Int. J. Electrochem. Sci., 12 **(2017)** 4107 – 4119, doi: 10.20964/2017.05.03

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

A novel Amperometric Gallic acid Sensor based on Polymelamine entrapped Graphene Composite

Tse-Wei Chen[1], *Selvakumar Palanisamy*[1], *Shen-Ming Chen*[1,*], *Vijayalakshmi Velusamy*[2],
Sayee Kannan Ramaraj[3]

[1] Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei 106, Taiwan (ROC)
[2] Division of Electrical and Electronic Engineering, School of Engineering, Manchester Metropolitan University, Manchester – M1 5GD, United Kingdom.
[3] PG & Research department of Chemistry, Thiagarajar College, Madurai-09, Tamilnadu, India.
[*]E-mail: smchen78@ms15.hinet.net

Received: 25 January 2017 / *Accepted:* 5 March 2017 / *Published:* 12 April 2017

The present work describes an amperometric determination of gallic acid (GA) using a glassy carbon electrode (GCE) modified with graphene (GR) and polymelamine (PM) composite. The GR/PM composite modified electrode was prepared by electropolymerization of melamine on GR modified GCE. The as-prepared GR/PM composite was characterized by scanning electron microscopy, elemental mapping and Fourier transform infrared spectroscopy. The GR/PM composite modified GCE was used as electrocatalyst for oxidation of GA, and the composite modified electrode shows an enhanced catalytic activity than electrodes modified with GR and PM. Under optimum conditions, amperometric i-t was used to determine the GA, and the amperometric response of GA was linear over the concentration ranging from 0.1 to 728.9 µM. The limit of detection and sensitivity of the sensor was estimated as 0.027 µM and 0.697 $\mu A \mu M^{-1}$ cm^{-2}, respectively. The GR/PM composite modified electrode exhibits high selectivity in the presence of range of potentially interfering polyphenol compounds, dopamine, uric acid and ascorbic acid. As a proof of concept, the practicality of the sensors was examined in green tea samples, and shows acceptable practicality for the determination of GA.

Keywords: Graphene; Poly(melamine); Gallic acid; Electro-oxidation; Amperometry

1. INTRODUCTION

Gallic acid (GA) is a type of polyphenolic compound and naturally occurring in green tea, black tea, white tea, banana, grapes and plants [1]. GA is widely known for its excellent antioxidant and antiradical activity in multiple biological and pharmaceutical applications, including anti-mutagenicity, anti-inflammation, anticancer and protection against cardiovascular diseases [2]. GA is widely used as a standard for determination of total polyphenolic content in different analytes by the Folin–Ciocalteu assay [3]. Owing to the aforementioned properties of GA, the accurate determination of GA in real samples is of great interest. So far, various analytical methods have been used for the determination of GA, such as high performance liquid chromatography (HPLC), ultrahigh performance liquid chromatography, flow injection analysis, reverse flow spectrophotometry photoelectrochemical method and electrochemical methods [4–10]. Compared with traditional analytical methods, electroanalytical methods has many advantages such as low cost, high sensitivity, fast response and portability [10, 11]. The electrochemistry of GA at different modified electrodes have been studied and documented. For example, carbon nanomaterials [12], metal oxides [11] and redox polymers [13] have been widely used for accurate and selective detection of GA. Since, the unmodified glassy carbon, indium-tin oxide, graphite, screen-printed carbon (SPC) and Pt electrodes are not suitable for the determination of GA due to their poor sensitivity, selectivity and poor reproducibility [14].

Graphene is a 2D carbon nanomaterial, possessing high surface area and electrochemical properties in comparison to other carbon nanoforms [15, 16]. Owing to its unique properties, GR is widely used as a building block for range of applications, such as solar cells, data storage, OLED displays, photocatalysis, electrochemical sensors and biosensors [17–21]. On the other hand, polymelamine (PM) is a new class of conducting polymer, which is widely used for different applications owing to its extreme stability and presence of abundant nitrogen functional groups [2]. Recently, PM with carbon and activated carbon electrodes have used for electrochemical detection of range of analytes including neurotransmitters and polyphenols [2, 22–26]. However, most of the reported PM based composites used with reduced graphene oxide, and the use of commercial GR is still limited in PM based composites. The motivation of the present work is to utilize the special properties of PM with GR, and use it as an electrocatalyst for oxidation of GA.

In the present work, we report an amperometric GA sensor using GR/PM composite modified electrode for the first time. The GR/PM composite exhibits an enhanced electrocatalytic activity towards GA than other modified electrodes. The selectivity of the sensor was evaluated in the presence of range of polyphenolic compounds and other potentially interfering compounds. The practicality of the sensor was evaluated using green tea samples and discussed.

2. EXPERIMENTAL

2.1. Materials

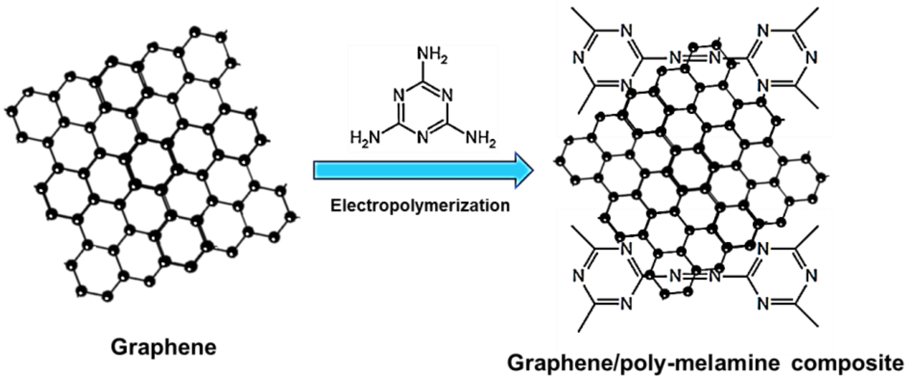
Graphene nano flakes (8 nm, product number UR-GNAPHENE) were obtained from UniRegion Bio-Tech, Taiwan. Melamine and gallic acid were purchased from Sigma-Aldrich. All other chemicals were received from Sigma-Aldrich and used as received. The green tea samples were purchased from a local supermarket in Taipei, Taiwan. The supporting electrolyte, pH 3.0 phosphate buffer solution (PBS) was prepared using 0.1 M Na_2HPO_4 and NaH_2PO_4 in doubly distilled water. All other chemicals were prepared using doubly distilled water without any additional purification.

