Int. J. Electrochem. Sci., 13 **(2018)** 3948 – 3957, doi: 10.20964/2018.04.63

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

On-Surface Formation of Polyarginine/Reduced Graphene Oxide Film and Its Application in Measuring Puerarin in Healthcare Products

Yufei Li[1,2], *Xiaotong Feng*[1], *Lei Zhao*[1], *Qiaosheng Pu*[1*]

[1]Department of Chemistry, Lanzhou University, Lanzhou, Gansu 730000, China
[2]Gansu Food Inspection and Research Institute, Lanzhou, Gansu 730000, China
[*]E-mail: puqs@lzu.edu.cn

Received: 20 November 2017 / *Accepted:* 14 February 2018 / *Published:* 6 March 2018

Graphene was an ideal material for various applications including electrochemical sensing. To improve the selectivity of graphene-based sensors, preparation of composites of graphene is an effective way. In this work, films of polyarginine/reduced graphene oxide were in-situ formed on the surface of the electrode and used as an electrochemical sensor of puerarin. The films were systematically characterized and the established sensor gave a linear response range from 2.3×10^{-7} to 5.5×10^{-6} mol·L^{-1} and a limit of detection of 4.0×10^{-8} mol·L^{-1}. The sensor was successfully applied for measuring puerarin in dietary supplements with a relative standard deviation of 2.4% and a standard recovery of 96.5%.

Keywords: graphene oxide, arginine, on-surface polymerization, modified electrode, puerarin

1. INTRODUCTION

As a semiconductor with zero-gap, graphene has been taken as an ideal 2D electronic material due to its unique electronic band structure and properties. Its higher electron mobility comparing with silicon and super big theoretical specific surface area make it a challenging component of electronic and electrochemical sensors. Reduced graphene oxide (rGO) is a kind of graphene normally manufactured by reduction of graphene oxide (GO) and it contains certain amounts of chemical groups, including epoxy, carboxyl, and hydroxyl, provides relatively high hydrophilicity and water solubility and is therefore more suitable for building aqueous solution based sensors[1]. Exploring functionalized graphene or its composites is still an important way to extend the application of graphene derivatives. Combination of rGO and biological materials with molecular recognition characteristics can fully take

advantage of the electronic characteristic of graphene and the selectivity of bio-molecules [2-7].

Puerarin (7,4-dihydroxyisoflavone-8-glucopyranoside) is an active compound extracted from the plants of Pueraria (Fabaceae) family. Pueraria is a traditional herb has long to be used for the treatment of fever, headache, diarrhea, dizziness and other related symptoms. Pharmacological effects of puerarin include the inhibition of β-adrenal activation of adenylatecyclase, expansion of the coronary artery, and treatment of angina pectoris and myocardial infarction. Puerarin can also be used to treat deafness caused by migraine and the occlusion of the retinal artery [8-12]. Detection of trace amount of this compound is important for either quality control of medicines or food safety aspects. Puerarin can be measured with techniques including liquid chromatography [13,14], liquid chromatography-mass spectrometry [15-21], capillary electrophoresis [22-25], and flow-injection chemiluminescence, but instruments of these techniques are bulky and expensive. Electrochemical sensors for puerarin have attracted wide attention due to the advantages including low-cost instruments, easy operation, high speed and excellent sensitivity. Some functional materials such as nano-cerium oxide/multi-walled carbon nanotube composites [26-31], cadmium telluride quantum-dot/functionalized graphene oxide (GO) composites [32], and zirconium oxychloride-doped carbon paste electrodes have been used for this purpose [33], but preparation of these materials on the electrode is rather complicated. Long period stability of metal based nano-particles used in these works may also a concern.

In this study, we optimized a procedure of layer-by-layer assembling of polyarginine/rGO through controlled on-surface polymerization of L-arginine to obtain densely uniformly distributed rGO. Polyarginine possess a protein - like structure and exhibits positive charge in a wide range pH due to its strong basicity of its side-chain guanidine groups (pKa = 12.48), its strong interaction with rGO firmly anchored both of the components on a glassy carbon electrode to stabilize rGO and provided necessary chemical functionality. The prepared sensor exhibits excellent sensitivity and selectivity toward puerarin.

