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# Sensitive and selective determination of gallic acid in green tea samples based on an electrochemical platform of poly(melamine) film

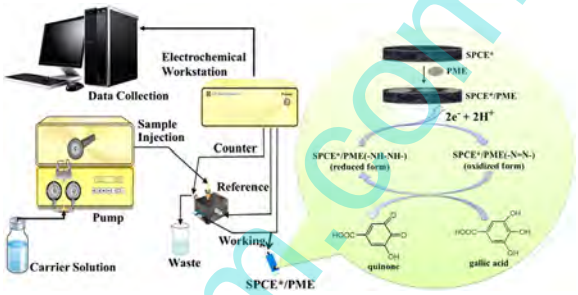
Ya-Ling Su, Shu-Hua Cheng*

*Department of Applied Chemistry, National Chi Nan University, Puli, Nantou Hsien, 545, Taiwan*

## HIGHLIGHTS

- A nitrogen-rich conducting polymer was used for electroanalysis of gallic acid.
- The sensor exhibits excellent electrochemical activity in both acidic and neutral media.
- Good analytical results in terms of low detection limit and wide linear range are obtained.
- The flow-injection amperometric assay is highly stable for continuous 57 replicates measurement (RSD = 3.9%).
- The assay shows good recovery for green tea samples.

## GRAPHICAL ABSTRACT

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## ABSTRACT

In this work, an electrochemical sensor coupled with an effective flow-injection amperometry (FIA) system is developed, targeting the determination of gallic acid (GA) in a mild neutral condition, in contrast to the existing electrochemical methods. The sensor is based on a thin electroactive poly(-melamine) film immobilized on a pre-anodized screen-printed carbon electrode (SPCE*/PME). The characteristics of the sensing surface are well-characterized by field emission scanning electron microscopy (FE-SEM), X-ray photoelectron spectroscopy (XPS) and surface water contact angle experiments. The proposed assay exhibits a wide linear response to GA in both pH 3 and pH 7.0 phosphate buffer solutions (PBS) under the optimized flow-injection amperometry. The detection limit (S/N = 3) is 0.076 μM and 0.21 μM in the pH 3 and pH 7 solutions, respectively. A relative standard deviation (RSD) of 3.9% is obtained for 57 successive measurements of 50 μM GA in pH 7 solutions. Interference studies indicate that some inorganic salts, catechol, caffeine and ascorbic acid do not interfere with the GA assay. The interference effects from some orthodiphenolic compounds are also investigated. The proposed method and a conventional Folin–Ciocalteu method are applied to detect GA in green tea samples using the standard addition method, and satisfactory spiked recoveries are obtained.



## 1. Introduction

Gallic acid (3,4,5-trihydroxybenzoic acid, GA) and its structurally related compounds, such as ellagic acid (a cyclized dimer) and hydrolysable tannins (a type of polyphenol), are comprehensively

* Corresponding author.
*E-mail address:* shcheng@ncnu.edu.tw (S.-H. Cheng).

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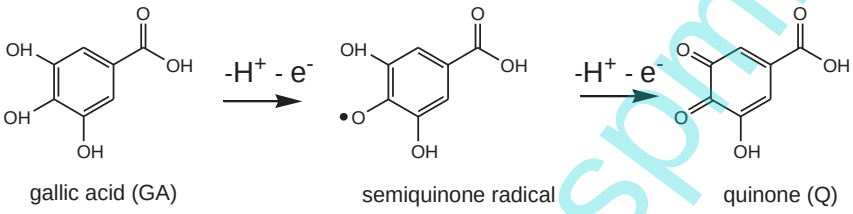
present in green tea, black tea, grapes and plants [1]. Due to its powerful antioxidant and antiradical activity, GA has received considerable attention because of its multiple biological and pharmaceutical applications, such as anti-mutagenicity, anti-inflammation, antibacterial properties, antiviral properties, anti-cancer and its protection against cardiovascular diseases [1]. GA solution is generally accepted as a reference standard when determining the total polyphenolic content in plants by the Folin–Ciocalteu assay, and the resultant GA equivalents are used to indicate the antioxidant level of the plant extracts [2]. Owing to the bioactive and pharmacological importance of GA, the development of robust, sensitive and fast methods for GA determination of various types of samples is of great interest.

Several techniques have been used to investigate the determination of GA and its derivatives, such as high performance liquid chromatography [3–5], ultrahigh performance liquid chromatography [6,7], flow injection chemiluminescent analysis [8,9], reverse flow spectrophotometry [10], photoelectrochemical platform [11] and electrochemical techniques [12–24]. Electroanalytical methods have certain advantages over other analytical methods, such as the low cost of instrumentation, high sensitivity, high accuracy, wide dynamic range and fast response time [13,17]. Direct electrochemical oxidation of electroactive GA is facile at glassy carbon electrodes [12,13], indium-tin oxide electrodes [13], and Pt electrodes [14]. The detailed ECEC-first order mechanisms were explored by Abdel-Hamid et al., and are summarized as Equation (1) [12].




(1)

In acidic solutions (pH 2.5), the 4-hydroxyl groups of GA are oxidized/deprotonated irreversibly at about 0.4–0.6 V to form semiquinone radicals, followed by irreversible oxidation/deprotonation to form quinone (Q) at about 0.8–0.9 V at carbon electrodes [12,13]. The oxidation of GA has also been reported in acidic solutions at electrodes modified with Zn/Al-layered double hydroxide film [16], $SiO_2$ nanoparticles [17], multi-walled carbon nanotubes [18,19], polyepinephrine [20], polyethyleneimine-functionalized graphene oxide [13], dinuclear copper (II) octaazamacrocyclic complex [21], thionine/nickel hexacyanoferrate composites [22] and boron-doped graphene [23]. Generally, acidic solutions (pH 2–3) are used as the optimal electrolytes for analysis of GA using an electrochemical oxidation approach [16–21]. According to the study of Ghoreishi et al., the peak current for GA oxidation decreased with an increasing pH, and the current response vanished in pH > 5 [19]. Abdel-Hamid et al., also reported finding no defined peaks for GA oxidation in solutions of pH > 6.22 [12]. A tyrosinase-gold nanoparticle biosensor has been used to detect GA in pH 7.4, where an electrochemical reduction method was employed [24]. Only a few studies have investigated the analysis of GA using non-acidic working media [14,22]. Narayanan et al. investigated the electrocatalytic oxidation of GA in $NaNO_3$ electrolyte solutions, where a bimediator-modified electrode based on polythionine and nickel hexacyanoferrate was employed [22]. Kunitake et al. reported the electrochemical oxidation of GA with an indium tin oxide electrode in a water-hexane microemulsion solution [14]. Another aspect of the electrochemical oxidation of GA is the serious surface fouling accompanied by the adsorption of oxidized products. This typically occurs when determining the phenol derivatives by means of electrochemical oxidation, and the same electrode cannot be used for the next measurement [25]. Recently, a synthesized copper complex was used to modify a graphite electrode for GA determination, and the surface fouling problems were significantly reduced [21]. The above electrochemical tools are effective for GA determination; however, the electrode preparation is tedious and the use of acidic media or nonaqueous solvents is not environmentally friendly. Therefore, it is worth investigating the use of an alternative platform and a mild aqueous solution for the continuous determination of GA.