2.2. Methods

Amperometric i-t and cyclic voltammetry (CV) measurements were carried out using CHI 1205B electrochemical station from CH instruments. Scanning electron microscopic images were obtained using Hitachi S-3000 H scanning electron microscope (SEM). Elemental mapping was performed using Hitachi S-3000 H SEM attached HORIBA EMAX X-ACT elemental analyzer. Fourier transform infrared (FTIR) spectroscopy were acquired using JASCO FTIR-6600 spectrometer. Amperometric *i-t* measurements were performed using a PRDE-3A (ALS Co., Ltd, Japan, www.bas.co.jp/). The three-electrode configuration was used for electrochemical studies in which GCE as a working electrode (only for CV measurements), saturated Ag/AgCl as reference electrode and Pt wire as a counter electrode. Rotating disc electrode (RDE) with an electrochemically active surface area (EASA) of 0.162 cm^2 was used for amperometric experiments. The EASA of the GR/PM composite modified electrode was calculated using Randles–Sevcik equation, as reported early [27].

2.3. Fabrication of GR/PM composite modified electrode

The GR dispersion was prepared by dispersing GR (5 mg mL^{-1}) into the dimethylformamide using ultrasonication about 30 min. Then, 8 µL of as-prepared GR dispersion (optimum) was dropped on pre-cleaned GCE and dried in an air oven. The resulting GR modified electrode was transferred into the electrochemical cell containing 0.5 mM melamine in 0.1 M HCl solution. To prepare GR/PM composite modified electrode, 10 cycles were performed in the potential between 0 to 1.5 V at a scan rate of 50 mV/s, as reported early [2]. After successive cycles, the PM was electropolymerized on GR surface. The schematic representation for the preparation of GR/PM composite is shown in Scheme 1. The as-prepared GR/PM composite modified electrode gently rinsed with doubly distilled water and dried at room temperature.



Scheme 1. Schematic representation for the preparation of GR/PM composite.

The PM modified electrode was prepared using the same procedure without GR. The GR modified electrode was prepared by drop coating of 8 µL of GR dispersion on GCE without PM. The electrochemical experiments were performed in N_2 atmosphere.

3. RESULTS AND DISCUSSION

3.1. Characterization of GR/PM composite

The surface morphology of the as-prepared GR and GR/PM composite modified electrodes were investigated using SEM. Fig. 1 displays the SEM images of GR (A) and GR/PM composite (B). The SEM image of GR shows its typical flake morphology. On the other hand, the GR/PM composite shows that dense layer of PM was uniformly covered on the surface of GR. The corresponding elemental analysis of GR/PM composite confirms the presence of carbon and nitrogen, as shown in Fig. 1C and D.

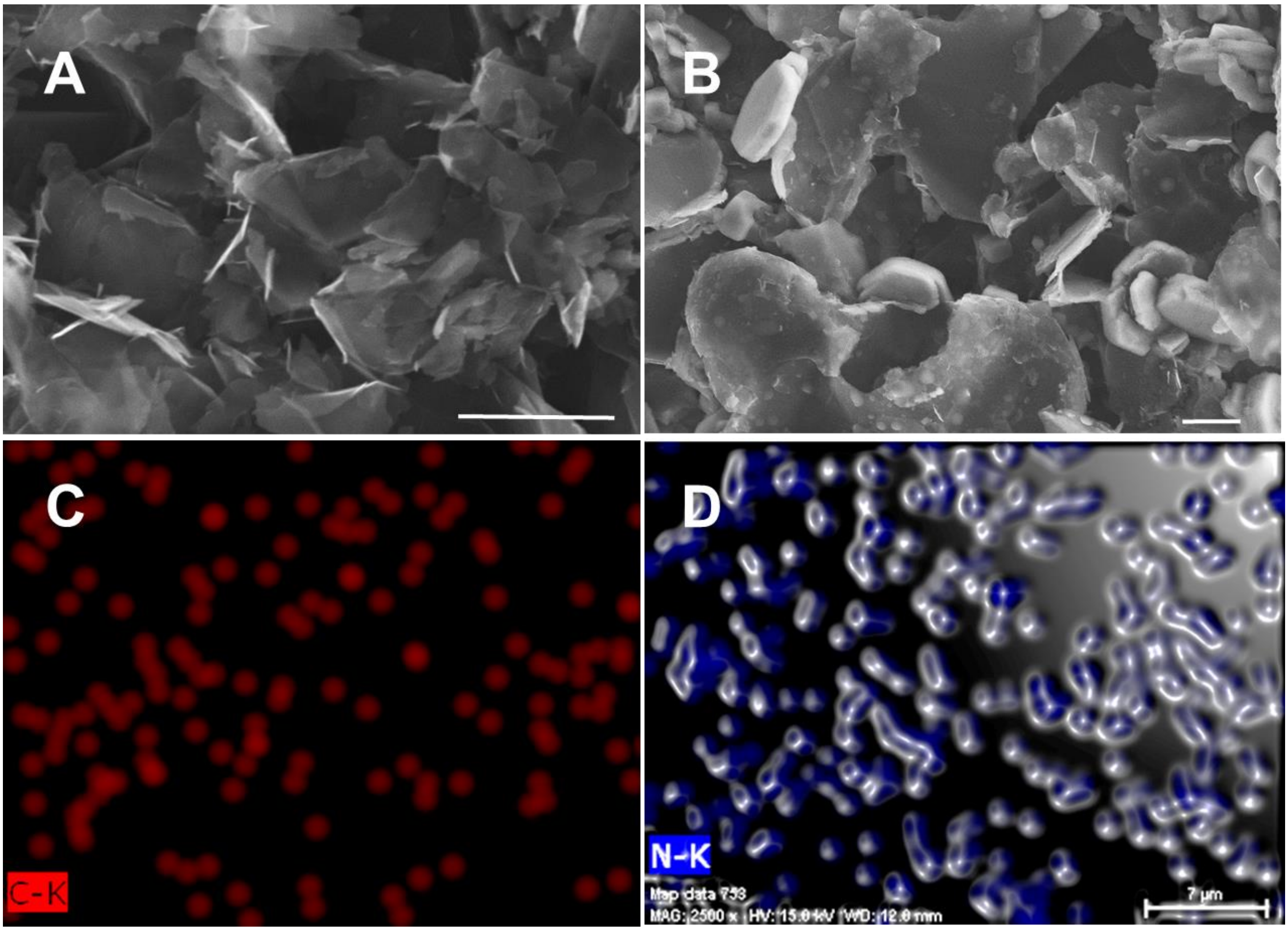


Figure 1. SEM images of GR (A), GR/PM composite (B), and corresponding elemental mapping of carbon (C) and nitrogen (D). Scale bar = 5 µM.

