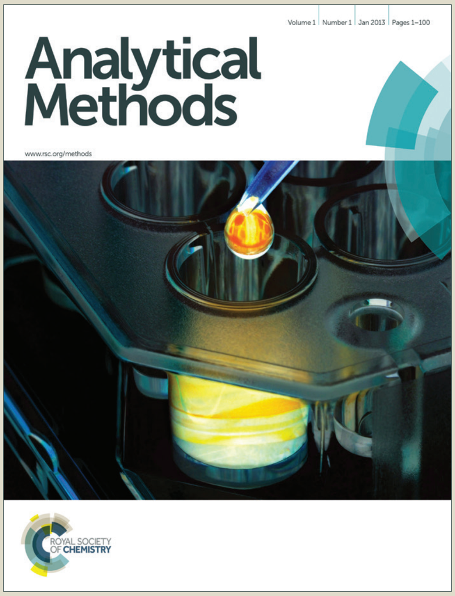
Analytical Methods



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Electrochemical behaviors of luteolin on chitosan-graphene modified glassy carbon electrode and its sensitive detection

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A simple and highly sensitive electrochemical method was developed for the determination of luteolin based on the chitosan-graphene (GR) modified glassy carbon electrode. Due to the specific characteristics of GR present on the electrode surface, electrochemical response of luteolin on the modified electrode was greatly enhanced with a pair of well-defined redox peak appeared. The results were attributed to large surface area and high conductivity of GR with the considerable improvement of the redox peak current, which allowed the development of a highly sensitive voltammetric sensor for the determination of luteolin. Under the optimized conditions the oxidative peak currents increased linearly with the concentration of luteolin in the range from 2.0 nmol L^{-1} to 60.0 μmol L^{-1} with a detection limit of 5.93×10^{-10} mol L^{-1} ($3S_0/S$). The analytical performance of this sensor has been evaluated for the detection of luteolin in Duyiwei capsule as a real sample with satisfactory results.

Introduction

Luteolin (3′,4′,5′,7′-tetrahydroxyflavone) is one kind of flavonoids that abundant in many plants such as celery, green pepper, parsley and perilla leaf [1]. Luteolin has many biochemical and pharmacological effects such as anti-oxidation, anti-bacteria, anti-virus, anti-inflammatory and anti-carcinogenic [2]. Recent reports indicate that luteolin exhibits anti-proliferative activity against cancer cells and suppress oxidative damage to DNA, lipid, proteins and carbohydrates [3, 4]. Therefore it is necessary to establish a sensitive, rapid and accurate method for the detection of luteolin in pharmaceutical samples. Up to now different kinds of analytical methods, such as HPLC [5, 6], capillary electrophoresis [7], gas chromatography [8], spectrophotometry [9] and electroanalysis [10, 11], have been reported for the detection of luteolin. Some of these methods involve complicated instruments with time-consuming procedure, insufficient selectivity or poor accuracy. Due to the electroactivity of luteolin with catechol group on the B ring, electrochemical behavior of luteolin had been investigated on different kinds of working electrodes [12, 13]. Some nanomaterials modified electrodes have been devised and used for the sensitive detection of luteolin. Zhao et al. fabricated a multi-walled carbon nanotube modified electrode for the sensitive electrochemical detection of luteolin in peanut hulls [11]. Zeng et al. applied a macroporous carbon modified glassy carbon electrode (GCE) for the electrochemical detection of luteolin [14]. Recently Pang et al. developed an electrochemical method for the luteolin detection based on graphene (GR) and hydroxyapatite nanocomposite modified GCE [15]. However, the reports about electrochemical detection of luteolin are relatively seldom.