Poly(melamine) film (PME) is a new type of conducting polymer, which has attracted considerable attention for analytical purposes because of its high stability and abundant nitrogen functional groups [26–28]. PME was first fabricated onto a glassy carbon electrode by Liu et al. for the electrochemical detection of guanine, adenine and epinephrine [26]. In 2014, Goyal et al. used edge plane pyrolytic graphite as an electrode substrate for PME modification to develop a serotonin sensor [27]. PME-modified screen-printed carbon electrodes have been applied for the electrochemical detection of free chlorine [28]. PME is also believed to provide binding sites for the accumulation of GA. Thus, the objective of the present study was to investigate the electrochemical detection of GA using PME-modified electrodes, the aim being to detect GA efficiently and to solve the problems described above. The results indicated that GA could be detected in aqueous solutions over a wide pH range using PME-modified electrodes and a flow-injection system. After optimizing the experimental conditions, such as PME amount, pH values of the aqueous solutions, hydrodynamic flow rates and applied electrode potentials, we developed a simple platform for the rapid determination of GA over a wide linear range and low detection limit in both acidic and neutral media. To validate the proposed assay, the determination of the total GA content in the green tea samples was performed and compared with the Folin–Ciocalteu assay, and satisfactory recoveries were obtained.

## 2. Experimental

### 2.1. Reagents

Melamine (ME, Sigma–Aldrich), gallic acid (GA, Acros Organics, USA) and all other compounds (Acros Organics, USA) were of the highest analytical grade available and were used without further purification. Folin–Ciocalteu reagent (2 N) was obtained from Sigma–Aldrich. All solutions were prepared using deionized water

from a Milli-Q ultrapure water system with a resistivity of 18 MΩcm. The phosphate buffer solutions (PBS) were prepared by dissolving sodium phosphate ($NaH_2PO_4$ and $Na_2HPO_4$) in distilled water, and the solution acidity (pH 2–10) was adjusted using concentrated HCl or NaOH solutions.

### 2.2. Instruments

Cyclic voltammetric and amperometric experiments were carried out using an electrochemical workstation (CH Instruments, model CHI-421A). A bare screen-printed carbon electrode (SPCE, geometric area 0.2 $cm^2$) from Zensor R&D, Taichung, Taiwan was employed as the working electrode. A platinum wire and a home-made Ag|AgCl|KCl (sat.) were used as counter and reference electrodes, respectively. All potentials were reported with respect to this reference electrode. The electrochemical experiments were carried out at room temperature (25 ± 2 °C). Atom force microscopy (AFM) images were performed on a *CSPM 5500* (Benyuan Co. Ltd., Beijing, China). An Hitachi S-4700I high-resolution scanning electron microscope (FE-SEM) was used to characterize the electrode surface morphology. The surface film compositions were investigated using a PHI 5000 VersaProbe/PHI Quantera SXM (XPS/ESCA) instrument with an $AlK_\alpha X$ source. The water contact angle was measured with a KRÜSS EasyDrop instrument. The pH values were measured with a Thermo Scientific Orion pH meter (model 420) and a Mettler Toledo pH electrode (model Inlab 439/120). UV/Vis spectra were obtained using an Agilent 8453 spectrophotometer. The measurement system for the flow-injection amperometry (FIA) consisted of a carrier reservoir, a microprocessor-controlled high-pressure pump PM-92e (BAS, USA), a Rehodyne 7725 sample injection valve (20 μL loop), a Zensor SP-100 thin-layer flow cell (Taiwan) specifically designed for SPCE and a BAS LC-4C amperometric detector.

### 2.3. Preparation of modified electrodes

The PME-modified electrodes were prepared according to the following two-step procedure. First, the SPCE was electrochemically cleaned by potential cycling between −1.0 and 1.0 V in 0.1 M PBS (pH 7.0), and then pre-anodized in the same solution by applying 2.0 V for 300 s under unstirred conditions. The pre-anodized electrodes were designated as SPCE*. Second, the PME was deposited on the SPCE* in an ME-containing 0.1 M HCl solution by cyclic potential scanning from 0.2 to 1.5 V at a scan rate of 0.05 V $s^{-1}$ for 10 cycles; the electrodes were then treated by repeated potential cycling (from −0.4 to +0.8 V at a scan rate of 0.1 V $s^{-1}$) in pH 7.0 PBS until a stable background was obtained [26–28]. The ME monomer solution was 0.03 mM, unless otherwise indicated.

### 2.4. Folin–Ciocalteu assay

The developed electrochemical determination of GA was compared with that of the Folin Ciocalteu assay [6]. Each test tube

