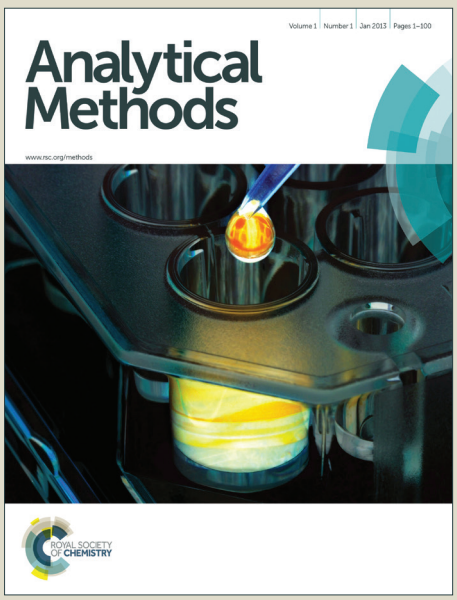
Analytical Methods

Accepted Manuscript



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Porous alumina microfibers-modified electrode as a highly-sensitive electrochemical sensor for quercetin

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Porous alumina microfibers were prepared *via* hydrothermal reaction using $Al(NO_3)$ and urea as the precursors. The resulting alumina microfibers were employed to modify the carbon paste electrode (CPE), and construct a new electrochemical sensor for quercetin. The modification of alumina microfibers greatly increased the oxidation signals of quercetin, showing strong signal enhancement effects. The influences of pH value, amount of alumina microfibers, accumulation potential and tine were examined on the oxidation peak currents of quercetin. As s result, a highly-sensitive, rapid and reliable electrochemical method was developed for the determination of quercetin. The linear range was from 25 nM to 1.5 μM, and the detection limit was 10 nM (3.02 μg L^{-1}) after 1-min accumulation. The proposed method was used in different tea and honeysuckle samples, and the detected results were in good agreement with the values that obtained by high-performance liquid chromatography.

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Introduction

Quercetin, a kind of flavonoids, is a major active ingredient that widely distributed in nature. Studies have proven that quercetin exerts strong anti-oxidative, anti-inflammatory, anti-pathogenic, and immune regulatory effects.[1-3] Apparently, it is interesting and important to develop simple, rapid and sensitive analytical methods for quercetin.

The commonly-used technique for the determination of quercetin was high-performance liquid chromatography (HPLC).[4,5] Thanks to short analysis time, high sensitivity, good handling convenience and low cost, electrochemical methods were also employed for the determination of quercetin because of its good redox features[6]. For example, activated silica gel-modified carbon paste electrode (CPE),[7] carbon nanotubes paste electrodes (CNTPE),[8,9] copper microparticles-modified CNTPE,[10] Co_3O_4 nanoparticles-modified glassy carbon electrode (GCE),[11] and carbon nanotubes/Nafion composite film-modified GCE[12] were successfully developed for the electrochemical determination of quercetin. However, to the best of our knowledge, electrochemical detection of quercetin using alumina microfibers-modified electrode is still missing.

Recently, aluminas have attracted much attention, and been widely used in the field of electrochemical detection due to its unique properties[13]. Carbon paste electrode (CPE), a mixture of an electrically conducting graphite powder and a pasting liquid, has obtained increasing attention, and been extensively applied as working electrode because it possesses following advantages: wide potential range,

easy preparation, convenient surface renewal, low residual current and low cost. The aim of this work was to develop a novel electrochemical sensor for quercetin using porous alumina microfibers as sensing material. Thus, alumina microfibers with porous structures were prepared through hydrothermal reaction, and then used to modify the surface of CPE. The electrochemical behaviors of quercetin were studied, and it was found that the oxidation signals of quercetin increased greatly on the surface of alumina microfibers-modified electrode. The notable peak current enlargements suggest that alumina mircrofibers are more sensitive for the electrochemical determination of quercetin. From the performance comparison of electrochemical sensors for quercetin that listed in Table 1, we clearly found that this new sensor exhibited higher sensitivity and shorter analysis time.