2. EXPERIMENTAL PROCEDURE

2.1 Formation of polyarginine stabilized rGO

Polyarginine stabilized rGO was formed in-situ on the surface of a glassy carbon electrode (diameter of 3 mm), which was polished with 0.05 μm alumina powder and ultrasonically cleaned in de-ionized water and ethanol, using a potentiostat (CHI660D CH Instrument, Shanghai, China) with a Pt wire counter electrode and a Ag/AgCl reference electrode. The electrodes were first placed in an L-arginine (Xi'an Zhou Dingguo Chemical Reagent Company) solution of 4 $mmol \cdot L^{-1}$ and scanned in a potential range from –2.0 to +2.5 V at a rate of 100 $mV \cdot s^{-1}$ for 8 cycles. The surface was rinsed with de-ionized water and dried naturally for later characterization. The rGO layer was assembled onto the polyarginine layer through a similar procedure using a 1.0 $mg \cdot mL^{-1}$ GO (Nanjing Xianfeng Nano-technology Company) solution with a scanning rate of 100 $mV \cdot s^{-1}$ and scanning range from 0.0 to +0.9 V for 16 cycles. The surface was rinsed with de-ionized water and dried by blowing nitrogen gas for

later use. The diagram of the electrode preparation is depicted in Fig 1. Scanning electron microscopy (SEM) measurements were performed on a MIRA3 TESCAN scanning electron microscope.

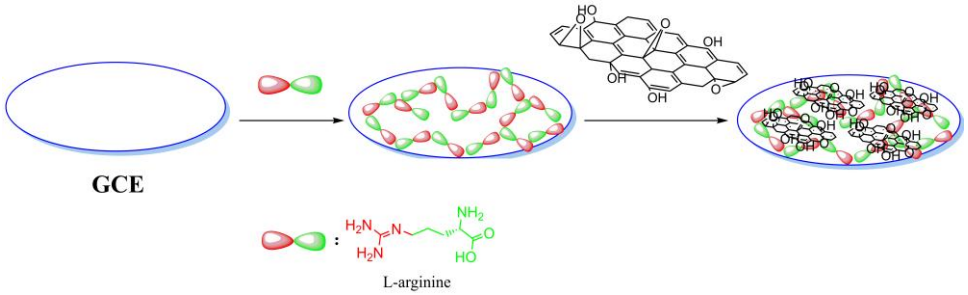

Figure 1. A schematic diagram of the preparation of the polyarginine/rGO layer.

2.2 Contact angle measurement

Contact angle (CA) measurement was performed by taking images of sessile drops of 3.0 µL of distilled water at the surface electrode with modified layer. The angle was calculated using a contact angle plugin for Image J as mentioned previously [34] with 5 replicates.

2.3 Cyclic voltammetry and Electrochemical impedance spectroscopy

Cyclic voltammetry (CV) was performed with the standard three-electrode setup as mentioned above. 2 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) was used as the probe molecule in 0.1 M phosphate buffer solution (pH = 7.0) containing 0.1 M KCl. Scanning rate was set to 100 mV s^{-1} for the electrode characterization. CV for puerarin was obtained in 0.5 mol·L^{-1} H_2SO_4 with 1 % KCl at a scanning rate of 50 mV·s^{-1}. Electrochemical impedance spectroscopy (EIS) was used to investigate the electron transfer on the modified layer. It was performed with the same setup in the same buffer solution.

2.4 Puerarin sensing

The puerarin standard solution of 11.5 mmol·L^{-1}.was prepared by accurately weighing 0.048 g of puerarin and dissolving it in 10.00 mL 70% methanol solution. The obtained standard solution was stored inside a brown bottle at 4 °C and diluted to desired concentrations ranging from 2.3 × 10^{-7} to 5.5 × 10^{-6} mol·L^{-1} just before use. Differential pulse voltammetry measurements were performed in the range from +0.6 to +1.0 V with a pulse magnitude of 0.05 V, sampling width of 0.0167 s, pulse width of 0.2 s, and pulse period of 0.5 s. 0.5 mol·L^{-1} H_2SO_4 solution was used as the sensing medium.

Commercially available Niubeile Pueraria soft capsules were used as the sample. 0.5 g of the capsule was placed in a 25 mL vial containing a proper amount of 70% methanol solution and then ultrasonicated for 20 min to extract puerarin. After cooled down to 20 °C the volume was supplemented to 10.00 mL with 70% methanol, and filtered through a 0.45 µm membrane after thorough mixing. The filtrate was used for the sensing.