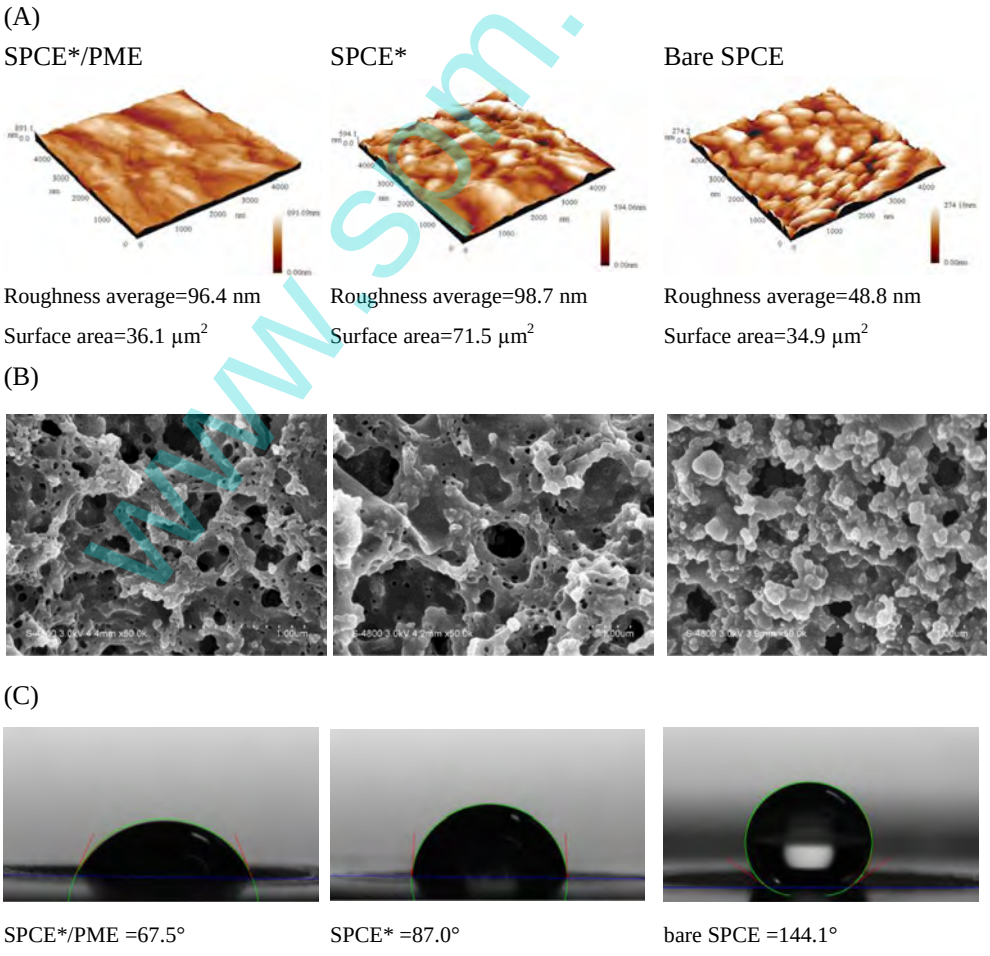



**Fig. 1.** (A) AFM image, (B) SEM image, and (C) water contact angle of SPCE*/PME, SPCE*, and bare SPCE.

contained a mixture of 700 μL of $Na_2CO_3$ (20% w/v), 100 μL of real sample extract, 100 μL methanol and 100 μL Folin–Ciocalteu reagent. The mixtures were stirred, incubated in the dark for 20 min and centrifuged at 13,000 rpm for 3 min. The absorbance of each mixture was measured at 735 nm using a spectrophotometer. This assay was repeated in quintuplicate for each real sample solution.

### 2.5. Analytical procedures for real samples

Two green tea samples were examined, including local tea drink and dry green tea leaves; their GA contents were determined using the standard addition method. The drink samples were prepared by filtration, and then diluted (20×) with a pH 7.0 PBS. The solution sample of tea leaves was prepared by adding deionized water to 2 g dry tea in a 100 mL Erlenmeyer flask, stirring on a hot plate at 100 °C for 10 min, cooling to room temperature, filtering and then diluting (10×) with a pH 7.0 PBS. A certain amount of each diluted sample was spiked, respectively, with different concentrations of GA standard, and the solution was either determined by the Folin–Ciocalteu assay or injected into the FIA system to record responses. The spiked calibration curves were established to acquire the GA concentration in the real samples.

## 3. Results and discussion

### 3.1. Electrode surface characterizations

Fig. 1 shows a comparison of the electrode surface characteristics by tapping mode AFM, SEM and water contact angle. As can be seen in the AFM images, the SPCE* showed significantly higher roughness average values ($R_a$) than that of the SPCE (98.7 nm vs. 48.8 nm). The treated SPCE* had a notably rough configuration of multilayers with large pores, as evidenced by the SEM images (50 K magnification) [25,29], and this was consistent with the high roughness average and surface area observed from the AFM images. A low concentration of ME monomer (0.03 mM) was used for the electrochemical deposition of a thin PME film, but the obtained PME would not able to completely cover the entire electrode surface. The SEM images of the SPCE*/PME confirmed the presence of nanoclusters deposited on a microporous surface. The resulting SPCE*/PME surface showed an insignificant change in $R_a$ values in comparison with the SPCE*; there was, however, a great difference in their respective surface areas (36.1 $\mu m^2$ vs. 71.5 $\mu m^2$). The decrease in surface area suggested that the grown PME was deposited and inserted into the cavity of the SPCE*; thus, the PME film thickness was not easily defined because of the roughness of the underlying SPCE* substrate. In fact, uncovered carbon particles/graphite sheets could be observed on the topmost surfaces of the SPCE*/PME, providing a unique surface that affected the electrochemical sensing at the electrode/solution interfaces. Water contact angle (θ) techniques were used to examine the effect of the surface modification on the hydrophobicity of the topmost layer. As shown in Fig. 1C, the bare SPCE, populated by the graphite sheets and hydrocarbon binding materials, was recognized as a hydrophobic surface ($\theta = 144.1°$), and the water contact angles of the SPCE* decreased to 87.0° because of the removal of the organic binder and the formation of oxygen groups [29]. The water contact angles were further lowered to 67.5° upon deposition of the PME film. The nitrogen-rich PME film was responsible for the enhanced hydrophilic characteristic of the topmost surface ($\theta < 90°$), which is an essential feature for electroanalysis in aqueous solutions. The surface compositions were further investigated by XPS wide-scan spectra (Fig. S1). The results confirmed the successful immobilization of PME on the electrode surface.

### 3.2. Electrochemical impedance measurements

The as-prepared SPCE*/PME was considered very suitable for analytical application in aqueous solution because of the hydrophilic characteristics; therefore, a model ferri/ferrocyanide redox couple was chosen as a probe [29]. As shown in Fig. 2A, a large peak-to-peak potential separation ($\Delta E_p$) value of 343 mV was observed at the bare SPCE, indicating the poor electron-transfer kinetics. Both the SPCE* and SPCE*/PME showed high electron-transfer kinetics by the improvement of the $\Delta E_p$ to 95 mV and 102 mV, respectively. Activation of electron transfer kinetics has been reported by the pre-anodization of the carbon surfaces to remove organic ink constituents or contaminants and to increase the surface roughness or oxygen functional groups [25,30]. A comparison of the peak currents showed very low currents for the bare SPCE ($E_{1/2} = 0.24$ V); the SPCE*/PME showed the highest redox peak current together with a small one at around 0.38 V. The cationic, nitrogen-rich PME surface was thought to concentration more anionic ferricyanide than that of the SPCE* due to the electrostatic attraction and, therefore, higher current responses were observed. The electrochemically active surface area was calculated to be 0.123 $cm^2$ based on the Randles–Sevcik equation [13].