Experimental sections

Reagents

All chemicals were of analytical grade and used as received. Quercetin was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Stock solution of quercetin (1.0 mM) was prepared with ethanol and stored at 4^{o}C. Urea, aluminium nitrate, graphite powder (spectral reagent) and paraffin oil were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Ultrapure water (18.2 MΩ) was obtained from a Milli-Q water purification system and used throughout.

Instruments

Electrochemical measurements were performed on a CHI 660E electrochemical workstation (Chenhua Instrument, Shanghai, China). The working electrode was a alumina microfibers-modified CPE, the reference electrode was a saturated calomel electrode (SCE), and the auxiliary electrode was a platinum wire. Transmission electron microscopy (TEM) measurements were taken on a Tecnai G220 microscope (FEI Company, Netherlands). Scanning electron microscopy (SEM) images were conducted with a Quanta 200 microscope (FEI Company, Netherlands). Nitrogen adsorption-desorption isotherms were obtained on an ASAP 3000 nitrogen adsorption apparatus. The Brunauer-Emmett-Teller (BET) specific surface areas (S_{BET}) were calculated using the BET equation. The desorption isotherm was used to determine the pore size distribution using the Barret-Joyner-Halender (BJH) method.

Preparation of electrochemical sensor

Alumina microfibers were prepared by hydrothermal reaction using $Al(NO_3)_3$ as the precursor.[14,15] In a typical synthesis, $Al(NO_3)_3 \cdot 9H_2O$ was dissolved in doubly distilled water to form a clear solution and urea was then added. The molar ratio of Al : Urea : H_2O was 1: 9: 90. After being totally dissolved, the mixture was transferred to a Teflon-lined stainless-steel autoclave, and reacted at 100 ^oC for 24 h. Finally, the solid was filtered off, washed with doubly distilled water, dried at 80 °C for 24 h, and calcined at 500 ^oC for 2 h.

The resulting alumina microfibers (0.15 g) and graphite powder (0.85 g) were

exactly weighed, and put in a carnelian mortar. The total mass was controlled at 1.0 g and the mass content of alumina microfibers was 15%. After that, 0.25 mL paraffin oil was added into the powder and then mixed homogeneously. Finally, the resulting carbon paste was tightly pressed into the end cavity (3 mm in diameter, 1 mm in depth) of electrode body, and the electrode surface was polished on a smooth paper. The unmodified CPE was also prepared without addition of the prepared alumina microfibers.

Sample preparation

Different sea and Honeysuckle samples were purchased from a local market, and treated as follows. Firstly, the sample was dried at 60 ^{o}C, and then crushed into powder. Secondly, 0.20 g sample was exactly weighed and 5.0 mL methanol was added into. After 30-min ultrasonication and 5-min centrifugation at 5000 rpm, the clear liquid phase was collected. The extraction was repeated, and the extracted solution was finally diluted to 10.0 mL for further measurement.

Analytical procedure

0.1 M phosphate buffer solution with pH of 6.0 was used as supporting electrolyte for the determination of quercetin. After 1-min accumulation at -0.30 V, the differential pulse voltammograms were recorded from -0.30 to 0.50 V, and the oxidation peak currents at 0.15 V were measured for quercetin. The pulse amplitude was 50 mV, the pulse width was 40 ms and the scan rate was 40 mV s^{-1}.

Results and discussion

Characterization of prepared alumina microfibers

The prepared alumina samples were characterized using TEM and SEM, and the images were shown in Fig. 1. It was clearly found that the obtained alumina samples consisted of regular fibers. The diameter was about 200 nm, and porous structures were clearly observed on the surface. These abundant and regular pores certainly provide larger response area and numerous adsorption sites.

