivity of part electroactive biomolecules can be improved by the carbon nanotube (CNT), which can also accelerate the electron-tranfer reactions. Furthermore, the electrodes modified with CNT exhibits the ability to enhance the sensibility of biomolecule accumulation on electrode. In recent years, graphene has attracted extensive interest in science and technology, because of its high surface area, remarkable electric and thermal conductivity as well as high mechanical strength, which are related to its particular physicochemical properties. For the graphitic materials in other dimensionalities, it is just the essential building block.

Herein, we reported the first use of multi-walled carbon nanotube (MWCNT) and graphene to modify the electrode in the electrochemical quantification of liquiritin. We studied the electrochemical behavior of the modified electrodes as well as the performance of liguiritin on various electrode

surface. In addition, we also investigated the practical performance of the modified electrode in the detection of liguiritin for real liquorice samples.

## 2. EXPERIMENTAL

### *2.1. Chemicals*

Graphite powder (99.95%, 325 mesh), ammonia solution (28 wt%) and hydrazine solution (50 wt%) were commercially obtained (Shanghai Chemical Reagent Co., Ltd., Shanghai, China). The MWCNTs were purchased from TECO Nanotech Co., Ltd., Taiwan, where the outer diameter was in the range of 40 to 60 nm, the inner diameter ranged from 2 to 5 nm and the length reached up to several micrometers. A certain amount of acetic acid, boric acid and phosphoric acid were mixed to prepare the BR buffer solution, where the pH was tuned by sodium hydroxide with a concentration of 0.2 M. All the other reagents, which were analytical grade, were used directly without any process unless stated otherwise. Moreover, deionized water with a resistivity of more than 18 MΩ, which was produced by the Milli-Q water purification system (Milli, USA), was used to prepare all the solutions in this work.

### *2.2. Apparatus*

For the electrochemical research, CHI660A electrochemical workstation (CH Instruments, USA) with a standard three-electrode cell was used in this work, where a platinum wire was used as auxiliary electrode, a saturated calomel was employed as reference electrode (SCE) and a modified electrode was utilized as working electrode. All the recorded potential values, which will be described below, refer to SCE.

### *2.3. Acidification of MWCNT*

First, in 50 mL mixture of concentrated $H_2SO_4$ and HCl mixture (3:1, v/v), 50 mg MWCNT were oxidized under sonication for 6 h. Then, the obtained mixture was centrifuged at 14680 rmp for 15 min. Noted the centrifugation was repeated twice. Subsequently, the solution was water, where NaOH was used here to tune the pH of the solution to neutral. At last, after being dried overnight in oven at 70 $^{o}$C, the solid oxidized MWCNT was obtained.

### *2.4. Carboxylic acid functionalized graphene preparation*

Based on the Hummers and Offeman method, Graphene oxide was synthesized using graphite as precursor [20]. In a typical procedure, 100 mL dispersion of the obtained graphene oxide was added into the mixture of 70 μL hydrazine solution (50 wt%) and 0.7 mL 28% ammonia solution, where the graphene oxide would chemically convert to graphene. Subsequently, the resulting mixture was stirred

at 95 $^{o}$C for 1 h. Then, the mixture was filtered and dried under vacuum to obtain a black hydrophobic powder of graphene. Moreover, in order to synthesize the graphene functionalized with carboxylic acid, graphene oxide was washed first with 5% HCl solution and then with water to adjust the pH of the filtrate to neutral. At last, the hydroxyl or carboxylic group functionalized graphene sheets, which served as C-GO, was prepared by ultra-rapid heating and splitting of graphene oxide.

### *2.5. Electrode modification*

The initial glassy carbon electrode was first polished with alumina slurry of 0.3 and 0.05 μm and then washed with water and ethanol before surface modification. A definite amount of C-GO (0.5 mg/mL) or MWCNT dispersion, in which the adsorbed impurities had been removed, was deposited on the surface of GCE and dried at room temperature. Then, the electrodes modified with MWCNT and C-GO was endowed as MWCNT/GCE and C-GO/GCE, respectively.