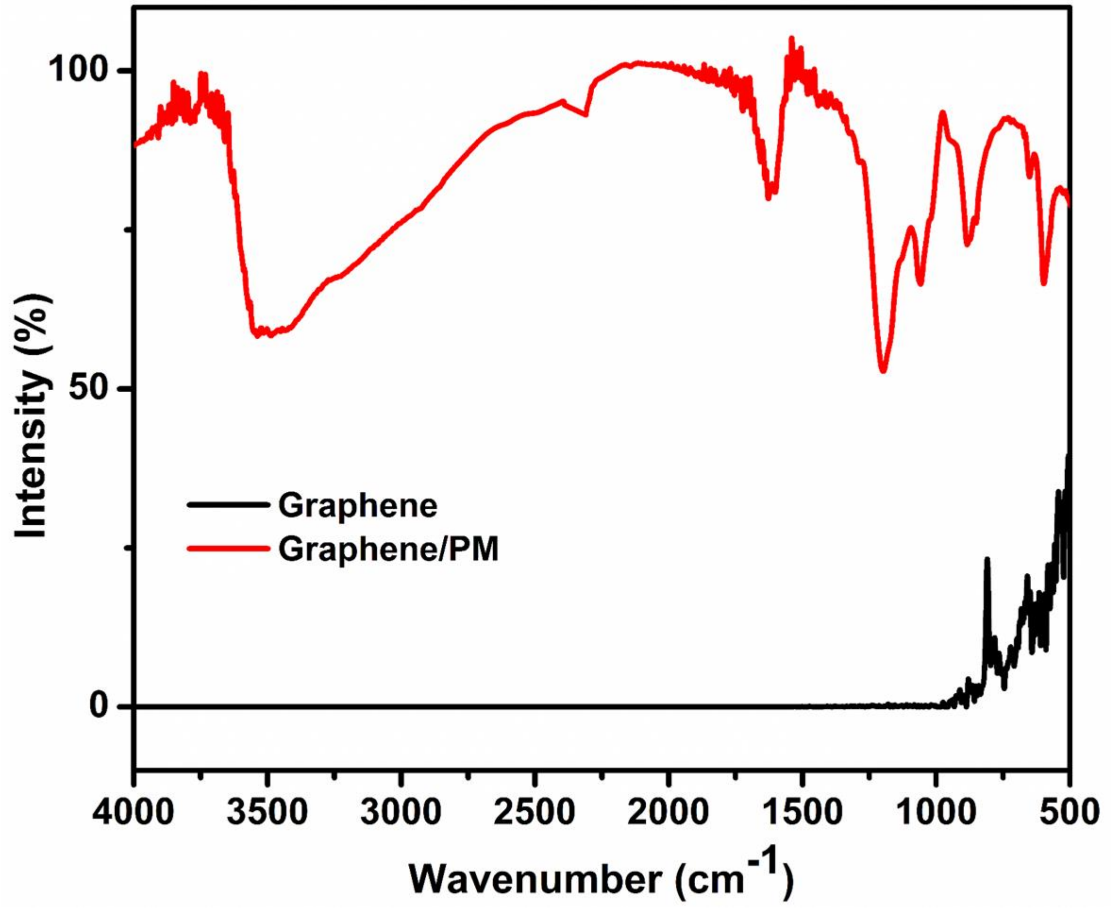


Figure 2. Typical FTIR spectra of GR and GR/PM composite.

The result confirms the formation of GR/PM composite. The FT-IR was used to further support the presence of PM on GR/PM composite. Fig. 2 shows the FT-IR spectra of GR (black line), and GR/PM composite (red line). The FT-IR spectrum of GR is found featureless in the finger print region. While, three distinct bands were observed at 3485, 1618 and 745 cm^{-1}, which are attributed to the vibrations of –NH and C=C in PM [25, 26]. In addition, the bands related to GR also appeared in the FT-IR spectrum of GR/PM composite. The result confirms the presence of GR and PM in GR/PM composite.

3.2. Electrochemical behavior of GA

The electrocatalytic ability of different modified electrodes toward oxidation of GA was studied using CV. Fig. 3 shows the CV response of bare (a), GR (b), PM (c), and GR/PM composite (d) modified electrodes in PBS containing 500 µM of GA at a scan rate of 50 mV/s. It can be seen that the bare and GR modified electrodes show same electrocatalytic activity against the oxidation of GA. While, the oxidation peak potential of GA at GR modified electrode was observed at quite low potential (0.532 V), when compared with un-modified electrode (0.591 V). On the other hand, the PM modified electrode shows an enhanced catalytic activity towards the oxidation of GA than GR modified electrode. The oxidation potential of GA was also found less positive potential (0.491 V) than the response observed at GR modified electrode, is attributed to the strong interaction between the PM with GA via hydrogen bonding.