As a single layer of carbon nanomaterial, GR has aroused great interests due to the specific characteristics such as unique geometrical structure, mechanical and electronic properties [16]. GR has been widely used in different fields such as supercapacitor, sensors and so on [17]. Because of the high conductivity of GR, GR and its related composite have been used in electrochemical sensors, which can accelerate the electron transfer between the analyte and the electrode surface [18, 19]. GR modified electrode have been used for the detection of electroactive substances such as rutin [20], dopamine [21], DNA [22] and bisphenol A [23]. However, GR nanosheets tend to aggregate back due to the strong π-π interaction and *Van der Waals*, which limits its real applications [24]. As a natural biopolymer, chitosan (CS) is a linear hydrophilic polysaccharide that has been widely used in the field of electrochemical sensors [25]. The mixture of CS with GR can result in a stable composite and the positively charged CS can interact with the negatively charged GR nanosheets to prevent the aggregation [26]. Therefore the CS-GR composite modified electrode has been used for the electrode modification and electrochemical applications. Wang et al. reported the electrochemical detection of dopamine by a CS-GR modified glassy carbon electrode (GCE) [27]. Yin et al. fabricated a CS-GR composite film modified GCE for the voltammetric determination of 4-aminophenol [28]. Han et al. applied a CS-GR modified electrode for the simultaneous voltammeteric determination of ascorbic acid, dopamine and uric acid [29]. The CS-GR composite had also been applied to the investigation of the direct electrochemistry of redox proteins such as glucose oxidase [30] and Cytochrome C [31]. However, there are no reports about the CS-GR modified electrode for luteolin detection.

In this paper a CS-GR modified GCE was fabricated and applied to the investigation on the electrochemical behaviors of luteolin. Under the selected conditions a pair of well-defined redox peaks appeared on the cyclic voltammograms, and the presence of GR resulted in the increase of the redox peak currents due to its large surface area that adsorbed more luteolin on the electrode surface. A sensitive differential pulse voltammetric method was further established for the luteolin and drug samples detection with satisfactory results.

Experimental

Instruments and reagents

All the electrochemical measurements including cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy (EIS) were performed on a CHI 660D electrochemical workstation (Shanghai CH Instrument, China). A conventional three-electrode system was used with GCE or modified GCE as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. Scanning electron microscopy (SEM) was conducted with a JSM-7100F scanning electron microscope (JEOL, Japan). Raman spectrum was recorded on a LabRAM HR800 laser confocal micro-Raman spectroscopy (Horiba Jobin Yvon Co., France).

1.0×10^{-3} mol L^{-1} luteolin (Xi'an Yuquan Biotech Ltd. Co., China) solution was prepared with anhydrous ethanol and used as the stock solution. Chitosan (CS, Dalian Xindie Ltd. Co., China), graphite powder (particle size of 30 μm, Shanghai Colloid Chem. Co., China) and GR (Taiyuan Tanmei Ltd. Co., China) were used as received. 0.04 mol L^{-1} Britton-Robinson (B-R) buffer solution with various pH values were used as the supporting electrolyte. All the other chemicals used were of analytical reagent grade and doubly distilled water was used in all the experiments.

Electrode preparation and modification

Prior to modification GCE was polished successively with 0.3 μm and 0.05 μm Al_2O_3 powder to obtain mirror-like surface. Then it was rinsed thoroughly with ethanol and water each for 5 min, and dried with blowing N_2. A homogeneous dispersion of CS-GR aqueous solution was prepared by dissolving 5 mg GR and 10 mg CS into 10 mL acetic acid (mass fraction of 1%) with ultrasonic treatment for 30 min. Then 5 μL of the CS-GR solution was dropped onto the surface of the freshly polished GCE and dried in ambient air to get the CS-GR/GCE. For comparison CS and graphite powder mixture was also prepared and applied on the GCE surface to get the CS-graphite modified GCE.

Procedure

The standard solution or sample solution of luteolin was added into 0.04 mol L^{-1} B-R buffer solution (pH = 4.0). Cyclic voltammograms was recorded from -0.2 to 0.8 V at a scan rate of 0.1 V s^{-1}. Differential pulse voltammetry was performed in the potential range from 0.15 to 0.55 V with pulse amplitude of 50 mV, pulse width of 50 ms and pulse period of 0.2 s.