### *2.6. CV, EIS and SWV parameters*

Cyclic Voltammetry (CV) was used for analyzing the target molecule behaviors at electrode surface. CV was conducted in 0.1 M BR buffer solution (pH 8.0) at scan rate of 50 mV/s. Electrochemical impedance spectroscopy (EIS) was used for characterizing the electrode resistance performance. 5 mM $[Fe(CN)_6]^{3-/4-}$ was used as probe, 0.1 M KCl was used as supporting electrolyte. Frequency range was set as $10^1$ to $10^5$ Hz and the amplitude was set as 5 mV. CV was performed in 0.1 M pH 7.0 PBS from -0.2 to -1.2 V at scan rate of 50 mV/s. To optimize the conditions in determining acetaminophen through square-wave voltammetry method, numerous instrumental parameters were investigated, including amplitude, frequency and step potential. Noted that the best condition was amplitude of 50 mV, step potential of 1 mV and frequency of 8 Hz.

### *2.7. Real sample analysis*

First, the commercially available liquorice was dried for 4 h in oven at 60 $^{o}$C. Then, around 1 g powder, which had been pulverized, was dispersed in 50 mL 70% ethanol solution and refluxed at 80 $^{o}$C for 1h. Subsequently, the solution was cooled down and filtered through the paper filter. Then the as-prepared solution was reduced to 50 mL under reduced pressure. At last, 2.0 mL obtained solution was diluted to 50 mL by the BR buffer.

## 3. RESULT AND DISCUSSION

The change in the surface property of electrodes after modification was studied in detail by Electrochemical impedance spectroscopy (EIS). Two parts, such as the linear and the semicircle portion, were included in the impedance spectra. The semicircle diameter at high frequency was

corresponding to the electron-transfer resistance ($R_{et}$), whereas at low frequency the linear portion represented the diffusion process. The impedance spectra of various electrodes were shown in Figure 1. It was obvious that the EIS of the bare GCE exhibited a relatively small well-defined semi-circle at high frequency. This indicated that the interface impedance was low. In contrast, a nearly straight line was observed in the Nyquist plot of the impedance spectroscopy when MWCNT was deposited on the GCE, which was corresponded to the diffusion-limited electron-transfer process. The electron transfer could be improved by MWCNT which could induced the decrease of $R_{et}$, owing to its remarkable electric conductivity. Moreover, the change in the process of modification suggested that the MWCNT was anchored on the surface of the electrode after modification. For C-GO/RGO, the obtained behavior was similar with MWCNT, which indicated that C-GO also was a remarkable electric conducting material. Thus, both MWCNT and C-GO can improve the capacity of the electrode and induce a better electrochemical activity. Similar results also been reported by other scholars. For example, Peña and co-workers claimed the enhancement of the electrode capacity after surface modification of multi-walled carbon nanotube [21]. Han and co-workers reported the clear enhancement of the electrode after modification of graphene [22].

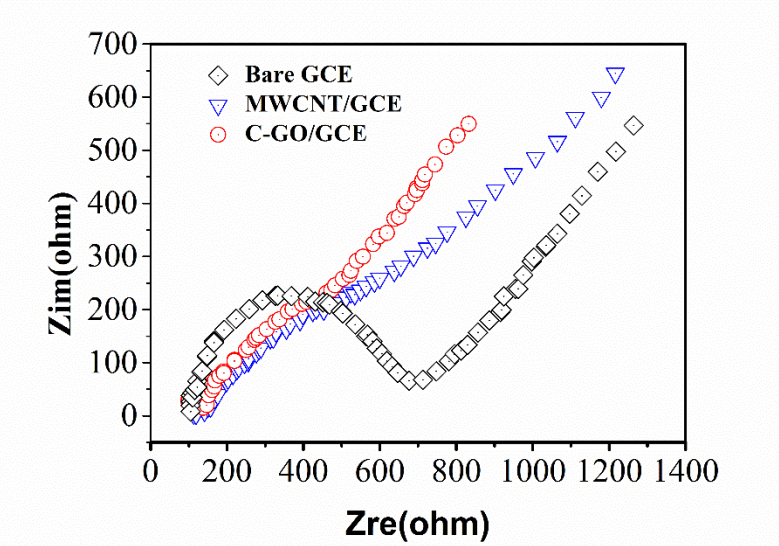


**Figure 1.** Nyquist plots of the diverse electrodes in a PBS (pH 7.0) solution containing 0.1 M KCl and 5.0 mM $Fe(CN)_6^{4-/3-}$. The frequency range was from $10^{-1}$ to $10^5$ Hz with perturbation amplitude of 5 mV.