In addition, the porous structure and properties of prepared alumina samples were studied. Fig.2 shows the N_2 adsorption-desorption isotherms and pore size distributions of alumina microfibers. The sample can be described to have the type IV gas adsorption isotherm with type H1 hyteresis loop at high relative pressure. The specific surface area and pore volume are 115.56 m^2 g^{-1} and 0.34 cm^3 g^{-1}, respectively. In addition, the average pore sizes is calculated to be 9.0 nm from the Barret-Joyner-Halenda (BJH) model derived from the desorption branches of the isotherms. The above results indicated that the prepared alumina microfibers were mesoporous and have high specific surface area, which will lead to excellent electrochemical properties.

Signal enhancement of alumina microfibers

The oxidation behaviors of quercetin on CPE and alumina microfibers-modified CPE were examined using differential pulse voltammetry (DPV), and the results were

depicted in Fig. 3. In pH 6.0 phosphate buffer solution containing 100 nM quercetin, an oxidation peak at 0.18 V appeared on the bare CPE surface (curve b). The peak currents were relatively lower, revealing that the oxidation activity of quercetin was much poorer on CPE surface. However, the oxidation wave was improved by 7.92-fold on the surface of alumina microfibers-modified CPE (curve d). The remarkable peak current enlargements proved the strong signal enhancement effects of alumina microfibers. From TEM and SEM measurements, we clearly found that the prepared alumina microfibers had abundant and regular porous structures, undoubtedly enhancing the response area and accumulation efficiency. Therefore, alumina microfibers-modified CPE displayed higher electrochemical reactivity towards quercetin oxidation, and greatly increased the oxidation peak currents of quercetin. Besides, the DPV curves on CPE (curve a) and alumina microfibers-modified CPE (curve c) in the absence of quercetin were also studied. Their blank curves were featureless, revealing that the oxidation wave was certainly due to the oxidation of quercetin. In conclusion, the comparison of Fig. 3 clearly demonstrates that the alumina microfibers surface is more active for the oxidation and detection of quercetin.

Electrochemical determination of quercetin

The oxidation responses of quercetin in 0.1 M phosphate buffer solutions with different pH values were investigated using DPV, and Fig. 4A demonstrates the effects of pH value on the oxidation signals of quercetin. As improving pH value from

5.0 to 6.0, the oxidation peak currents of quercetin on CPE and alumina microfibers-modified CPE gradually increased, suggesting that the oxidation of quercetin was easier at higher pH value. When further increasing pH value to 8.0, the oxidation peak currents of quercetin decreased gradually. For achieving high sensitivity, pH 6.0 phosphate buffer solution was applied for the determination of quercetin.

The influences of mass content of alumina microfibers on the oxidation peak current of quercetin were discussed. As shown in Fig. 4B, the oxidation signals of quercetin increased greatly when improving the mass content from 0 to 15%. During this period, the accumulation efficiency and response area of modified CPE enhanced obviously. As a result, the oxidation signals of quercetin increased remarkably. When further raising the mass content to 20%, no obvious peak current enlargement was noticed for quercetin, likely due to a saturation status. However, the oxidation peak currents of quercetin started to decrease when the mass content was higher than 25%. This may be due to the fact that too much alumina microfibers lower the conductivity and block the electron transfer of quercetin. In this work, the optimal content of alumina microfibers was selected as 15%.

In order to discuss the influences of accumulation potential, the oxidation peak currents of quercetin were individually measured after 1-min accumulation at different potentials of -0.50, -0.40, -0.30, -0.20, -0.10 and 0.00 V. It was found that the oxidation peak currents changed slightly, revealing that accumulation potential had no obvious impacts on the detection of quercetin. For the sake of handling

convenience, the accumulation step was applied at the initial potential. In addition, the effects of accumulation time were examined on the oxidation peak currents of quercetin. As seen in Fig. 4C, the oxidation peak currents of quercetin on alumina microfibers-modified CPE increased remarkably when extending the accumulation time from 0 to 1 min. Longer accumulation time than 1 min did not enhance the oxidation signals obviously, indicating that the surface amount of quercetin approached a limiting value. Considering sensitivity and efficiency, 1-min accumulation was employed.