3. RESULTS AND DISCUSSION

3.1 Characterization of polyarginine/rGO layer

Due to its guanidine groups, arginine is a typical zwitterion exhibits positive charge in a wide range of pH. It has been proven that arginine can form a polymer layer with the aid of electrochemical force Radical produced during electrochemical process make it firmly anchored to the surface and also guaranteed the chemical attachment of GO [35]. CVs obtained during the electro-polymerization process indicate that the redox peaks became stable after 8 cycles of scan. Deposition of GO gave decreasing reduction current, indicating the assembling of GO and its reduction to rGO. The morphology of the polyarginine/rGO composite film was also confirmed by SEM. The surface of polyarginine/rGO is rough and presents a kind of squamaceous structure, while the surface of the bare electrode is smooth. The strong combination between the polyarginine and rGO may be favourable for the charge transfer inside the composite.

The modification was also confirmed by CA measurement. CAs were measured before and after the formation of polyarginine/rGO layer. The CA of the bare electrode surface was 63.6° (Fig. 2A), while after the polyarginine formation it decreased to 43.5° (Fig. 2B), after assembling of rGO, it became 54.2° (Fig. 2C). These results demonstrate the successful formation of polyarginine/rGO layer. The largest static contact angle among three materials was observed on the surface of bare electrode (63.6° for deionized water) due to its intrinsic hydrophobicity. The contact angle decreased to 54.2° after modified by polyarginine and GO, evidently reflecting the change of the hydrophilicity of the surface. These results proved that the surface of bare electrode has been successfully modified by polyarginine/rGO layer too.

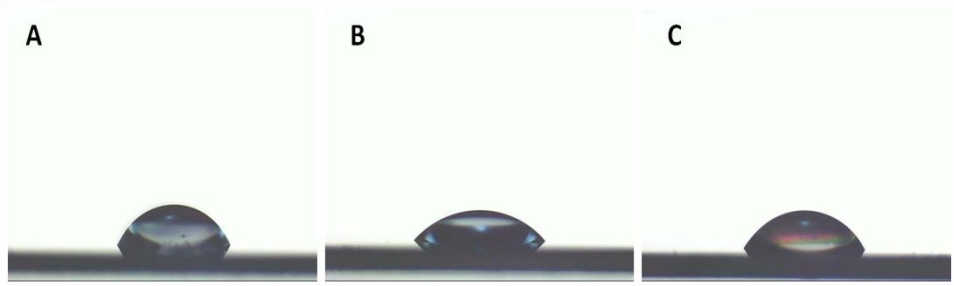

Figure 2. Contact angle measurements conducted for the (A) bare electrode, (B) polyargininelayer, and (C) polyarginine/rGO film.

3.2 Electrochemical behavior of polyarginine/rGO

The electrochemical behavior of the modified electrode was investigated with CV. Comparing with the bare electrode, both anodic and cathodic peaks increased evidently with the formed layers, indicating the conductivity of the film is good and can improve the electron transfer efficiency between the electrode and the surface of the formed layer. This was also confirmed by electrochemical

impedance spectroscopy. EIS is normally used to evaluate the characteristics of electron conduction processes on the modified surfaces.