Results and discussion

Characteristics of the modified electrode

The surface morphologies of GR and CS-GR on the GCE surface were recorded by SEM with the results shown in Fig. 1A and B. It can be seen that corrugated and scrolled sheets that resembled as crumpled silk veil waves appeared, which was the typical characteristic of GR nanosheets. The GR layers could interact with each other to form an open pore system with the increase of the surface area, through which electrolyte ions could easily access into the surface of GR to form electron transfer channels. Also the surface area was increased greatly due to the presence of GR on the electrode surface. Raman spectrum of GR used was recorded with the result shown in Fig.1C, which gave two prominent bands around 1340 and 1580 cm^{-1}. Those bands are assigned to the D and G bands of GR, respectively.

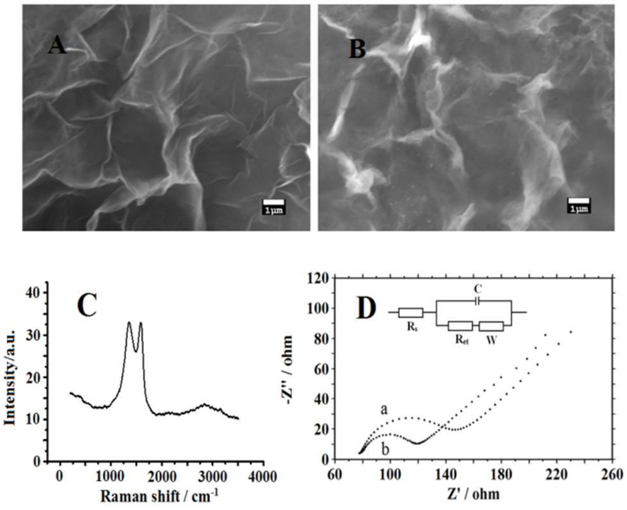


Fig. 1 SEM images of GR (A) and CS-GR (B) on the surface of GCE; (C) Raman spectrum of GR; (D) EIS of (a) GCE and (b) CS-GR/GCE in a 10.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol L^{-1} KCl solution with the frequencies swept from 10^5 to 1 Hz (Insert is the Randles circuit model in the cell) .

Electrochemical impedance spectroscopy (EIS) can exhibit the impedance changes during the modification processes, which is further used for the investigation on the modified electrode. The value of the electron transfer resistance (R_{et}) depends on the dielectric and insulating features at the electrode/electrolyte interface with different modification processes. The Randles circuit is used to fit the impedance data obtained in the experiment with the scheme added as the insert of Fig. 1D, where R_s is solution resistance, C is interface capacitance, R_{et} is electron transfer resistance and W is dispersion resistance. Fig. 1D showed the EIS results of bare GCE (curve a) and CS-GR/GCE (curve b) in a 10.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1 mol L^{-1} KCl solution with the frequency range swept from 10^5 to 1 Hz. The AC voltage amplitude was 5 mV and the applied potential was 0.202 V. Here Z' and Z'' are the real variable and the negative value of the imaginary variable of impedance. On GCE (curve a) the R_{et} value was got as 87.4 Ω, and on CS-GR/GCE (curve b) the R_{et} value was decreased to 44.4 Ω, which was due to the presence of high conductive GR nanosheet on the electrode surface that could decrease the interfacial resistance.