The successive measurements using one same alumina microfibers-modified CPE were examined. The oxidation peak currents of quercetin decreased continuously, maybe due to the severe surface sorption and fouling. So alumina microfibers-modified CPE was just employed for single measurement, and the reproducibility between multiple electrodes was evaluated by parallel determining the oxidation peak current of 100 nM quercetin. The relative standard deviation (RSD) was 3.9 % for fifteen alumina microfibers-modified CPEs. The low RSD value suggested that the fabrication reproducibility and detection precision were good.

The potential interferences on the determination of quercetin were also evaluated. In pH 6.0 phosphate buffer containing differently-concentrated interferents, the oxidation peak currents of quercetin were individually measured, and the peak current change was then checked. The results indicated that 1 mM glucose, starch, Ca^{2+}, Mg^{2+} and Fe^{3+}; 0.5 mM glycine, tyrosine, phenylalanine, vitamin C and vitamin E; 0.1 mM phenol; 50 μM resorcinol, protocatechualdehyde; 30 μM chlorogenic acid, gallic acid,

catechol, quinolinic acid and caffeine; and 10 μM hydroquinone, did not interfere with the determination of 100 nM quercetin since the peak current change was lower than 10%.

The linear range and detection limit were evaluated under the optimized conditions. As shown in Fig. 5, the oxidation peak currents of quercetin (I_p, μA) increased linearly with its concentration (C, μM) over the range from 0.025 to 1.5 μM. The linear regression equation was $I_p = 6.508\ C$, and the correlation coefficient was 0.998. After 1-min accumulation, the detection limit was evaluated to be 10 nM (3.02 μg L^{-1}) based on three signal-to-noise ratio.

Analytical application

In order to evaluate the practical application of the proposed method, it was used to measure the content of quercetin in different tea and honeysuckle samples. After adding 100.0 μL sample solution into a 10.0 mL pH 6.0 phosphate buffer solution, the DPV curves from -0.30 to 0.50 V was recorded after 1-min accumulation. Each sample was determined by three parallel detections, and the RSD was lower than 5%, suggesting the precision is good. The content of quercetin was determined by the standard addition method, and the results were given in Table 2. In addition, the concentration of quercetin was also detected using HPLC to test the accuracy. The obtained results by HPLC and alumina microfibers-based electrochemical sensor were in good agreement, and the relative error was below 8%, indicating that the proposed method is effective and reliable.

Conclusions

The prepared alumina microfibers exhibited high accumulation efficiency toward quercetin, and greatly increased the oxidation signals of quercetin. Utilizing the strong signal enhancement of alumina microfibers, a highly-sensitive and rapid electrochemical method was successfully developed for the determination of quercetin. The low detection limit (10 nM), short accumulation time (1 min) and low relative error (8%) strongly demonstrated that the newly-proposed method was fascinating and feasible.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 61361009), the Open Foundation of Key Laboratory of Biologic Resources Protection and Utilization of Hubei Province (PKLHB PKLHB1313), the Student Research Training Project of Hubei Minzu University (No. 201310517018), and the Project of New Strategic Industries for Fostering Talents in Applied Chemistry of Higher Education of Hubei Province, China.

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Captions for figures and tables

Fig. 1 TEM (A) and SEM (B) images of prepared alumina samples.

Fig.2 N_2 adsoption-desorption isotherms and pore size distribution (inset) of the prepared alumina samples.

Fig. 3 DPV curves of 100 nM quercetin on CPE (b) and alumina microfibers-modified CPE (d). Curves (a) and (c): blank curves in pH 6.0 phosphate buffer solution. Accumulation time: 1 min. Mass content of alumina microfibers: 15%. Insert plot: molecular structure of quercetin.

Fig. 4 Influences of pH value (A), mass content of alumina microfibers (B) and accumulation time (C) on the oxidation peak currents of 100 nM quercetin. Other conditions were the same as in Fig. 3. Error bar represents the standard deviation of triple measurements.