Furthermore, cyclic voltammetry was employed to study the electrochemical performance of the MWCNT/GCE and C-GO/GCE. Figure 2A, 2B and 2C illustrated the cyclic voltammograms of liquiritin with a concentration of 0.05 mM at the bare GCE, MWCNT/GCE and C-GO/GCE respectively, where the liquiritin was dispersed in 0.1 M BR buffer solution with a pH of 8.0. In prior to add liquiritin, the bare GCE, MWCNT/GCE and C-GO/GCE described above had not exhibited any responses. However, the background current of MWCNT/GCE and C-GO/GCE observed in cyclic voltammograms were higher than that of the bare GCE, which suggested that larger effective electroactive surface area was obtained with these two materials compared ot the bare GCE. After

adding the liquiritin, for the bare GCE and MWCNT/GCE, an oxidation peak at about 0.54 and 0.52 V were observed respectively. However, two definite redox peaks were obtained for C-GO/GCE. It was obvious that the oxidation and reduction peaks were obtained at 0.51 and 0.46 V respectively, where the oxidation peak current of C-GO/GCE exhibited 100 times higher than that of the bare GCE. The significant increase of the peak current of C-Go/GCE suggested that C-GO exhibited a high electroactive surface and an excellent electrocatalytic capacity to liquiritin. Especially, liquiritin at C-GO/GCE showed a lower oxidation peak potential than that at either the bare GCE or the MWCNT/GCE, which indicated that C-GO/GCE was an efficient electrocatalyst for liquiritin redox reaction.

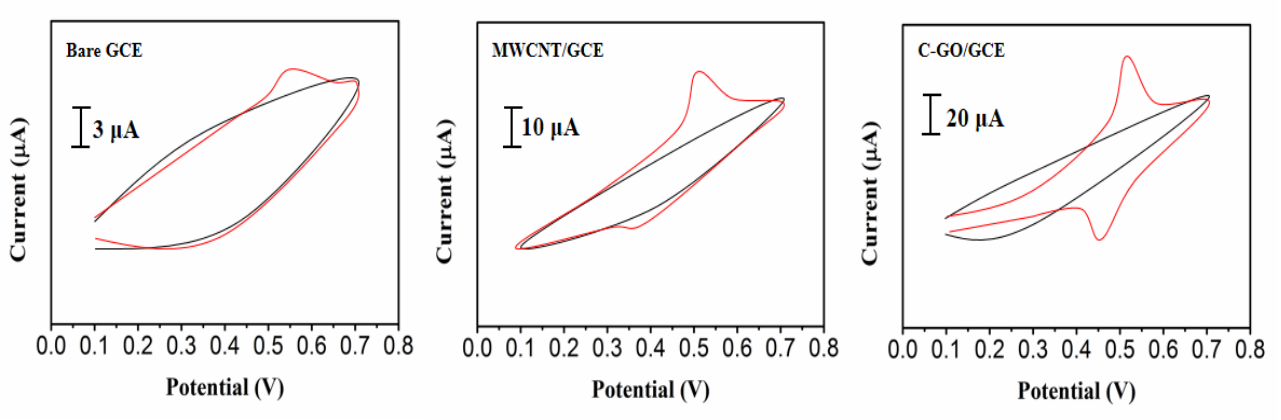


**Figure 2.** Cyclic voltammograms of the bare GCE, C-GO/GCE and MWCNT/GCE with and without 0.05 mM liquiritin in 0.1 M BR buffer solution (pH 8.0) at. Scan rate: 50 mV/s.