Electrochemical impedance spectroscopy (EIS) is an extremely efficient technique for providing the electron-transfer properties of the electrochemical interface [16,27]. Fig. 2B shows the Nyquist

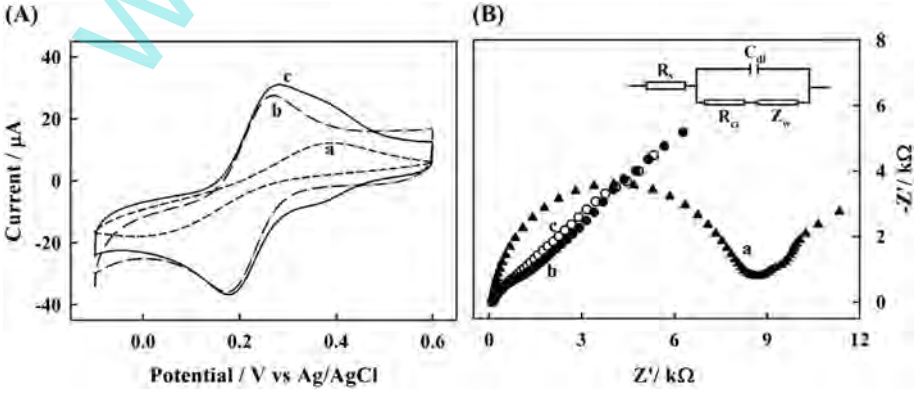
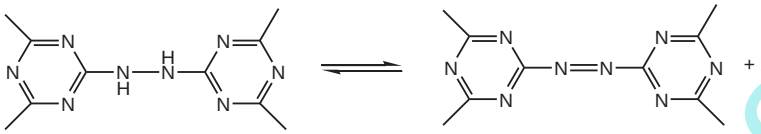



**Fig. 2.** (A) Cyclic voltammograms and (B) Nyquist plots of (a) bare SPCE, (b) SPCE*, and (c) SPCE*/PME in 0.1 M KCl containing 1.0 mM $[Fe(CN)_6]^{3-/4-}$. Inset: An equivalent circuit representing the electrode/electrolyte solution interface. $R_s$ is the solution phase resistance; $C_{dl}$ is the double-layer capacitance; $R_{ct}$ is the electron transfer resistance; $Z_w$ is the Warburg impedance.

plots of the bare SPCE, SPCE* and SPCE*/PME in the presence of the $K_3Fe(CN)_6$ probe. The semicircular part at the high frequencies indicated that the reaction was controlled by an electron-transfer limited process. The electron transfer resistance ($R_{ct}$), showing the barrier for interfacial electron transfer of the redox probe, could be roughly estimated from the semicircle's diameter. The linear portion at the low frequencies resulted from the diffusion limiting step of the electrochemical process. A Randle equivalent electrical circuit model (inset in Fig. 2B) was used to fit the EIS results [16,27], and an $R_{ct}$ of 8.40 kΩ was obtained for the bare SPCE, while the $R_{ct}$ decreased to 1.39 and 0.90 kΩ for the SPCE* and SPCE*/PME, respectively. The results indicated that the pre-anodized and the PME-deposited electrodes accelerated the electron communication between the redox couple $[Fe(CN)_6]^{3-}$ and the electrode surface. The fast electron-transfer kinetics demonstrated the potential of the both electrodes for sensing applications.

### 3.3. Electrochemical characteristics of SPCE*/PME

The electroactive character of the PME film was investigated by means of cyclic voltammetry. As showed in Fig. 3A (solid lines), a well-defined and reversible redox peak was observed, corresponding to the oxidation and reduction of the surface of the PME film. In pH 3 PBS, the half-wave potential ($E_{1/2}$) of the PME film was centered at 0.48 V, and the peak-to-peak potential separation was 0.025 V. The redox reaction can be described as Equation (2). Similar electroactive features of the PME film were observed using an edge plane pyrolytic graphite substrate [27]. There were no redox couples for the SPCE* or bare SPCE (Fig. 3B and C, solid lines). The surface coverage of the PME film was calculated by the equation of $\Gamma_{PME} = Q/nFA$, where $n = 2$, $Q$ is the peak area, $F$ the Faraday constant and $A$ the geometrical surface area of the working electrode [30]. The $Q$ value was obtained by averaging the anodic and cathodic peak areas measured by integrating the cyclic voltammogram at a scan rate of 0.1 V $s^{-1}$. A surface coverage of $4.37 \times 10^{-10}$ mol $cm^{-2}$ was obtained. The low surface coverage was consistent with the porous structure of the SPCE*/PME as found in the SEM images.

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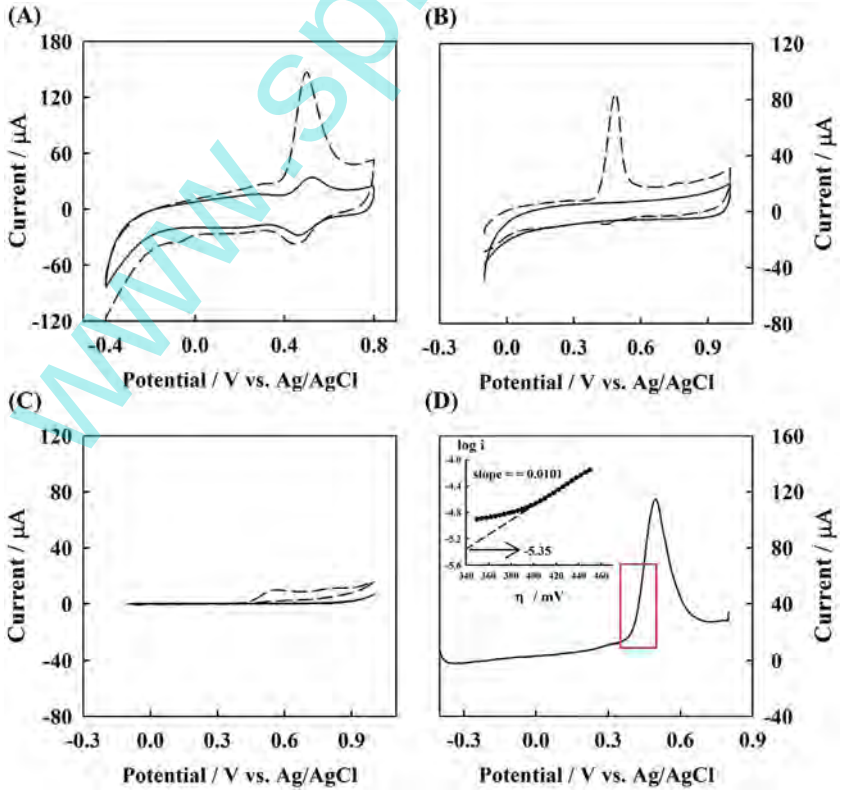



**Fig. 3.** Cyclic voltammograms of (A) SPCE*/PME, (B) SPCE*, (C) bare SPCE in 0.1 M pH 3 PBS in the (a) absence (solid line) and (b) presence (dash line) of 100 μM GA. (D) Corrected voltammogram of SPCE*/PME in the presence of 100 μM GA. Inset: Tafel plot obtained from the data of the square region.