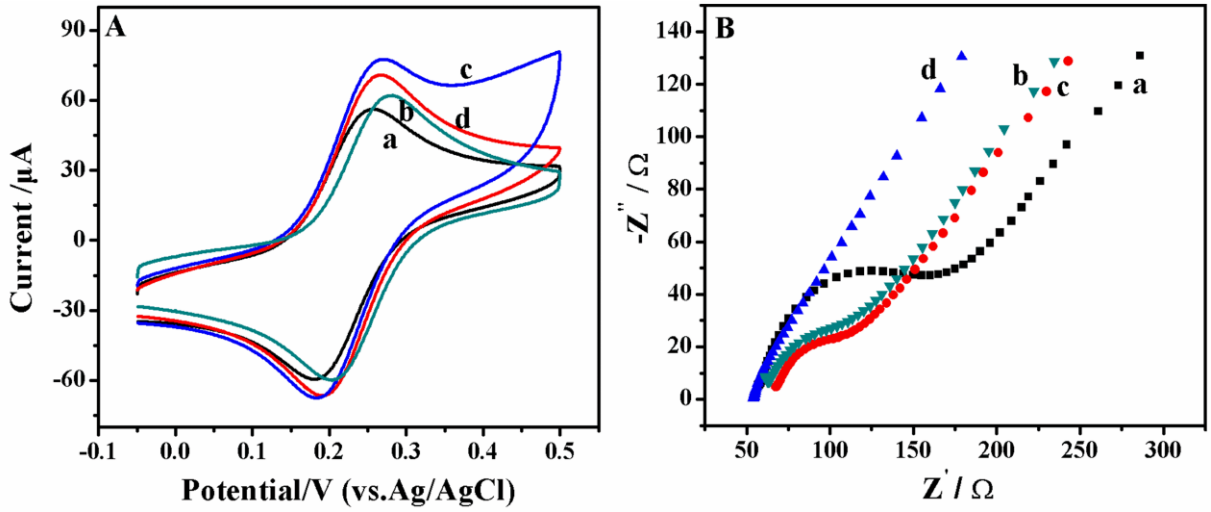

Figure 3. (A) CV and (B) alternating current impedance plots recorded for the (a) bare electrode, (b) GO layer, (c) polyarginine layer, and (d) polyarginine-rGO film in the 0.1 mol·L^{-1} phosphate solution containing 0.1 mol·L^{-1} KC1 and 2 mmol·L^{-1} $[Fe(CN)_6]^{3-/4-}$ (1:1).

The diameter of the semicircle at the high-frequency end corresponds to the electron transfer resistance, and the linear part at the low-frequency region is related to the diffusion process [36]. With polyarginine and GO species, this resistance represented by the semicircle is much smaller. Therefore, the formed layer possesses good electrochemical performance and suitable to be used as a sensor.

3.3 Electrochemical sensing of puerarin

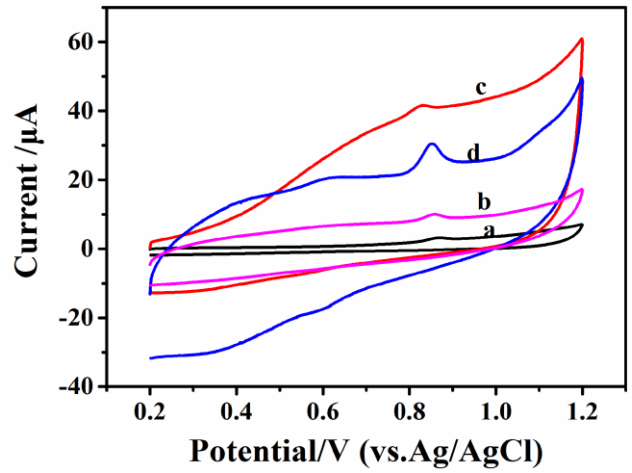

Figure 4. CV recorded for the puerarin solutions in 0.5 mol·L^{-1} H_2SO_4 using the (a) bare electrode, (b) GO layer, (c) polyarginine layer, and (d) polyarginine-rGO film.

The CVs of puerarin dissolved in a mixture of 0.5 mol·L^{-1} H_2SO_4 and 1% KCl were obtained at a scanning rate of 50 mV·s^{-1} using the ployarginine and ployarginine/rGO layer covered electrodes as well as the bare ones. Fig. 4 shows the results of puerarin at different electrodes. The order of the oxidation peak currents of puerarin on these electrodes are as follows: polyarginine-rGO film > polyarginine layer > GO layer > bare electrode, which implies that electrochemical oxidation behavior of puerarin on the bare GCE was improved significantly by polyarginine-rGO. At a puerarin concentration of 0.01 mmol·L^{-1}, the peaks of oxidation and reduction are low on the bare electrodes. While with polyarginine/rGO layer, there is about ten times of boost of the current, indicating good sensing performance for puerarin.

The CVs of puerarin in 0.5 mol·L^{-1} H_2SO_4 containing 1% KC1 at scanning rates of 5, 10, 15, 20, 30, 40, 50, 60, 70, and 80 mV·s^{-1} (Fig. 5A) showed linear dependence of both anodic and cathodic currents on the scanning rate, I_{pa} (μA) = 1.473 + 0.076V (mV·s^{-1}) (R = 0.981), indicating that the reaction of puerarin at polyarginine/rGO layer is adsorption controlled.