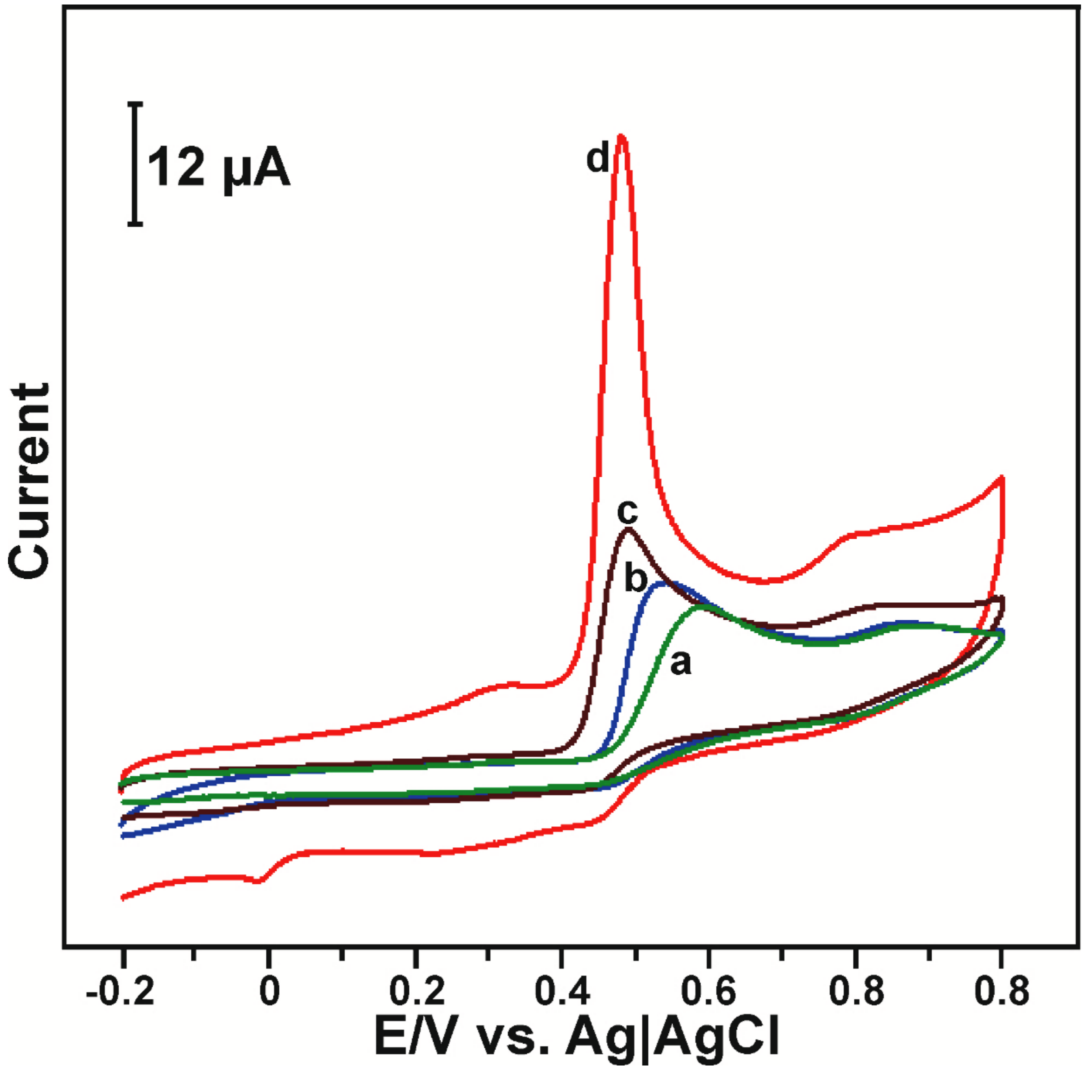


Figure 3. Cyclic voltammetry response obtained for bare (a), GR (b), PM (c), and GR/PM composite (d) modified electrodes in PBS containing 500 µM of GA at a scan rate of 50 mV/s.

However, the GR/PM composite modified electrode shows 3 folds enhanced oxidation peak current response to GA than the electrodes modified with PM and GR. The oxidation potential of GA is observed at 0.501 V, which is very close to those observed at PM modified electrode. The high conductivity and high surface area of GR combined PM is result into the high catalytic activity towards GA than other modified electrodes. Hence, GR/PM composite is more suitable for sensitive detection of GA than other modified electrodes.

The effect of scan rate and pH on the oxidation of GA at GR/PM composite modified electrode was investigated by CV.