Cyclic voltammetric behaviors of luteolin on the modified electrode

Electrochemical behaviors of 1.0 μmol L^{-1} luteolin at different electrodes were further recorded in 0.04 mol L^{-1} pH 4.0 B-R buffer solution with the voltammograms shown in Fig.2. It can be seen that a pair of redox peaks appeared on all the electrodes, which indicated that electrochemical reaction of luteolin was realized on different electrodes. Luteolin is an electroactive compound that can take place redox reactions directly on the electrode with a pair of well-defined redox peaks appeared [11]. As shown in Fig.2A, no electrochemical responses were observed in the buffer solution (curve a), and on GCE (curve b) the redox peak potentials of luteolin were got as 0.47 V (Epa) and 0.44 V (Epc) with the redox peak currents as -1.02 μA (Ipa) and 0.72 μA (Ipc). While on CS-GR/GCE (curve c) the redox peak currents of luteolin increased greatly with the values as -5.14 μA (Ipa) and 4.10 μA (Ipc), which could be attributed to the presence of high conductive GR film on the electrode surface. GR has been elucidated with many specific characteristics such as high conductivity and big surface area, which can act as an effective mediator to promote the electrochemical reaction of luteolin. Therefore the GR modified electrode exhibit excellent electrochemical performance and can be used for the sensitive detection of luteolin. Recently Figueiredo-Filho et al. [32] explored the origins of the apparent electrocatalytic activity of GR modified electrode with kojic acid as the model, which indicated that graphite on the electrode surface also exhibited certain electrocatalytic activity. So the electrochemical behaviors of luteolin were further investigated on CS-graphite/GCE by cyclic voltammetry with the results shown in Fig.2B. It can be seen that a pair of well-defined redox peaks also appeared on cyclic voltammogram. However, with the addition of different amounts of CS-graphite composite on the GCE surface, the redox peak current decreased gradually, which indicated that the presence of graphite powder on the electrode surface exhibited negative effects for the electrochemical reaction of luteolin. The presence of CS-graphite on the electrode surface increased the thickness of interface and the distances for electron transfer, which resulted in the decrease of the redox peak currents. Also the result further proved that the presence of GR was superior to that graphite powder, which maybe due to the presence of CS that could disperse GR and avoid the aggregation with its specific electrochemical properties remained.

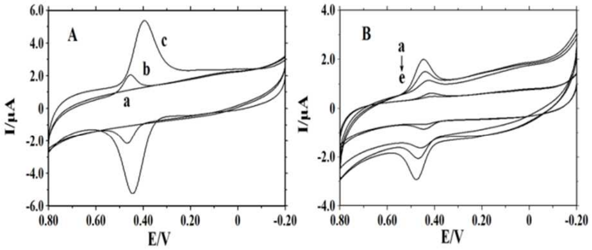


Fig. 2 (A) Cyclic voltammograms of pH 4.0 B-R buffer solution (a), and 1.0 μmol L^{-1} luteolin (b) on GCE and (c) CS-GR/GCE; (B) cyclic voltammograms of 1.0 μmol L^{-1} luteolin on CS-graphite/GCE with the increasing amounts of graphite as 0, 8, 12, 16, 20 μg on the electrode surface (curves a to e). Scan rate:100 mV s^{-1}.

Optimization of GR amount for the electrode modification

The amount of GR applied on the electrode surface was optimized by recording the electrochemical responses of luteolin solution. As shown in Fig.3, with the volume of CS-GR composite increased on the electrode surface the redox peak currents increased first until it was up to 5 μL and then decreased gradually. The results indicated the less modified electrode cannot adsorb enough luteolin on the electrode surface, while over modified electrode results in the thick interface with the long electron transfer path. Therefore 5 μL of 1.0 mg mL^{-1} CS-GR solution was selected as the optimal amount for modification.

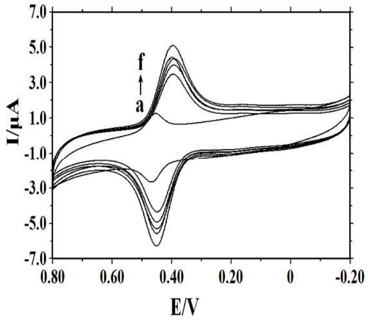


Fig. 3 Cyclic voltammograms of 1.0 μmol L^{-1} luteolin on CS-GR/GCE with different amounts of CS-GR at the scan rate of 100 mV s^{-1} (from a→f: 0, 2, 3, 4, 6, 5 μL)