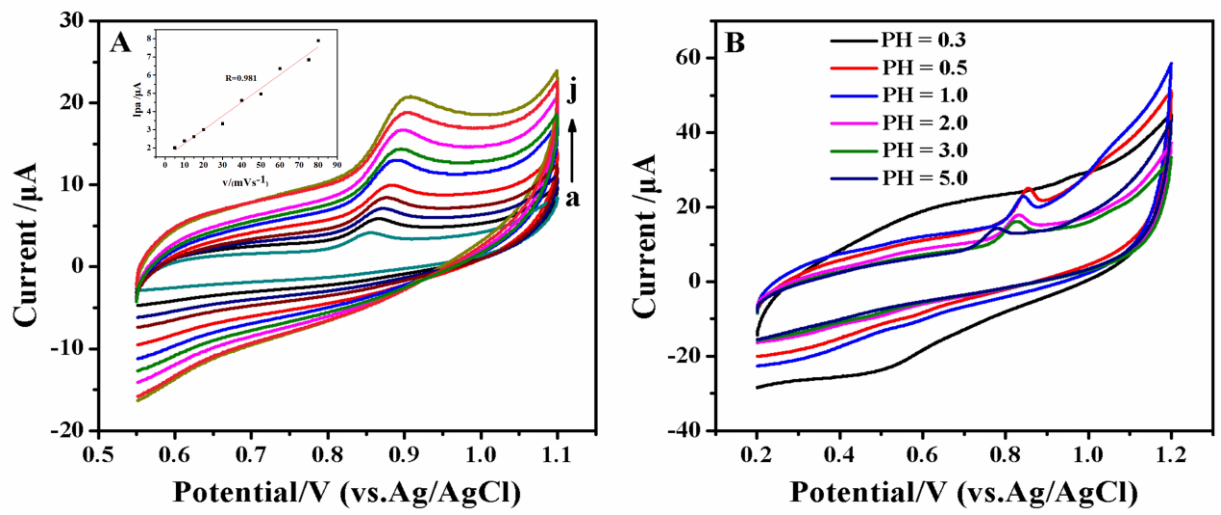

Figure 5. (A) CV obtained for the puerarin solutions in 0.5 mol·L^{-1} H_2SO_4 at scanning rates of 5 - 80 mV·s^{-1} (a - j). (B) CV obtained for the puerarin solutions at pH values of 0.3, 0.5, 1.0, 2.0, 3.0, and 5.0.

The effect of pH on the sensing performance was studied (Fig. 5B). No peak can be observed if the pH value is 7.0 or higher, but if it is too acidic (pH < 0.3) the peaks disappears too. The highest peak was obtained at pH 0.5. Under the optimized condition, the oxidation peak current versus the puerarin concentration is linear from 2.3 × 10^{-7} to 1.84 × 10^{-6} mol·L^{-1} I_{pa} (μA) = 1.11C – 0.185 (μmol·L^{-1}) (R = 0.965) with a slope of 1.11 μA per μmol·L^{-1} (Fig. 6). If the concentration is higher, the slope is lower, I_{pa} (μA) = 0.361C + 1.32 (μmol L^{-1}) (R = 0.958), for concentrations from 1.85 × 10^{-6} to 5.5 × 10^{-6} mol·L^{-1}, suitable for sensing puerarin at a wide concentration range. These results are consistent with the adsorption controlled behaviour mentioned above. At lower concentrations, the electrode surface is not fully occupied by the puerarin, the sensitivity is higher. At higher concentrations, the surface is fully occupied by the analyte molecules, which led to an evident decrease of the slope of the calibration curve.