Fig. 5A DPV curves of quercetin with different concentration in pH 6.0 phosphate buffer solution using alumina microfibers-modified CPE. Fig. 5B Calibration curve for quercetin. Other conditions were the same as in Fig. 3.

Table 1 Comparison of electrochemical sensors for quercetin.

Table 2 Determination of quercetin in tea and honeysuckle samples.

Fig. 1

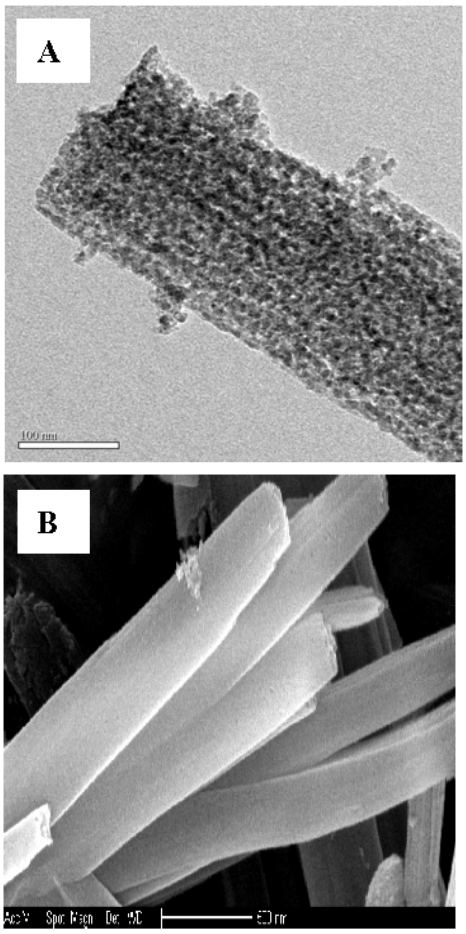

Fig. 2