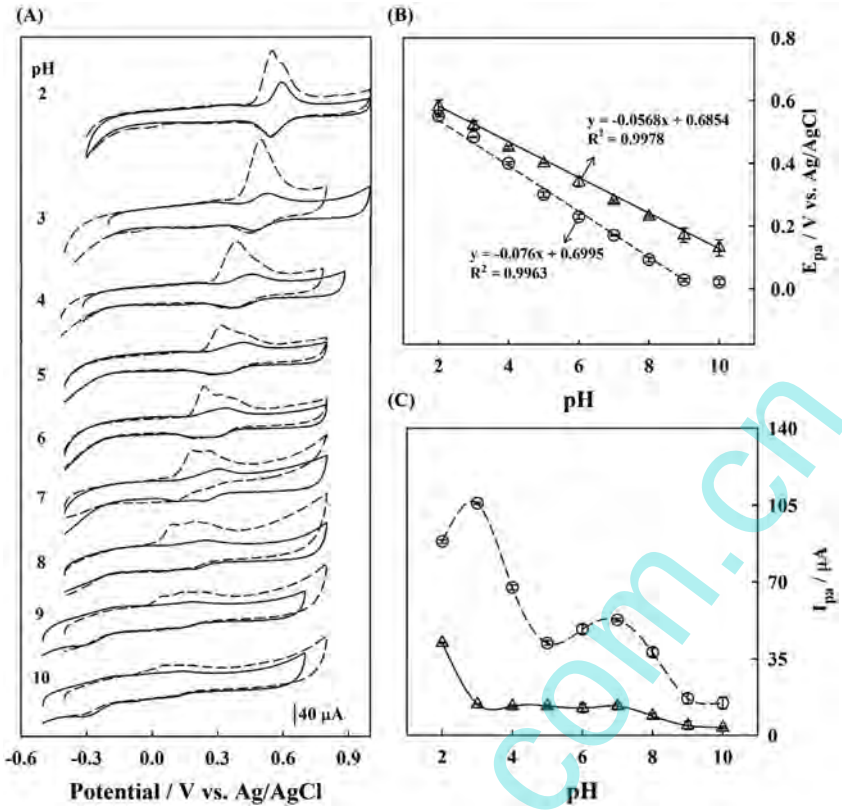
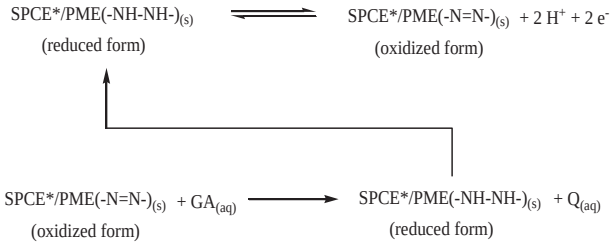



**Fig. 4.** (A) Cyclic voltammograms of SPCE*/PME in the absence (the solid line) and presence (the dashed line) of 100 μM GA in various phosphate buffer solutions. Scan rate = 0.1 V $s^{-1}$. (B) Plot of anodic peak potential ($E_{pa}$) *vs.* pH. (C) Plot of anodic peak current ($I_{pa}$) *vs.* pH. In the absence (△) and presence (○) of 100 μM GA.

The pH effects on the cyclic voltammetric responses of the SPCE*/PME were further examined (Fig. 4A, solid lines). The redox couples of the PME shifted to negative potentials as the pH values increased, and a slope of −56.8 mV $pH^{-1}$ in the pH range of 2–10 was obtained (Fig. 4B, white triangle). The slope was very close to the theoretical Nernstian value of −59.2 mV $pH^{-1}$, and was consistent with the two electron-two proton process for the hydroazo/azo redox reaction (Equation (2)) [28]. The redox wave became broad in pH 9–10, and the anodic peak currents decreased sharply as the pH of the solution increased (Fig. 4C, white triangle), suggesting the slow electron transfer kinetics of the PME film in weak base solutions.




**Scheme 1.** Schematic representation for the electrocatalytic oxidation mechanism of GA at the SPCE*/PME.

### 3.4. Electrochemical oxidation of GA

The electrochemical oxidation responses of 100 μM GA in pH 3.0 PBS at various electrodes were compared using cyclic voltammetry (Fig. 3, dash lines). In contrast to the very small faradaic current obtained at the bare SPCE, both the SPCE* and SPCE*/PME showed large anodic current responses for GA oxidation at about 0.5 V. As reported previously, the SPCE* contains abundant oxygen elements, such as carboxylic acid (−COOH) and hydroxyl (−OH) functional groups, and, thus, possesses the electrocatalytic properties for the oxidation of phenol derivatives [25]. These oxygen functional groups of SPCE* exhibited a strong interaction with GA and, thus, obtained good current responses (80 μA) for GA oxidation via Equation (1). On the other hand, higher oxidation current responses were observed at the SPCE*/PME (160 μA). It has been reported that polyethyleneimine-functionalized graphene oxide exhibits a strong accumulation of GA, thus having a good sensitivity for GA detection [13]. The PME film possessed aromatic nitrogen heterocycles (1,3,5-triazine) substituted with amino groups. The nitrogen-rich and aromatic features of the PME film are believed to interact heterogeneously with GA through hydrogen bonding and π–π interactions, respectively. The combination of the both effects is suggested to be responsible for the enhanced current responses. Hydrogen bonding interactions have been found in the hydrogel products between ME and GA [31]. The electrochemical kinetic parameters, such as the electron transfer coefficient (α) and exchange current density ($j_0$) for the SPEC*/PME were determined for GA oxidation in pH 3 PBS. The logarithm of anodic peak current (log i) after correction for the background current was plotted as a function of potential in the Tafel region. The plot (Fig. 3D) revealed a linear relationship with an increase in potential. Based on Tafel equation [19], the electron transfer coefficient was calculated as 0.40; the exchange current density was 36.3 μA $cm^{-2}$, which was higher than the results in the literature [19].