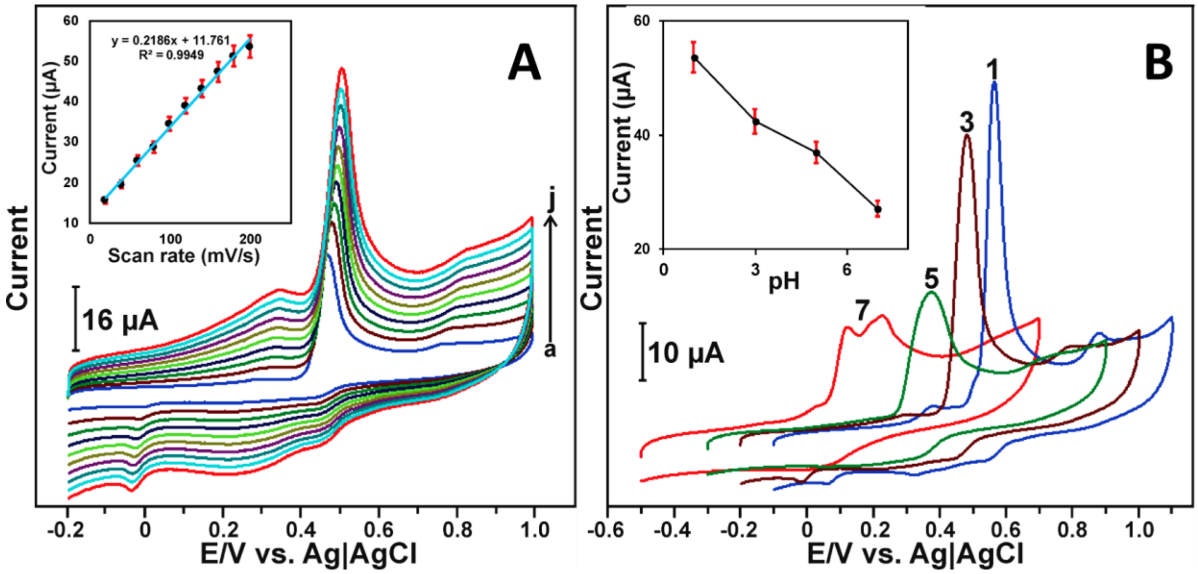
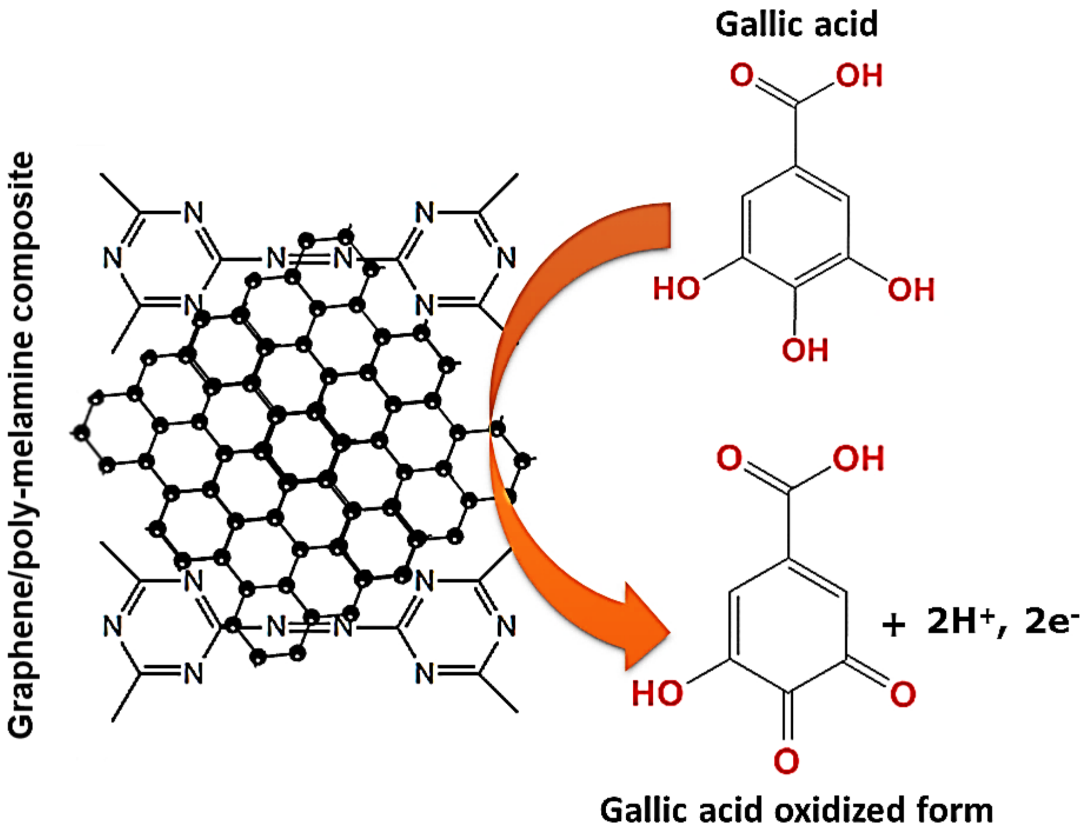


Figure 4. A) Cyclic voltammetry response of GR/PM composite modified electrode in 500 µM of GA containing PBS for different scan rates. Scan rates: 20–200 mV/s (a-j). Inset shows the linear plot for scan rate vs. current. B) At the same conditions, the cyclic voltammetry response of GR/PM composite in different pH at a scan rate of 50 mV/s. Inset is pH vs. current response.

Fig. 4A shows the CV response of GR/PM composite modified electrode in 500 µM GA containing PBS for different sweeping scan rates, and the scan rates were in the range from 20 to 200 mV/s. It can be seen that the oxidation peak current of GA increases with increasing the scan rates with small positive potential shift, and the anodic peak current of GA had linear relationship with the scan rates from 20 to 200 mV/s (a-j) with the correlation coefficient of 0.9949 (inset). The result indicates that the electrochemical oxidation behavior of GA at GR/PM modified electrode is controlled by typical adsorption controlled electrochemical process. The pH is a crucial factor for study the electrochemical behavior of GA on GR/PM composite modified electrode. Fig. 4B shows the CV response of GR/PM composite modified electrode in 500 µM of GA containing different pH at a scan rate of 50 mV/s. A maximum current response of GA was observed at pH 1 than other pH (inset). However, pH 3 was selected for the electrochemical experiments in order to use it in practical applications. In addition, the observed current response in pH 3 was found similar to the response observed in pH 1. We have also found that the anodic peak potential of GA had linear dependence with pH, and the peak potentials were shifted towards negative potential upon increasing the pH from 1 to 7. The anodic peak potential of GA vs. pH shows the slope value of 57.8 mV/pH with the correlation coefficient of 0.9911(not shown). The obtained slope value (57.8 mV/pH) is close to the previously reported slope value for an equal number of electron and proton transferred electrochemical redox process [2]. According to early reports, the oxidation of GA is involving of two protons and electrons transferred electrochemical redox process [2, 9]. The oxidation mechanism of GA at GR/PM composite modified electrode is shown in Scheme 2.



Scheme 2. Scheme for the plausible electrochemical oxidation mechanism of GA at GR/PM composite.

3.3. Amperometric determination of GA

Amperometric *i-t* method was used to determine the GA using GR/PM composite modified electrode. Fig. 5A shows the typical current-time response of GR/PM composite modified RDE for the addition of different concentration of GA (0.1–978.9 µM) into the constantly stirred PBS. The working potential held at 0.48 V. As shown Fig. 5A inset, the GR/PM composite modified electrode shows a sharp amperometric response for the addition of 0.1 (a), 0.3 (b), 0.5 (c), 1.0 (d), 3.0 (d) and 5 µM (e) GA into the constantly stirred PBS. The result indicates the high catalytic activity of composite modified electrode towards GA. The response time of the sensor was calculated as 3 s, which indicates the fast adsorption of GA on the composite modified electrode surface. As shown in Fig. 5B, the GR/PM composite modified electrode shows a stable response from 0.1 to 728.9 µM with the detection limit (LOD) of 0.027 µM based on S/N=3. The sensitivity of the sensor calculated as 0.697 $\mu A \mu M^{-1}$ cm^{-2} based on slope value/EASA of the composite electrode. The analytical comparison of our sensor with previously reported GA sensors is shown in Table 1. It can be seen that the obtained LOD of the sensor was lower than previously reported GA sensors including PM modified electrodes [2, 10–14]. In addition, the linear response range of the sensor was comparable with previously reported GA sensors as shown in Table 1. The excellent analytical features (lower LOD and wide linear response range) of the developed sensor can used for a sensitive detection of GA.