Effect of buffer pH

The effect of buffer pH on the electrochemical responses of 1.0 μmol L^{-1} luteolin on CS-GR/GCE was investigated in the pH range from 2.0 to 8.0. Fig. 4A showed the cyclic voltammograms of luteolin in different pH solution from 2.0 to 8.0, which gave a pair of redox peaks. However cyclic voltammetric curves were deformed gradually with the increase of the buffer pH, indicating that the electrochemical reaction of luteolin was difficult to be realized gradually. The relationship of the redox peak current and the formal peak potential ($E^{0'}$) with buffer pH were plotted with the results shown in Fig. 4B and Fig. 4C. It can be seen that maximum peak current appeared at pH 4.0 and then decreased with further increase of pH. So pH 4.0 was selected as the optimal buffer pH. The value of $E^{0'}$ shifted negatively with the increase of pH value (Fig. 4C), indicating that protons were involved in the electrode reaction. A good linear relationship between $E^{0'}$ and pH was constructed with the linear regression equation as $E^{0'}$(V) =0.72 - 0.064 pH (r = 0.999). The slope value of −64.0 mV pH^{-1} was close to the theoretical value of −59.0 mV pH^{-1} at 25°C, indicating that an equal number of protons and electrons involved in electrode reaction.

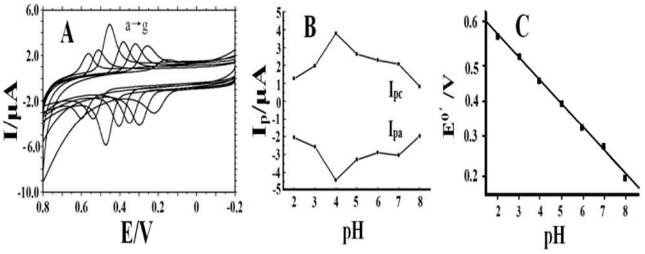


Fig. 4 (A) Cyclic voltammograms of 1.0 μmol L^{-1} luteolin on CS-GR/GCE in different pH B-R buffer solution with the scan rate as 100 mV s^{-1} (from a to g: 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0); (B) the relationship of I_p against pH; (C) the relationship of $E^{0'}$ against pH.

Effect of scan rate

The influence of scan rate on the electrochemical response of 1.0 μmol L^{-1} luteolin was investigated on CS-GR/GCE by cyclic voltammetry in pH 4.0 B-R buffer solution with the results shown in Fig. 5. A pair of well-defined redox peaks appeared in the scan rate range from 20 to 500 mV s^{-1} and the redox peak currents increased gradually with the increase of scan rate. The relationship of log I_p with log υ was obtained with two linear regression equations as log I_{pa} (μA) = 0.77 log υ (V s^{-1}) + 1.75 (r = 0.999) and log I_{pc} (μA) = 0.82 log υ (V s^{-1}) + 1.76 (r = 0.999). The slope values were got as 0.77 and 0.82, respectively, which were between 0.5 and 1.0, the ideal value of diffusion-controlled and surface-controlled electrode process [33]. So the electrochemical reaction of luteolin on CS-GR/GCE was the combination of diffusion-controlled and surface-controlled process. The presence of GR can greatly enhance the surface area that can absorb more luteolin on the electrode surface, also the high conductivity of GR can enhance the electron transfer rate of luteolin with electrode. So the electrode process of luteolin on CS-GR/GCE was controlled by diffusion and adsorption simultaneously.

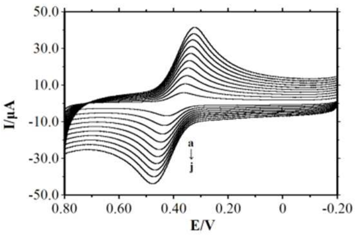


Fig.5 Cyclic voltammograms of 1.0 μmol L^{-1} luteolin on CS-GR/GCE in pH 4.0 B-R buffer solution at various scan rates (a-j: 20, 40, 60, 80, 100, 150, 200, 300, 400, 500 mV s^{-1}, respectively).