The effect of the scan rate (υ) on the cyclic voltammetric responses of the SPCE*/PME in the presence and absence of

100 μM GA in pH 3 PBS was examined (Fig. S2). The double logarithmic plots of $i_{pa}$ vs. υ were analyzed, and the obtained slope values indicated the redox reaction of SPCE*/PME to be a specific adsorption-controlled electron-transfer process [30] and the GA oxidation at the electrode surface of the SPCE*/PME to be a diffusion-controlled reaction. The results also implied that the oxidation of GA by the SPCE*/PME was a reversible electron transfer reaction coupled to an irreversible chemical reaction ($E_rC_i$ mechanism) [32]. Therefore, the electrocatalytic reaction of GA by the use of the SPCE*/PME was proposed and shown in Scheme 1.

The pH effects on the cyclic voltammograms of the SPCE*/PME in the presence of GA were systematically investigated in pH 2–10 solutions (Fig. 4A, dash lines). The electrocatalytic GA oxidation peak potentials were pH-dependent. The peaks were apparent and sharp in the acidic media (pH 2–4), however, the peak shape became broad in pH 5, then split into 2 waves in pH 6–9 and then became vague in pH 10. This trend is very similar to the pH effects on the voltammetric responses of PME film (Fig. 4A, solid lines). Therefore, the transfer of 2-electron, 2-proton in the one-step oxidation reaction is assumed to be separated as 2 steps of one-electron, one-proton reactions in pH 5–10 The current responses of GA were also highly related to the acidity of the solution media. As can be seen in Fig. 4C (white circles), the oxidation currents were high in acidic solutions, observable in neutral solutions and very low in alkaline solutions. The observed high current responses in acidic solutions were attributed partially to the interaction between the surface oxygen functional groups of oxidized carbon particles and the GA [12], while the deposition of a thin PME film provided extra interactions, as discussed above. It was most interesting to observe the oxidation current responses of GA at the SPCE*/PME in neutral aqueous solutions, a subject as yet undiscovered in the literature. Usually, very vague oxidation current responses can be observed at carbon electrodes in pH 7 or alkaline solutions [12], because of the electrostatic repulsion exerted by the negative charge of the carboxylate groups on the surface of the carbon particles [29] toward the anionic GA (the acid dissociation constants, $pK_a$ for GA are 4.4, 8.7, 11.4 and 13.0 [33]). The PME-modified electrode surfaces contained uncovered carbon particles, and so not useful for attracting for GA in pH 7 or alkaline solutions. However, the deposition of a thin PME film would compensate for the current responses by the electrostatic attraction of anionic GA to the protonated (or neutral) nitrogen functional groups of PME film. The concept was confirmed by the observation of the broad oxidation waves at around 0.0–0.2 V for GA at the PME-modified electrodes in pH 7–10 solutions (Fig. 4A, dash lines).

The amount (or thickness) of the PME was expected to influence the current responses for GA oxidation; therefore, the ME monomer concentrations were varied for the preparation of the modified electrode. The effects of the various ME concentration on the oxidation current responses of GA in pH 3 and pH 7 solutions are recorded in Fig. 5. A low concentration of 0.03 mM was found to exhibit the highest current responses for both solutions. The higher the ME concentration, the more PME film was deposited onto the SPCE*; however, higher ME concentrations were not favorable for the GA oxidation current. Because the contribution from the exposed oxygen functionality of the SPCE* was significantly hindered by a thick PME film, a significant decrease in the current response was observed, especially in pH 3 solution. Another reason for the decrease of the GA oxidation current was the possible aggregation for a thick PME film, leading to fewer binding sites for GA detection. Considering the sensitivity and practical analytical applications for GA standards and real samples, a concentration of 0.03 mM ME was chosen for the electropolymerization processes, and solution pH values of 3.0 and 7.0 were chosen as the working media.

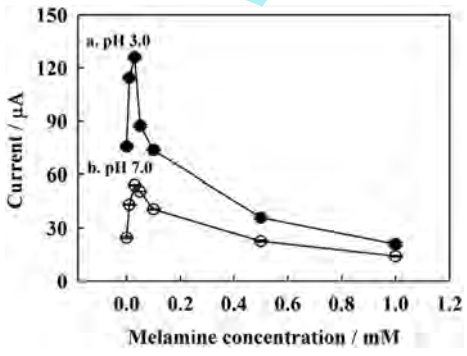



**Fig. 5.** Plot of peak current ($I_{pa}$) *vs.* melamine concentration at the SPCE*/PME in the pH 3 (●) and pH 7 (○) solutions containing 100 μM GA.

### 3.5. Electrochemical oxidation of GA by flow-injection amperometry

In order to prevent surface adsorption and to guarantee a fresh electrode surface for the continuous, sensitive and accurate detection of GA, a flow-injection system was coupled to the prepared modified electrodes. The parameters for the amperometric measurement of the current responses of GA were optimized, including flow rates and oxidation potentials. The effects on the current responses for 50 μM GA at the SPCE*/PME in pH 7.0 PBS were recorded (Fig. S3). Accordingly, a flow rate of 500 μLmin$^{-1}$ and a potential of 0.24 V were selected for further study.

Fig. 6 shows the FIA responses for various GA concentrations at the SPCE*/PME under the optimized conditions. When pH 3 PBS was used as a carrier solution, a linear calibration curve was obtained for 0.5–2500 μM. There was a little suppression of current responses for the high concentration range, probably due to kinetic limitations. The obtained slope was 0.1118 μA μM$^{-1}$. The detection limit (S/N = 3) was estimated as 0.076 μM. The RSD for three replicate determinations of 1 μM, 100 μM and 1000 μM of GA were 0.3, 1.8 and 1.6%, respectively, an indication of the highly reproducible responses. A mild aqueous condition was achieved by the use of pH 7 PBS; a linear calibration curve was obtained for 1–1000 μM, and the obtained slope was 0.0123 μA μM$^{-1}$. The detection limit (S/N = 3) was estimated as 0.21 μM. As listed in Table 1, the proposed assay showed a good analytical performance in comparison with the reported electrochemical sensors [16–22] in terms of detection limit and linear calibration range. To be noted, the electrolytic medium in this study could be a neutral aqueous solution.