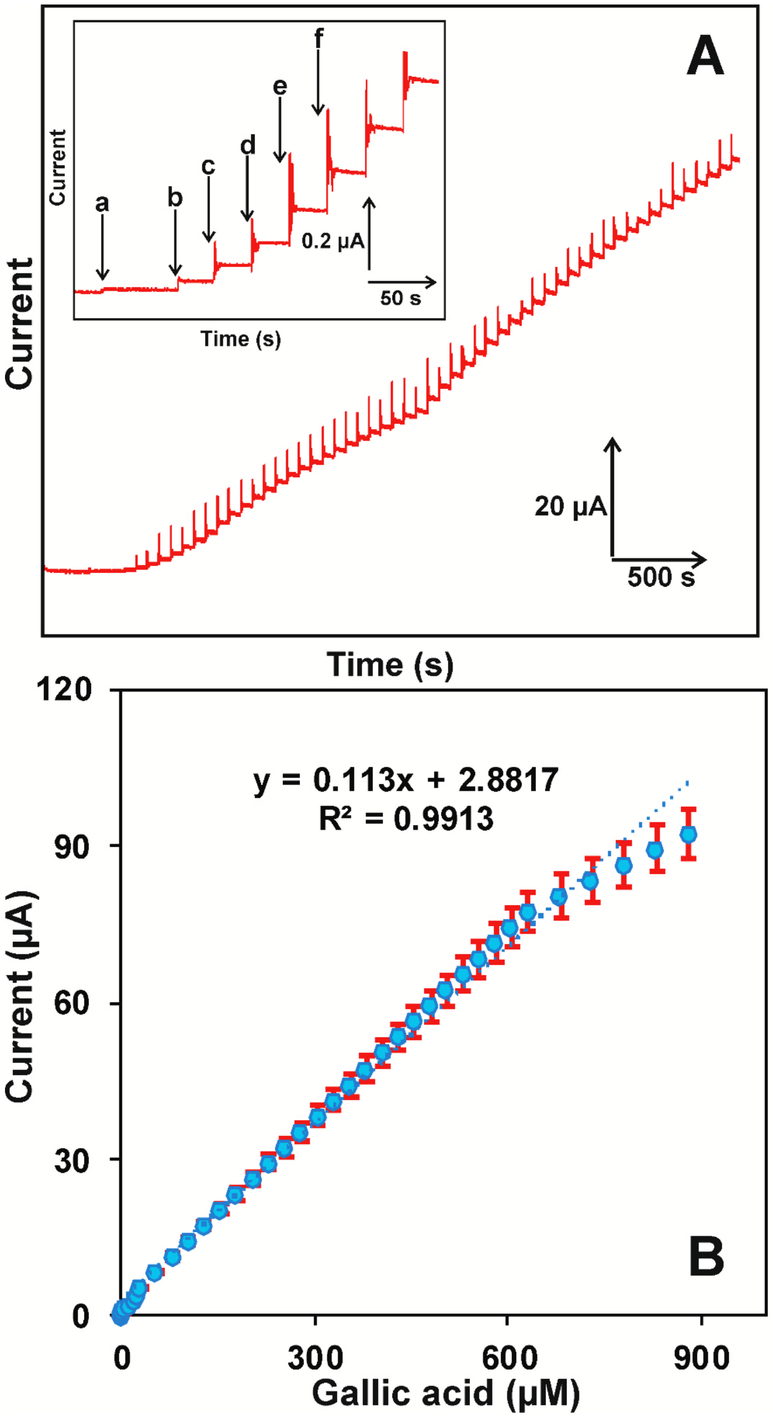


Figure 5. Amperometric i-t response obtained for GR/PM composite modified RDE for different concentration addition of GA into the constantly stirred N_2 saturated PBS. Working potential = 0.48 V. Inset shows the amperometric response of the GR/PM composite modified electrode for addition of 0.1 (a), 0.3 (b), 0.5 (c), 1.0 (d), 3.0 (d) and 5 µM GA into the constantly stirred PBS. Other working conditions are similar to Fig. 6A. B) Calibration plot for [GA] vs. amperometric current response.

Table 1. Analytical comparison (LOD and linear response range) of the as-prepared GA sensor (GR-PM modified GCE) with the previously reported GA sensors.

Modified electrode	**Method of detection**	**LOD (μM)**	**Linear range (μM)**	**pH**	**Ref.**
[1]ASPCE/PME	FIA*	0.21	up to 1000.0	7.0	**[2]**
[2]MIP-GCE	DPV**	3.5	up to 480.0	6.4	**[10]**
[3]SiO_2/CPE	DPV	0.25	up to 100.0	1.7	**[11]**
[4]CNT/CPE	DPV	0.5	up to 15.0	2.5	**[12]**
[5]TH/NiHCF/GE	DPV	1.66	up to 1200.0	1.12	**[13]**
[6]Zn-Al-NO_3 LDHf/GCE	DPV	2.6	up to 180.0	3.0	**[14]**
GR-PM/GCE	Amperometry	0.027	up to 728.9	3.0	This work

*Flow-injection amperometry
**Differential pulse voltammetry
[1] Poly(melamine) film immobilized on a pre-anodized screen-printed carbon electrode.
[2] Molecularly imprinted polypyrrole modified glassy carbon electrode.
[3] SiO_2 modified carbon paste electrode.
[4] carbon nanotubes modified carbon paste electrode.
[5] Thionine and nickel hexacyanoferrate modified graphite electrode.
[6] Zn-Al-NO_3 layered double hydroxide film on a glassy carbon electrode.