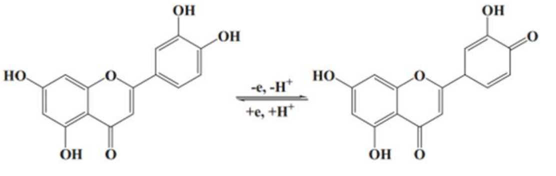
With the increase of scan rate the oxidation peak potentials moved to the positive direction and the reduction peak potentials moved to the negative direction with the increase of the ΔE_p value, indicating a quasi-reversible process. The relationship of the redox peak potentials with ln υ were further obtained with two linear regression equations as E_{pa}(V) = 0.048 lnυ (V s^{-1}) + 0.51 (r = 0.997) and E_{pc}(V) = -0.039 lnυ(V s^{-1}) + 0.030 (r = 0.997). Based on the following equations [34] :

$$E_{pa} = E^{0'} + m[0.78 + \ln(D^{1/2}k_s^{-1}) - 0.5\ln m] + \frac{m}{2}\ln\upsilon,\ m = \frac{RT}{(1-\alpha)nF} \qquad (1)$$

$$E_{pc} = E^{0'} - m'[0.78 + \ln(D^{1/2}k_s^{-1}) - 0.5\ln m'] - \frac{m'}{2}\ln\upsilon,\ m' = \frac{RT}{\alpha nF} \qquad (2)$$

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF\upsilon} - \frac{(1-\alpha)\alpha F\Delta E_p}{2.3RT} \qquad (3)$$

The electrochemical parameters were calculated with the results of the charge transfer coefficients (*α*) as 0.48, the apparent heterogeneous electron transfer rate constant (k_s) as 2.1 s^{-1} and the electron transfer number (*n*) as 0.84. The results indicated that about one electron was involved in the electrochemical reaction. Based on the reference [15], the electrode reaction process of luteolin can be expressed with the following equation, which involvs the oxidation of 4′-hydroxyl that is more active than the 3′-hydroxyl on the between ring.



Scheme 1. Electrochemical reaction process of luteolin

Calibration curve

Under the selected conditions a series of luteolin solutions were measured by differential pulse voltammetry (DPV) with the typical voltammograms shown in Fig. 6A and C. The oxidation peak currents increased linearly with luteolin concentration in the range from 2.0 nmol L^{-1} to 6.0 μmol L^{-1} and 6.0 to 60.0 μmol L^{-1} with the linear regression equations as I_{pa}(μA) = 0.002 - 1.20 C (μmol L^{-1}) (r

= 0.998) and I_{pa}(μA) = -7.54 - 0.458 C (μmol L^{-1}) (r = 0.999). The detection limit of luteolin with CS-GR/GCE was estimated to be 5.93 × 10^{-10} mol L^{-1} ($3S_0/S$), where 3 is the factor at the 99% confidential level, S_0 the standard deviation of the blank measurements without luteolin (n = 9) and S the slope of the calibration curve. Also the calibration curve for the detection of luteolin was established by using GCE as the working electrode. On GCE the oxidation peak current of luteolin was proportional to the concentration in the range from 8.0 nmol L^{-1} to 6.0 μmol L^{-1} with the detection limit as 2.97 nmol L^{-1}. The analytical parameters for the electrochemical detection of luteolin on different modified electrodes were summarized in Table 1. It can be seen that CS-GR/GCE exhibited broader detection range and lower detection limit, which had better sensitivity for the luteolin detection.