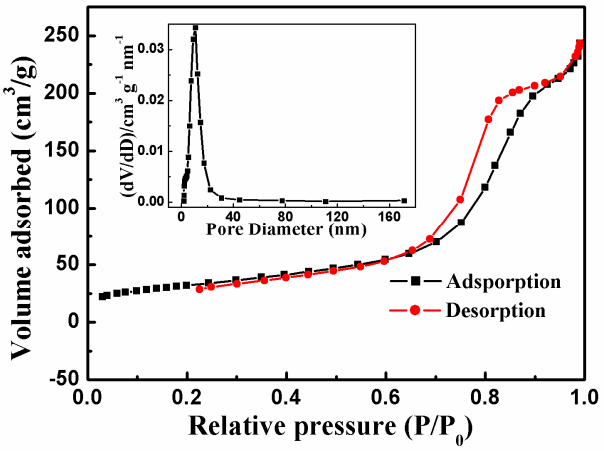

Fig. 3

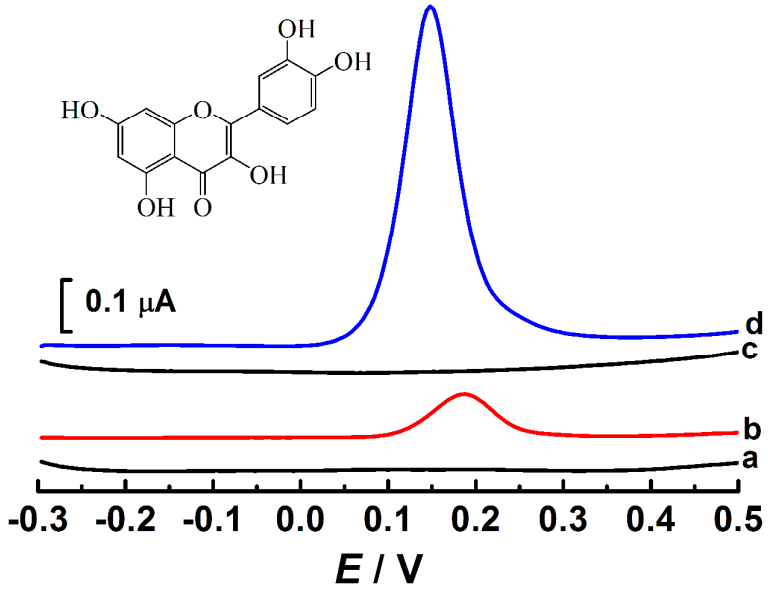

Fig. 4

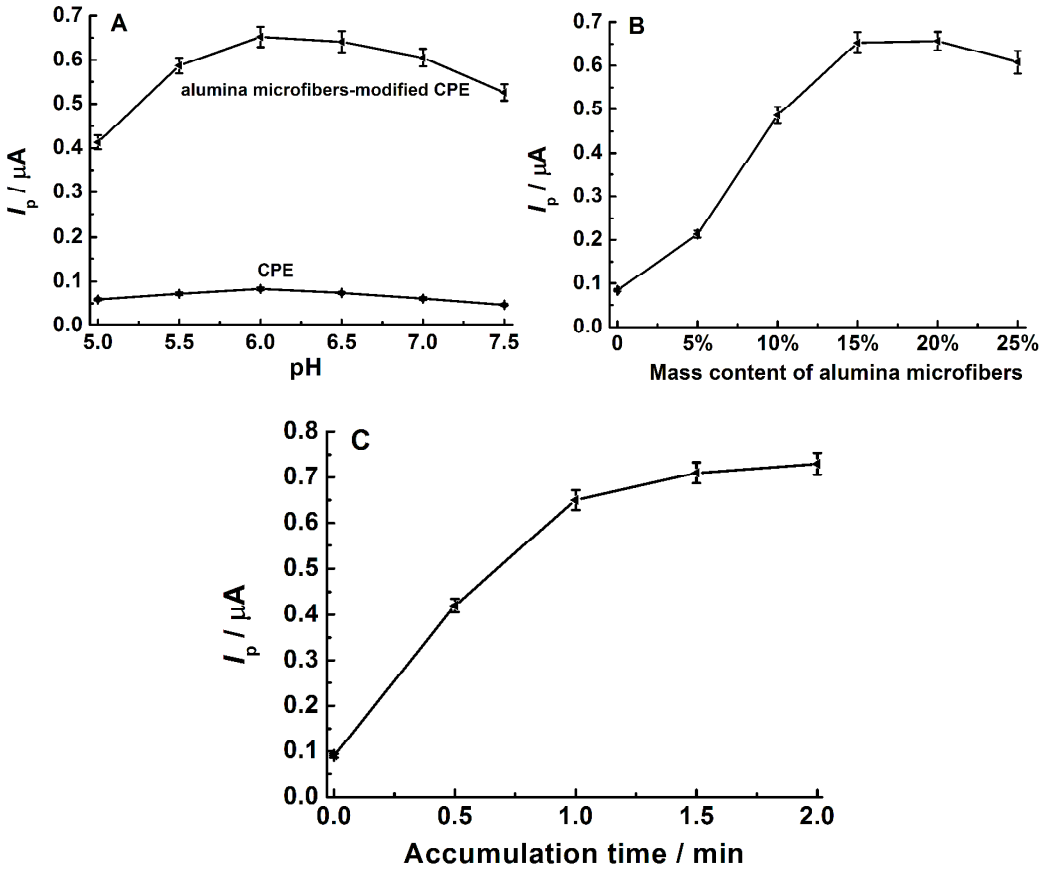

Fig. 5

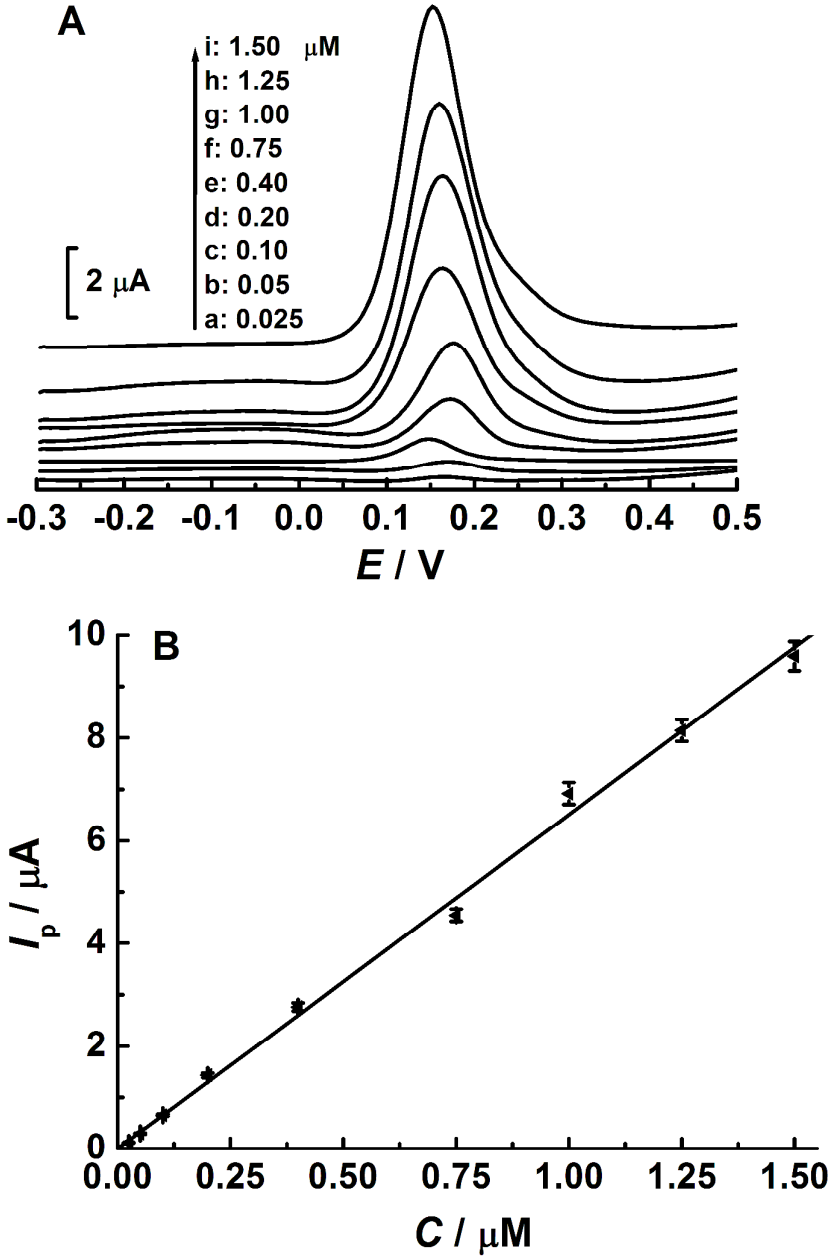

Table 1 Comparison of electrochemical sensors for quercetin.

Sensors	Detection limit (nM)	Time (s)	Ref.
Activated silica gel modified-CPE	11.7	120	6
CNTPE	30	250	7
CNTPE	20	300	8
Cu microparticles-modified CNTPE	236	-	9
Co_3O_4 nanoparticles-modified GCE	100	-	10
CNT/Nafion modified GCE	20	120	11
Alumina microfibers-modified CPE	10	60	This work

Table 2 Determination of quercetin in tea and honeysuckle samples.

Samples	by HPLC (mg g^{-1})	by this method (mg g^{-1})	Relative Error
Tea A	0.346	0.319	-7.8%
Tea B	0.637	0.605	-5.0%
Tea C	0.262	0.279	6.5%
Honeysuckle A	0.891	0.928	4.2%
Honeysuckle B	0.937	0.879	-6.2%
Honeysuckle C	0.683	0.722	5.7%

Graphical abstract