As the protons in the whole electrode reaction might be attributed to the liquiritin, the pH value of the solution could influence the redox system. Thus, the pH value in the range of 3 to 11 was employed to study the effect of the solution pH on the redox reaction. Moreover, the cyclic voltammograms of liquiritin with a concentration of 0.05 mM under various solution pH was collected, where the liquiritin was dispersed in the BR buffer solution with a concentration of 0.05 mM. The values of solution pH exhibited a negative relationship with both the oxidation and reduction peak potentials of the C-GO/GCE redox couple. As shown in Figure 3A, the oxidation peak potential ($E_{pa}$) of liquiritin showed a negative linear relationship with the pH values ranging from 3 to 11, where the slope was calculated to be −61.2 mV/pH. This result was approximate to the theoretical data of −57 mV/pH, which was in accordance with the Nernst equation for the transfer reaction of the two-electron and two-proton. In Figure 3B, it was obvious that the anodic peak currents of the liquiritin was enhanced when the values of the solution pH were increased from 3 to 11. However, the sensor exhibited a decreased response when the pH value of the solution was beyond 8.

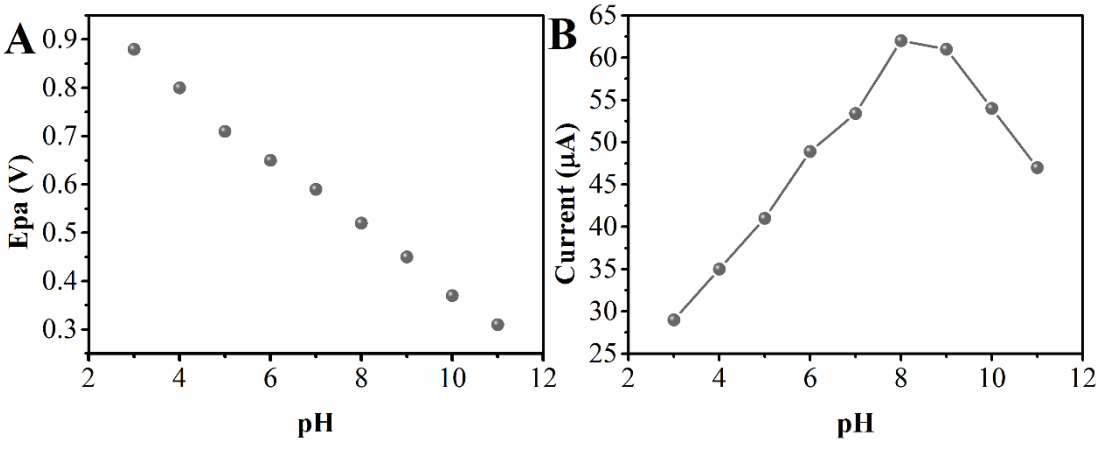


**Figure 3.** (A) The relationship between $E_{pa}$ vs. and the pH value and of the solution; (B) the relationship between anodic peak current response and the pH value of the solution recorded in cyclic voltammograms of 0.05 mM liquiritin in 0.1 M BR buffer solution at the C-GO/GCE with solution pH values.

Moreover, the square-wave voltammetry of liquiritin was performed to enhance the sensitivity and decrease the limit of liquiritin detection, where the liquiritin was dispersed in 0.1 M BR buffer solution with a pH of 8.0. Figure 4 showed the responses of square-wave voltammetry on the CGO/GCE in the process of successively adding liquiritin described above. As shown in Figure 4, the anodic peak current exhibited a positive linear relationship with the concentration of liquiritin ranging from 0.05 to 100 μM. For the C-GO/GCE, the equation of linear regression was $I_{pa}$ (A) = 24.9$C_{liquiritin}$ (M) + 1.57 × $10^{-3}$ (mA) ($r$ = 0.998). The limit of the detection was calculated to be 0.02μM. The limit of the liquiritin determination with the electrode after modification was lower than that with a ultra-performance liquid chromatography (0.25 μM) [23], a high-performance liquid chromatographic technique (0.25 μM) [24] and a liquid chromatography-electrospray ionization-tandem mass spectrometry [25]. This analytical performance can be compared with those recently reported in the literature for Table 1. As compared with different determination methods, due to the high sensitivity of the proposed electrochemical performance, the C-GO/GCE could be used for potentially detecting liquiritin at real samples.