The surface fouling problem, which usually occurs for the existing electrochemical methods, limits the number of replicate measurements at the same electrode [17–19,22]. The use of different, independently prepared electrodes for GA detection is a way to present the reproducibility of the sensors [16]. This limitation could be solved by the use of one electrode coupled to a flow-injection system. By the use of the bare SPCE, the peak signals were very low for the injection of 50 μM GA (pH 7 PBS) under the optimized conditions. The current responses at the SPCE* were higher, but decayed apparently; the current decreased by 35% and 75% at the second and the tenth injection, respectively. In contrast, the proposed system at the SPCE*/PME was highly stable for a continuous 57 injections with an allowable loss of oxidation current (RSD = 3.9%). The result showed the good stability of the FIA-SPCE*/PME system for GA detection. Besides, the flow buffer removed the adsorbed GA and the oxidized products effectively to provide a refreshed electrode surface for continuous measurements.

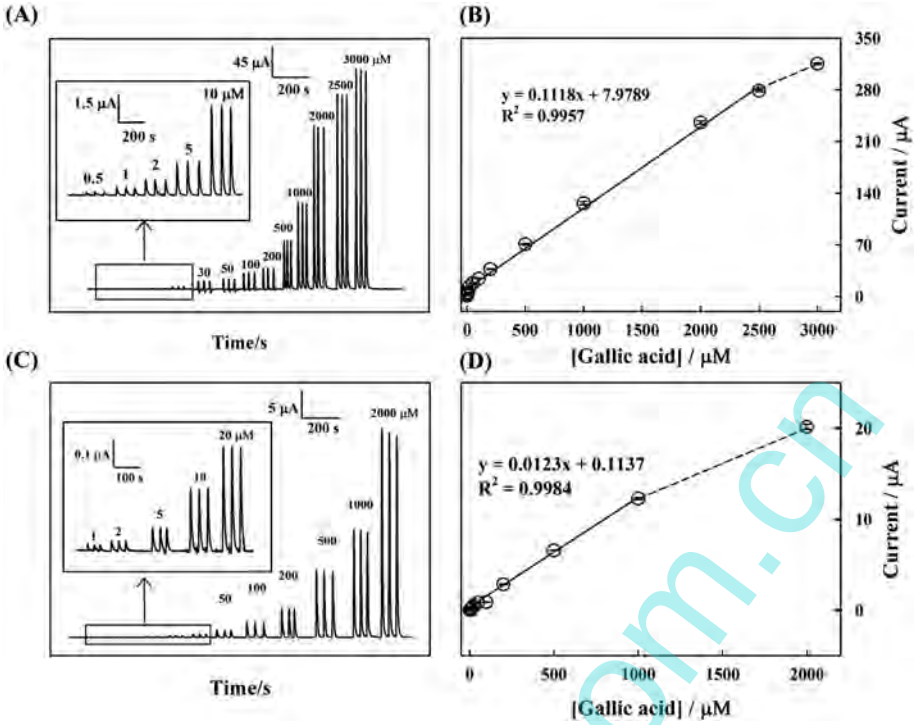



**Fig. 6.** FIA responses for various concentration of GA at SPCE*/PME in (A) pH 3, and (C) pH 7 PBS. Plot of $I_{pa}$ *vs.* GA in (B) pH 3, and (D) pH 7 PBS. The injection volume was 20 μL. Carrier solution was 0.1 M PBS (pH 3 or pH 7). Applied potential = 0.50 V (pH 3) and 0.24 V (pH 7). Flow rate = 500 μL/min.

**Table 1**
Comparison of analytical performance for GA between the proposed sensor and published results.

| Electrodes | Technique | Solution | Linear range (μM) | LOD (μM) | Ref. |
|---|---|---|---|---|---|
| GCE/LDHf[a] | DPV | pH 3 | 4–600 | 1.6 | [16] |
| CPE/$SiO_2$ | DPV | pH 1.7 | 0.8–100 | 0.25 | [17] |
| CPE/MWCNT[b] | DPV | pH 2 | 1–6.25; 6.25–33.75 | 0.27 | [18] |
| CPE/MWCNT-Fe[c] | DPV | pH 2.5 | 0.5–15 | 0.3 | [19] |
| GCE/PEP[d] | SWV | pH 1.88 | 1–20 | 0.663 | [20] |
| GE/[$Cu_2$tpmc][e] | DPV | pH 2 | 0.25–1; 5-100 | 0.148 | [21] |
| GE/TH/NiHCF[f] | CV | 0.1 M $NaNO_3$ | 4.99–1200 | 1.66 | [22] |
| SPCE*/PME | FIA | pH 3.0 | 0.5–2500 | 0.076 | This work |
| SPCE*/PME | FIA | pH 7.0 | 1–1000 | 0.21 | This work |

[a] Glassy carbon electrode modified with Zn/Al layered double hydroxide film.
[b] Carbon paste electrode modified with multi-walled carbon nanotubes.
[c] Carbon paste electrode modified with multi-walled carbon nanotubes containing iron oxides.
[d] Glassy carbon electrode modified with polyepinephrine.
[e] Graphite electrode modified with a dinuclear copper (II) octaazamacrocyclic complex in PVC matrix.
[f] Graphite electrode modified with thionine and nickel hexacyanoferrate.

**Table 2**
Interference effects on the determination of $5 \times 10^{-5}$ M gallic acid in pH 7.0 PBS.

| No. | Interference | Concentration (M) | Signal change (%) | RSD[a] (%) |
|---|---|---|---|---|
| 1 | $CaCl_2$ | 0.01 | −0.02 | 1.6 |
| 2 | $NaNO_2$ | 0.01 | −0.01 | 1.0 |
| 3 | $Na_2CO_3$ | 0.01 | +0.03 | 2.3 |
| 4 | Catechol | $5 \times 10^{-5}$ | +3.49 | 2.1 |
| 5 | Ascorbic acid | $5 \times 10^{-5}$ | +0.75 | 2.6 |
| 6 | Catechin | $5 \times 10^{-5}$ | +7.31 | 1.1 |
| 7 | Caffeine | $5 \times 10^{-5}$ | −0.05 | 1.2 |
| 8 | Caffeic acid | $5 \times 10^{-5}$ | +29.72 | 2.4 |
| 9 | Quercetin | $5 \times 10^{-5}$ | +26.92 | 1.3 |
| 10 | EGCG[b] | $5 \times 10^{-5}$ | +29.11 | 2.4 |
| 11 | Rutin | $5 \times 10^{-5}$ | +14.49 | 0.9 |

[a] n = 3.
[b] Epigallocatechin gallate.