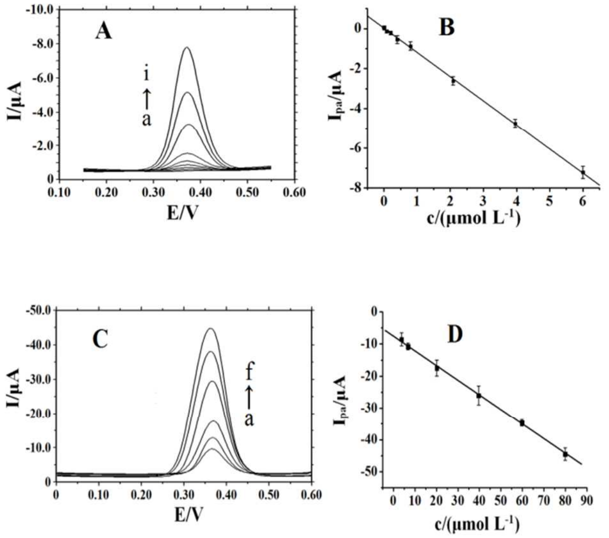


Fig. 6 (A) DPV of luteolin on CS-GR/GCE at lower range (from a to i: 0.002, 0.008, 0.08, 0.2, 0.4, 0.8, 2.0, 4.0, 6.0 μmol L^{-1}, respectively); (B) Linear relationship of the redox peak current (I_{pa}) at lower concentration range of luteolin; (C) DPV of luteolin on CS-GR/GCE at higher concentration range (from a to f: 6.0, 8.0, 20.0, 40.0, 60.0, 80.0 μmol L^{-1}, respectively); (D) Linear relationship of the redox peak current (I_{pa}) at higher concentration range of luteolin.

Stability and reproducibility of the modified electrode

To evaluate the repeatability of CS-GR/GCE, four intermittent determinations of a luteolin solution (1.0 μmol L^{-1}) were performed and the obtained relative standard deviation (RSD) value was 4%. The stability of CS-GR/GCE was carried out by continuous scanning for 40 cycles from -0.2 to 0.8 V in pH 4.0 B–R buffer solution containing 1.0 μmol L^{-1} luteolin. The anodic peak current was descended gradually with cyclic time increasing and trended to be stable after 10 cycles. The long-term stability of the CS-GR/GCE was tested after being stored in dry conditions at room temperature for 10 days. The current responses decreased less than 12% of its original response detected for the same luteolin solution. These experiments demonstrate that CS-GR/GCE has good stability, repeatability and reproducibility for the determination of luteolin.

Table 1. Comparison of the analytical parameters for the luteolin detection on different electrodes.

Electrode	Linear range (mol L^{-1})	LOD (mol L^{-1})	Ref.
GCE	1.0 × 10^{-8} - 1.0 × 10^{-6}	5.0 × 10^{-9}	10
MWNTs/GCE[a]	2.0 × 10^{-10} - 3.0 × 10^{-9}	6.0 × 10^{-11}	11
MPC/GCE[b]	3.0 × 10^{-7} - 3.0 × 10^{-5}	1.3 × 10^{-9}	14
GNs/HA/GCE[c]	2.0 × 10^{-8} - 1.0 × 10^{-5}	1.0 × 10^{-8}	15
HPLDE[d]	4.0 × 10^{-9} - 1.0 × 10^{-6}	1.0 × 10^{-9}	35
Au-BMIPF$_6$-CPE[e]	1.0 × 10^{-7} - 5.8 × 10^{-6}	2.8× 10^{-8}	36
CS-GR/GCE	2.0 × 10^{-9} - 6.0 × 10^{-5}	5.93 × 10^{-10}	This work

[a] Multi-walled carbon nanotubes modified glassy carbon electrode
[b] Macroporous carbon modified glassy carbon electrode
[c] Graphene nanosheets and hydroxyapatite nanocomposite modified glassy carbon electrode
[d] Heated pencil lead disk electrode
[e] Au nanoparticles -1-butyl-3-methylimidazolium hexafluoro-phosphate modified carbon paste electrode

Interference studies

The influences of some coexisting substances on the determination of 1.0 μmol L^{-1} luteolin were investigated by differential pulse voltammetry with the results listed in Table 2, which was performed by the addition of these substances in the luteolin solution and measured the electrochemical responses. It can be seen that few of them disturbed the determination, which indicated that CS-GR/GCE showed good selectivity.