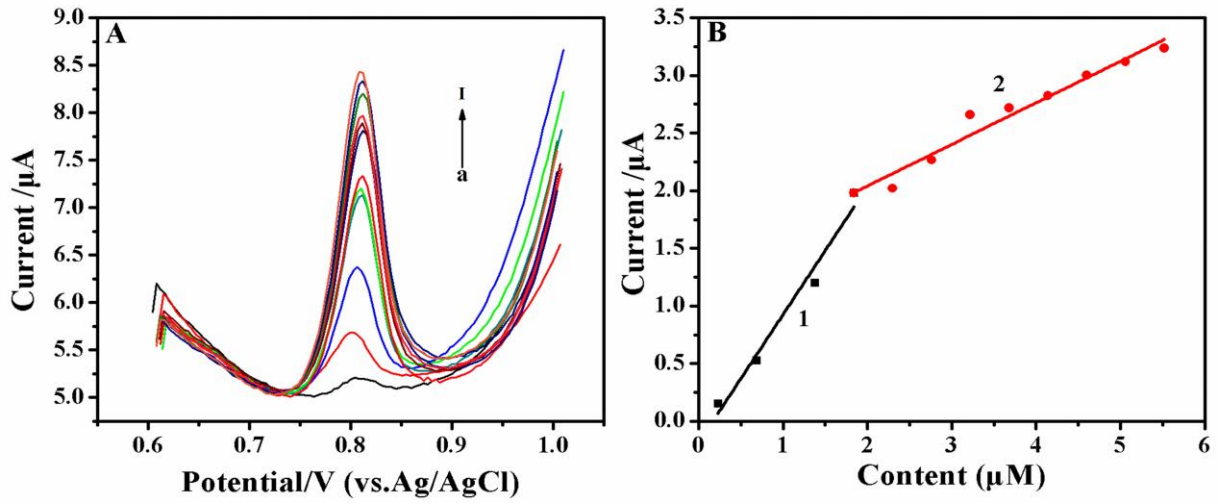

Figure 6. (A) Differential pulse voltammeogram obtained at various puerarin concentrations in the 0.5 mol·L^{-1} H_2SO_4 solution. (B) Oxidation peak current plotted versus the puerarin concentration.

Table 1 summarizes a comparison of some of the analytical performance achieved for the determination of puerarin with different methods. UPLC-MS/MS, LC–ESI-MS, UPLC–MS, RRLC–MS/MS[16,19-21] normally give good performance, but electrochemical methods is superior due to its low expense, simple system, and easy miniaturization. CL including CE–CL and FI-CL[22-24] methods exhibit higher sensitivity and lower detection limit, but their application may be limited by the reagents and instrumentation.

Table 1. Comparison of different methods for puerarin determination

Methods	Linear range (μM)	Detection limit (μM)	Toxicity	References	Date
HPLC	0.012-1.2	0.0072		[13]	2005
UPLC-MS/MS	0.06-8.9	0.009		[16]	2013
LC–ESI-MS	0.001-0.96	0.0009		[19]	2008
UPLC–MS	0.0236-5.9	0.0236		[20]	2009
RRLC–MS/MS	0.024-4.8	0.0008		[21]	2011
CE–CL	0.05-2.5	0.01		[22]	2009
CL	0.08-2.0	0.0075		[23]	2011
FI-CL	0.01-6.0	0.0028		[24]	2017
SWCNTs/poly-EB/GCE	0.3-46.0	0.12		[27]	2012
Poly(Alizarin Red S/Graphene)/ GCE	0.1-750	0.034		[28]	2017
nano-CeO2/MWCNTs/GCE	0.04 to 6.0	0.008		[29]	2011
PSS-GN/WO3/GCE	0.06-6.0	0.04		[30]	2017
CdTe@[emim]MP-amimRG/ GCE	0.01-4.0	0.0006		[31]	2017
CdTe-PDDA-Gr/GCE	0.001-1.0	0.0006		[32]	2015
This work	0.23-5.5	0.04			
China standard HPLC method GB/T22251-2008	12-120	0.048		[37]	2008

Comparing with other electrochemical methods, the proposed polyarginine/rGO/GCE displayed good sensitivity, wider linear range. The preparation process of the electrode is simple. Although its sensitivity is not the highest, the proposed approach displayed good stability and high reproducibility as five modified electrodes that were prepared employing the same process gave roughly same performance. It should be mentioned that some organic polymers, metal oxide nanoparticles, and semiconductor quantum dots have been reported to constructed modification electrodes [29-32], but the potential toxicity of these materials may be a concern.

3.4 Stability, reproducibility and selectivity studies

The stability of the polyarginine/rGO/GCE towards puerarin was investigated. It displays good stability and high reproducibility as five modified electrodes were respectively built by employing the same process. The relative deviation of 5 measurements of puerarin of 1.0 μmol·L^{-1} was 3.12%. The limit of detection is 4.0×10^{-8} mol·L^{-1}. The stability of the layer is good, the same electrode with the formed layer stored at 4 °C for 72 h gave 93.8% of the original value of a freshly prepared layer.