### 3.6. Interference and real samples analysis

The effect of potential interference on the current responses of GA was checked in pH 7 PBS using the flow-injection amperometry (Fig. S4); the results are shown in Table 2. The interference effect was studied by recording the current responses of 50 μM GA in the presence of equal or an excess amount of interference. The assay is considered to be free of interference if the current responses obtained from the two quantities (in the absence and presence of interference) differ by less than ±5%. The results indicated that the SPCE*/PME showed tolerance to the possible interference of inorganic and organic chemicals, such as $Ca^{2+}$, $Na^+$, $Cl^-$, $NO_2^-$, $CO_3^{2-}$, ascorbic acid, catechol (*o*-dihydroxybenzene), and caffeine. According to the reported works in seven references [16–22], a few studies reported the insignificant interference from ascorbic acid [18,20,21], catechol [22] and catechol derivatives [18–21]. The

**Table 3**
Results of the recovery tests for the determination of gallic acid in real samples with the proposed electroanalytical method and Folin–Ciocalteu method.

| Samples | Electroanalytical method | | | | | Folin–Ciocalteu method | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Spiked (μM) | Total[a] (μM) | Found[b] (μM) | Recovery[c] (%) | RSD[d] (%) | Spiked (μM) | Total[a] (μM) | Found[b] (μM) | Recovery[c] (%) | RSD[d] (%) |
| Tea drink | 0 | 67.17 | | | 1.9 | 0 | 77.56 | | | 4.5 |
| | 5 | 71.95 | 4.78 | 95.6 | 1.1 | 100 | 176.17 | 98.61 | 98.6 | 4.1 |
| | 10 | 76.75 | 9.58 | 95.8 | 0.8 | 200 | 279.85 | 202.29 | 101.1 | 3.8 |
| | 20 | 86.98 | 19.81 | 99.1 | 1.6 | 500 | 584.68 | 507.12 | 101.4 | 4.5 |
| | 30 | 97.12 | 29.95 | 99.8 | 1.2 | 1000 | 1102.4 | 1024.84 | 102.5 | 2.6 |
| | 50 | 116.95 | 49.78 | 99.6 | 1.6 | 1500 | 1631.56 | 1554.00 | 103.6 | 2.0 |
| | 100 | 170.29 | 103.12 | 103.1 | 0.8 | | | | | |
| Tea leaves | 0 | 248.45 | | | 3.5 | 0 | 285.76 | | | 3.1 |
| | 20 | 268.56 | 20.11 | 100.6 | 3.9 | 100 | 385.12 | 99.36 | 99.4 | 1.6 |
| | 50 | 298.64 | 50.19 | 100.4 | 1.4 | 200 | 487.82 | 202.06 | 101.0 | 1.8 |
| | 100 | 351.71 | 103.26 | 103.3 | 2.9 | 500 | 776.49 | 490.73 | 98.1 | 2.0 |
| | 150 | 401.18 | 152.73 | 101.8 | 2.5 | 1000 | 1296.39 | 1010.63 | 101.1 | 4.7 |
| | 200 | 457.38 | 208.93 | 104.5 | 3.0 | 1500 | 1859.14 | 1573.38 | 104.9 | 4.8 |

[a] The observed total concentration of sample and spiked amount.
[b] The found spiked amount.
[c] Recovery = Found/spiked × 100.
[d] n = 5.

present sensing surface exerted strong interactions for GA, and thus showed good selectivity in the discrimination of gallic acid with the easily oxidized organic chemicals, such as catechol and ascorbic acid. The RSD values of three replicate measurements were less than 5%, indicating good precision. Further studies on the interference effects from the orthodiphenol derivatives (caffeic acid, quercetin, catechin and rutin) and 3,4,5-trihydroxyphenyl derivatives (epigallocatechin gallate, EGCG) showed that there were about 7–30% increase in signals, in contrast to the result of catechol. The interference could be due to the existence of extra polar groups, such as carboxylic acid and hydroxyl groups of these polyphenolic compounds, which exhibited affinity toward the PME film, and thus showed high impact. Although the present assay showed interference from these polyphenols, the effect was less than a recent report [21], where caffeic acid and quercetin showed serious interference (more than +200%) on the determination of equal amount of GA.

The developed method was further applied to determine the GA of two green tea samples (Fig. S5). The GA concentrations of the diluted samples were estimated to be 67.2 μM and 248.5 μM for the tea drink and dry tea leaves, respectively. Table 3 summarizes the recovery results and RSD values for the GA determination using the proposed method and the spectrophotometric method based on Folin–Ciocalteu reagents [6]. The accuracy of the method us expressed in terms of percentage recoveries, which are estimated as the ratios of the found amount of GA to that originally added [2]. As can be seen, the recoveries for different concentrations were in the range of 95.6–104.5% and 98.1–104.9% by the proposed method and the Folin–Ciocalteu method, respectively. The results indicated that the proposed working system was highly accurate for the quantitation of GA in the green tea samples. The GA amount in the real samples obtained from the Folin–Ciocalteu method was a little higher in comparison to the proposed method (77.56 μM vs. 67.17 μM). The overestimation of GA content in the real samples by the Folin–Ciocalteu method could be ascribed to reactions with the total phenol derivatives and non-phenolic organic substances in the test solutions [6,21], while the present method could detect GA selectively. Finally, the sensor stability determination was carried out by keeping the sensor at room temperature in a dry environment filled with nitrogen gas for several days, and then the current responses were examined. The percentage of current response was 93.7% and 75.4% after storage for 3 and 15 days, respectively. Therefore, further investigation is required as regards the long-term stability of the sensing film and adequate storage conditions.

## 4. Conclusion

We successfully developed a simple electrochemical platform for fast, sensitive and selective detection of GA in acidic and neutral media. The electrochemical sensor was a pre-anodized screen-printed carbon electrode covered with a thin PME film. In contrast to previous works using acidic solutions or nonaqueous solutions, the nitrogen-rich PME film proved beneficial in accelerating the electron transfer rate and enhancing the oxidation current responses of GA both in acidic and neutral solutions. In combination with a hydrodynamic flow-injection system, the electrochemical assay showed excellent analytical features, not only in increasing the current response, but also in improving signal stability. The present work resulted in a good analytical performance, including wide linear range, low detection limit and good selectivity. The accuracy of the proposed FIA assay was examined by recovery measurements on spiked samples in comparison to the reference Folin–Ciocalteu method; satisfactory spiked recoveries were obtained. These analytical characteristics combined with the ease of sensor fabrication makes the proposed method an alternative for the successful determination of GA in biological research and pharmaceutical applications.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2015.10.026.

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