Tabel 2. Influence of coexisting substances on the determination of 1.0 μmol L^{-1} luteolin (n=3)

Coexisting substance	Concentration	Relative error (%)	Coexisting substance	Concentration	Relative error (%)
L-Threonine	100.0 μmol L^{-1}	1.15	L-Proline	100.0 μmol L^{-1}	1.35
L-Arginine	100.0 μmol L^{-1}	-2.87	Ni^{2+}	100.0 μmol L^{-1}	2.24
L-Cystine	100.0 μmol L^{-1}	1.97	Ba^{2+}	100.0 μmol L^{-1}	3.01
L-Alanine	100.0 μmol L^{-1}	0.98	Ca^{2+}	100.0 μmol L^{-1}	3.59
L-Leucine	100.0 μmol L^{-1}	0.56	K^{+}	100.0 μmol L^{-1}	-3.24
L-Tryptophan	100.0 μmol L^{-1}	-0.88	N^{2+}	100.0 μmol L^{-1}	4.53
L-Lysine	100.0 μmol L^{-1}	1.57	Cr^{3+}	100.0 μmol L^{-1}	1.29

Analytical application

The CS-GR/GCE was further applied to determine luteolin in the Duyiwei soft capsules, which were purchased from Gansu Duyiwei Pharmaceutical Ltd. Co. (Z10970053). Five capsules were finely pulverized and then the powder was dissolved and diluted with ethanol. After sonicated for 30 min and refluxed for 2 hours, the turbid liquid was filtered. After that, the clear filtrate was diluted with pH 4.0 B-R buffer solution to prepare the sample solutions. The samples were detected by the experimental procedure with the results shown in Table 3. The recovery was measured by the addition of the standard luteolin solution. It can be seen that the results were satisfactory with the recovery in the range of 96.44 - 105.21%, which indicated that the proposed electrode could be efficiently used for the determination of luteolin content in the Duyiwei soft capsules. Also the analytical results in Table 3 indicated that the concentration of luteolin in the sample of Duyiwei capsules is relatively high and can be detected by the commonly used GCE. As a widely used working electrode, GCE exhibits the properties including excellent mechanical and electrical properties, wide potential window, chemical inertness and relatively reproducible performance. Therefore GCE is often used as the substrate electrode for the further modification. However, GR modified GCE gives the lower detection limit for luteolin and has the potential applications in the samples with lower concentrations of luteolin.

Table 3. Determination of luteolin in Duyiwei capsules

Samples	Detected (μmol L^{-1})	Added (μmol L^{-1})	Total (μmol L^{-1})	Recovery (%)	RSD (%)
1	8.96	3.65	12.80	105.21	3.21
2	9.62	3.65	13.14	96.44	1.96
3	9.50	3.65	13.32	104.66	2.78

Conclusion

In this paper a CS-GR modified electrode was prepared and used for the electrochemical detection of luteolin. Directly casting of GR on the electrode surface may result in the aggregation of GR nanosheets, and the specific properties of GR cannot be realized. After the ultrasonication of GR in CS solution, GR nanosheet can be dispersed homogeneously. Electrochemical behaviors of luteolin on CS-GR/GCE were carefully investigated with the electrochemical parameters calculated. Based on the oxidation peak current luteolin can be detected in the concentration range from 2.0×10^{-9} mol L^{-1} to 6.0×10^{-5} mol L^{-1} with the detection limit as 5.93×10^{-10} mol L^{-1} ($3S_0/S$). The proposed method was applied to the detection of luteolin content in Duyiwei capsules with satisfactory results. The fabricated electrode showed the advantages including high sensitivity, wide linear calibration range, good reproducibility and stability.

Acknowledgements

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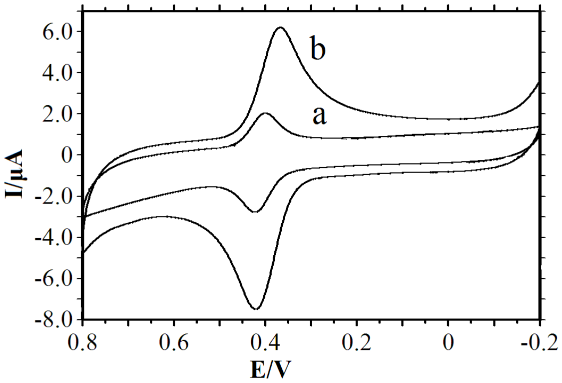
Graphical abstract



Electrochemical responses of luteolin are greatly enhanced on graphene modified glassy carbon electrode, which can be used for the sensitive detection of luteolin.