3.4. Selectivity, practicality, repeatability and reproducibility of the sensor

The selectivity of the sensor is more important when amperometric sensors are in positive working potentials. Hence, the selectivity of the sensor was evaluated in the presence of range of compounds including polyphenols and neurotransmitters, since these compounds have an oxidation potential very close where GA is oxidized. Fig. 6B shows Amperometric i-t response of GR/PM composite modified RDE for the addition of 0.5 µM GA (a), and 100 fold additions of catechol (CC), hydroquinone (HQ), resorcinol (RC), dopamine (DA), ascorbic acid (AA), uric acid (UA) and catechin (CT) into the constantly stirred PBS at a working potential of 0.48 V. It can be seen that a stable and sharp amperometric response was observed for the addition of 0.5 µM GA. The composite modified electrode did not show any apparent response for the addition of 50 µM of CC, HQ, RC and AA. While, a weak response was observed for the addition of 50 µM of DA, UA and CT. However, the observed response of these compounds are negligible when compared to the response of GA. The result indicates the high selectivity of GR/PM composite for detection of GA.

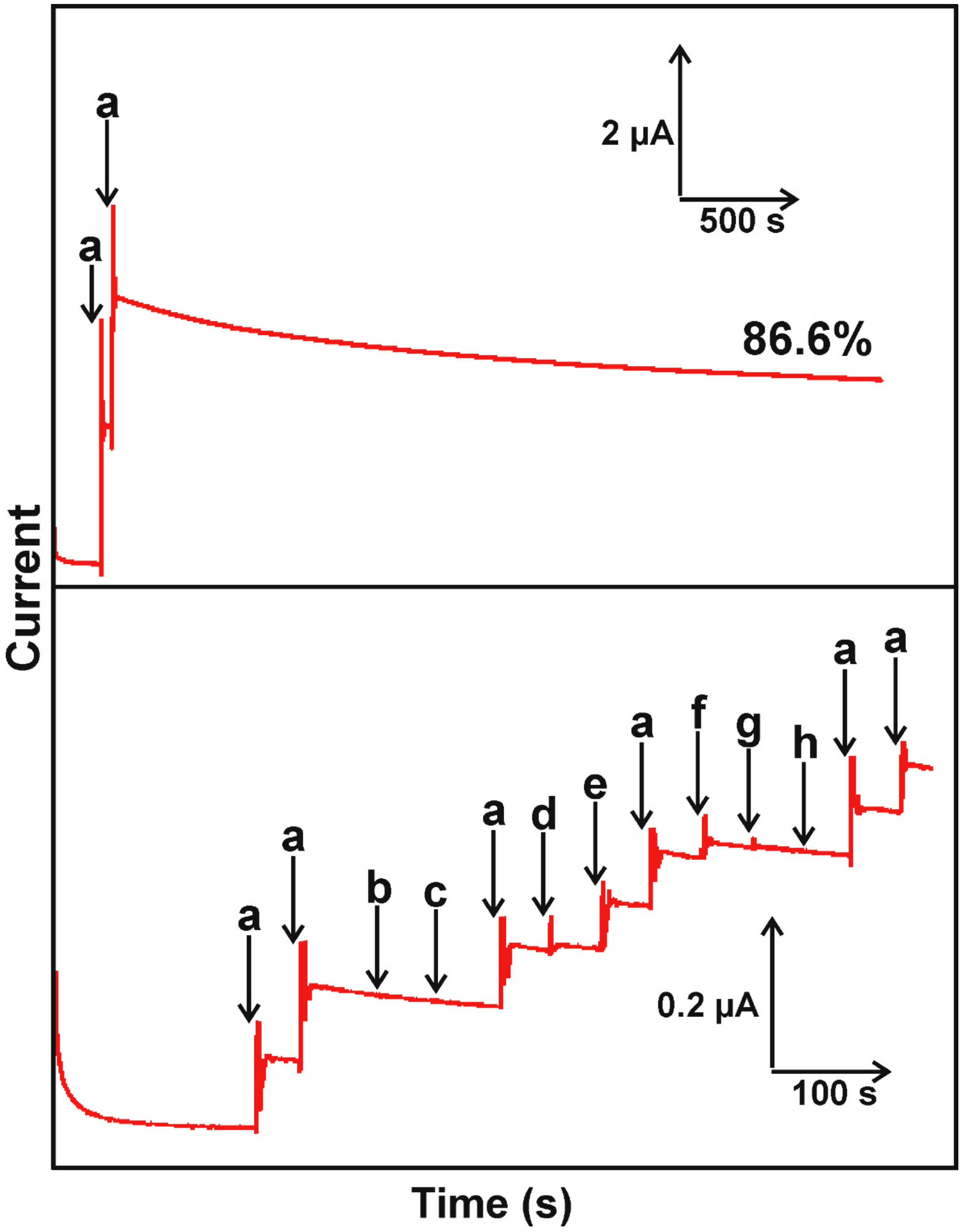


Figure 6. A) Amperometric i-t response of GR/PM composite modified electrode for the addition of 20 μM GA into the PBS, and its background current response up to 4000 s. B) Amperometric i-t response of GR/PM composite modified RDE for the addition of 0.5 μM GA (a) and 50 μM additions of CC (b), HQ (c), RC (d), DA (e), AA (f), UA (g) and CT (h) into the constantly stirred N_2 saturated PBS. Working potential = 0.48 V.

To evaluate the practicality of the sensor, we have determined the GA content in green tea samples using GR/PM modified electrode by amperometry. The experimental conditions are similar to Fig. 5A. The standard addition method was used for calculation of recovery of GA in green tea samples [28]. The concentration of GA in diluted green tea samples were found as 42.6 and 57.6 μM.

Then, a known concentration of GA containing green tea samples were injected into the electrolyte solution and the obtained recovery values of GA is summarized in Table 2. It can be seen that the average recoveries of GA in green tea samples were 97.2 and 96.7%, with acceptable relative standard deviation (RSD). Hence, the proposed GR/PM composite modified electrode can be used for accurate determination of GA in green tea samples.