**Table 1.** Comparison of our proposed liquiritin electrochemical sensor with other reports.

| Electrode | Linear range (μM) | Limit of detection (μM) | Reference |
|---|---|---|---|
| HPLC-PDA | 0.15-0.6 | 0.04 | [26] |
| High-performance liquid chromatographic method | 0.25-0.7 | 0.121 | [27] |
| Ultra-performance liquid chromatography–mass spectrometry | 0.00254–1.02 | — | [28] |
| High-performance liquid chromatographic method | 0.15-0.3 | 0.08 | [29] |
| C-GO/GCE | 0.05-100 | 0.02 | This work |

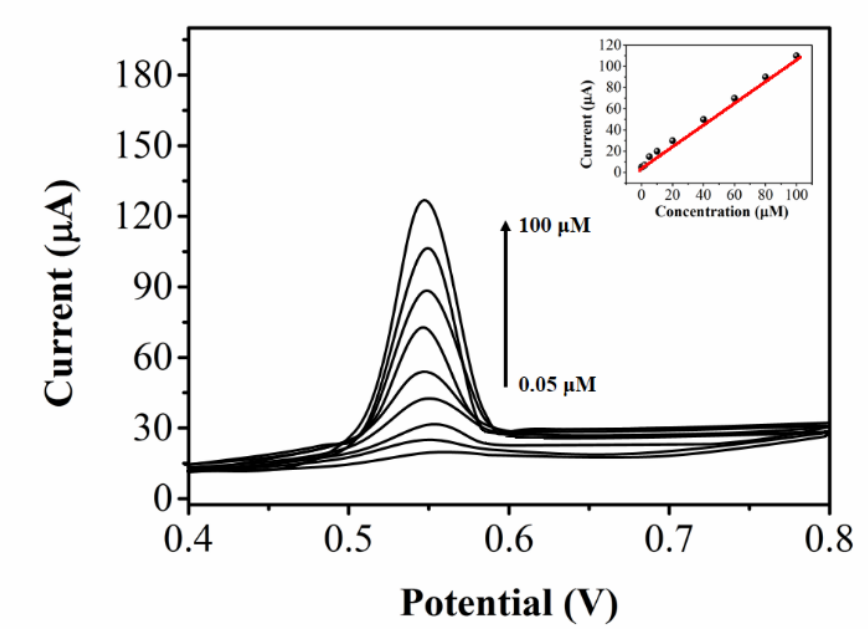


**Figure 4.** Square-wave voltammetric responses to the successive additions of liquiritin with a concentration in the range of 0.05 to 100 μM in 0.1 M BR buffer solution (pH 8.0) at the C-GO/GCE. The inset shows the corresponding calibration curve.

**Table 2.** Determination of the content of liquiritin in liquorice extract samples using C-GO/GCE.

| Sample | Addition (μM) | Found (μM) | Recovery (%) |
|---|---|---|---|
| 1 | 0 | 14.57 | — |
| | 10 | 54.54 | 99.88 |
| 2 | 0 | 15.02 | — |
| | 20 | 35.12 | 100.26 |
| 3 | 0 | 14.88 | — |
| | 50 | 65.33 | 100.69 |

At last, four extracts of grape fern herb were investigated to evaluate the practicability of the designed C-GO/GCE to determine the liquiritin in liquorice. To confirm the viability of the proposed approach, the spike and recovery process was employed. Table 2 illustrated the results of the designed electrochemical sensor. It was obvious that the designed C-GO/GCE exhibited a remarkable capacity in detect the liquiritin in the samples of liquorice extract, which indicated that the proposed approach was effective for detecting liquiritin in the real herb specimens.

## 4. CONCLUSION

In conclusion, we reported the modification of the electrode surface with the carbon materials such as MWCNT and C-GO. A reliable analytical approach to determine the liquiritin in the extraction

of liquorice with sensitivity and selectivity was firstly elaborated by the obtained GCE which was modified with MWCNT or C-GO. It was demonstrated that the GCE modified with C-GO showed a remarkable behaviour for the electrochemical oxidation and reduction of liquirtin, where a linear relationship was observed between the response and the concentration in the range of 0.05 to 100 μM.

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