Table 2. Determination of GA in green tea samples using GR/PM composite modified electrode by amperometry. (n=3)

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Green tea 1	–	42.6	–	–
	10.0	51.8	98.1	3.3
Green tea 2	–	57.6	–	–

We have also investigated the operational stability of the sensor by amperometry. Fig. 6A shows the amperometric *i-t* response obtained for GR/PM composite modified RDE for addition of GA into the constantly stirred N_2 saturated PBS at a working potential of 0.48 V and its background current response up to 4000 s. It is clear that the sensor retains 86.6% of its initial background current response after continues run up to 4000 s. The result indicates that the high operational stability of the sensor. The GR/PM composite modified electrode exhibit the acceptable reproducibility (RSD = 3.6%) for the detection of 500 μM GA using 5 different independently prepared GR/PM composite modified electrodes. The reproducibility of the sensor was examined by CV and the experimental conditions are similar as of in Fig. 3. The result indicates that high accuracy of the composite modified electrode for detection of GA. The promising features of the as-prepared GR/PM composite modified electrode can be used viable sensing of GA.

4. CONCLUSIONS

In summary, we have developed a sensitive and selective GA amperometric sensor using GR/PM composite for the first time. The physiochemical characterizations confirmed the formation of GR/PM composite. The unique combined synergetic effects of GR and PM in GR/PM composite resulted into the enhanced electrocatalytic activity towards oxidation of GA than pristine GR and PM modified electrodes. The GR/PM composite modified electrode exhibited an excellent analytical features towards detection of GA, such as low LOD (27 nM), wide linear response (up to 728.9 μM), fast response (3 s) and high selectivity. The excellent practicality of the sensor is further open for the determination of GA in green tea samples with high accuracy. As a future perspective, GR/PM composite can be used for construction of other electrochemical sensors..

ACKNOWLEDGEMENTS
The project was supported by the Ministry of Science and Technology (MOST), Taiwan.

References

1. M.A. Thompson and P.B. Collins, Handbook on Gallic Acid: Natural Occurrences, Antioxidant Properties and Health Implications, Nova Science Pub Inc, New York, 2013.
2. Y.L. Su and S.H. Cheng, *Anal. Chim. Acta*, 901 (2015) 41.
3. A. Blainski, G. Lopes and J. Mello, *Molecules*, 18 (2013) 6852.
4. W. Qu, A.P.B. Iii, Z. Pan and H. Ma, *Food Chem.* 132 (2012) 1585.
5. Z. Sun, L. Zhao, L. Zuo, C. Qi, P. Zhao and X. Hou, *J. Chromatogr. B*, 958 (2014) 55.
6. B.G.T. Corominas, J.V.G. Mateo, L.L. Zamora and J.M. Calatayud, *Talanta*, 58 (2002) 1243.
7. W. Phakthong, B. Liawruangrath and S. Liawruangrath, *Talanta*, 130 (2014) 577.
8. W. Ma, D. Han, S. Gan, N. Zhang, S. Liu, T. Wu, Q. Zhang, X. Dong and L. Niu, *Chem. Commun.* 49 (2013) 7842.
9. R. Abdel-Hamid and E.F. Newair, *J. Electroanal. Chem.* 657 (2011) 107.
10. J.H. Luo, B.L. Li, N.B. Li and H.Q. Luo, *Sens. Actuators B*, 186 (2013) 84.
11. J. Tashkhourian and S.F. Nami-Ana, *Mater. Sci. Eng. C*, 52 (2015) 103.
12. L.P. Souza, F. Calegari, A.J.G. Zarbin, L.H. Marcolino-Junior and M.F. Bergmann, *J. Agric. Food Chem.* 59 (2011) 7620.
13. N.S. Sangeetha and S.S. Narayanan, *Anal. Chim. Acta*, 828 (2014) 34.
14. M. Kahl and T.D. Golden, *Electroanalysis*, 26 (2014) 1664.
15. E.P. Randviir, D.A.C. Brownson and C.E. Banks, *Mater. Today*, 17 (2014) 426.
16. S. Palanisamy, S.K. Ramaraj, S.M. Chen, T.C.K. Yang, P.Y. Fan, T.W. Chen, V. Velusamy and S. Selvam, *Scientific Rep.* 7 (2017) 41214.
17. S. Palanisamy, A.T. Ezhil Vilian and S.M. Chen, *Int. J. Electrochem. Sci.*, 7 (2012) 2153.
18. S. Palanisamy, C. Karuppiah and S.M. Chen, *Colloids Surf., B*, 114 (2014) 164.
19. M.E. Khan, M.M. Khan and M.H. Cho, *RSC Adv.*, 5 (2015) 26897–26904.
20. M. Pumera, A. Ambrosi, A. Bonanni, E. L. K. Chng and H. L. Poh, *Trends Anal. Chem.*, 29 (2010) 954.
21. Y. Shao, J. Wang, H. Wu, J. Liu, I. A. Aksay and Y. Lin, *Electroanalysis*, 22 (2010) 1027.
22. J. Peng, Y. Feng, X.X. Han and Z.N. Gao, *Microchim. Acta*, 183 (2016) 2289.
23. P.S. Dorraji and F. Jalaliz, *J. Electrochem. Soc.* 162(9) (2015) B237.
24. X. Liu, L. Luo, Y. Ding, Q. Wu, Y. Wei and D. Ye, *J. Electroanal. Chem.* 675 (2012) 47.
25. S. Palanisamy, S.K. Ramaraj, S.M. Chen, T.W. Chiu, V. Velusamy, T.C.K. Yang, T.W. Chen and S. Selvam, *J. Colloid Interface Sci.* (2016) http://dx.doi.org/10.1016/j.jcis.2016.12.062.
26. S. Palanisamy, S.K. Ramaraj, S.M. Chen, V. Velusamy and T.C.K. Yang, *Microchim. Acta* (2016) DOI: 10.1007/s00604-017-2073-y.
27. S. Palanisamy, K. Thangavelu, S.M. Chen, B. Thirumalraj and X.H. Liu, *Sensors and Actuators B* 233 (2016) 298.
28. B. Unnikrishnan, S. Palanisamy and S.M. Chen, *Biosens. Bioelectron.* 39 (2013) 70.