At a puerarin concentration of 0.01 mmol·L^{-1}, the effect of possible interferences on the sensing was studied by adding 50-fold concentrations (0.50 mmol·L^{-1}) of lactic acid, cysteine, glutamic acid, valine, phenylalanine, tryptophan, tyrosine, glucose, and citric acid, the deviation caused by these compounds are negligible. 100-fold concentrations of metal ions including Zn^{2+}, Na^{+}, K^{+}, Mn^{2+}, Cu^{2+}, Ca^{2+}, Hg^{2+}, Pb^{2+}, and Cd^{2+} as well as NO_3^-, Cl^-, CO_3^{2-}, PO_4^{3-}, NO_2^-, and SO_4^{2-} anions gave no interference too, probably due to the high acidity of the medium. Only isoflavones, which have similar structure as puerarin showed response. These results proved the good selectivity of the sensor.

3.5 Real sample analysis

Table 2. Puerarin contents in the starch and puerarin samples.

Samples	Measured (μM)	Added (μM)	Found (μM)	RSD (%)	Recovery (%)
Starch soft capsule	0.00	0.80	0.77 ± 0.02	1.08	96.3
Puerarin soft capsule	1.40	0.60	1.98 ± 0.02	3.72	96.7

Puerarin in a dietary supplement Niubeile Pueraria soft capsule was measured via the proposed sensor through the standard addition method (Table 2), with a starch soft capsule as a control. The estimated puerarin recovery were 96.7% and 96.3% with relative standard deviations of 3.72% and 1.08%, respectively. The obtained results indicate that the proposed sensor can be used for real samples. The proposed method is suitable for determination of puerarin in Niubeile Pueraria soft capsule samples with advantages of higher efficiency, higher sensitivity, and lower detection limit. The

result is consistent with that obtained with the Chinese national standard method by HPLC (GB/T22251-2008)[37].

4. CONCLUSIONS

Polyarginine/rGO film was used as an electrochemical sensor for puerarin concentration in healthcare products. The film was characterized systematically. The fabricated sensor exhibited high electrochemical activity and can measure puerarin in a range of 2.3×10^{-7} to 5.5×10^{-6} mol·L^{-1} with a limit of detection of 40 nmol·L^{-1}. The significance of this work is on the facile detection of puerarin in real samples with easily prepared electrodes with arginine/rGO layers. The proposed sensor is an example to extend the sensing application of graphene and may be used for on-site quick evaluation the quality of foods or other related products because of easy miniaturization of electrochemical devices.

ACKNOWLEDGEMENTS
Assitance of Mr Derong. Zhang on the preparation of the manuscript was highly appreciated.

References

1. B. Nam, H.J. Lee, H. Goh, Y.B. Lee, W.S. Choi, *J. Mater. Chem*., 22 (2012) 3148.
2. T. Schrader, *Chem. Eur. J*., 3 (1997) 1537.
3. D. Zakim, Y. Hochman, W.C. Kenney, *J. Biol. Chem*., 258 (1983) 6430.
4. V. Jubian, R.P. Dixon, A.D. Hamilton, *J. Am. Chem. Soc*., 114 (1992) 1120.
5. A. Echavarren, A. Galan, J.M. Lehn, J. De Mendoza, *J. Am. Chem. Soc*., 111 (1989) 4994.
6. T. Kim, M. Ou, M. Lee, S.W. Kim, *Biomaterials*, 30 (2009) 658.
7. 7 .J.S. Choi, K. Nam, J.Y. Park, J.B. Kim, J.K. Lee, J.S. Park, *J. Control. Release*., 99 (2004) 445.
8. L.L. Fan, D.D. O'Keefe, W.J.Jr. Powell, *Acta. Pharmacol. Sin*., 19 (1984) 801.
9. X.P. Song, P.P. Chen, X.S. Chai, *Acta Pharmacol. Sin.*, 9 (1988) 55.
10. F.L. Hsu, I.M. Liu, D.H. Kuo, W.C. Chen, H.C. Su, J.T. Cheng, *J. Nat. Prod*., 66 (2003) 788.
11. K.H. Wong, G.Q. Li, K.M. Li, V. Razmovski-Naumovski, K. Chan, *J. Ethnopharma-col*., 134 (2011) 584.
12. M.C. Guerra, E. Speroni, M. Broccoli, M. Cangini, P. Pasini, A. Minghett, N.Crespi-Perellino, M. Mirasoli, G. Cantelli-Forti, M. Paolini, *Life Sci*., 67 (2000) 2997.
13. Z.Ma, Q. Wu, D.Y.W. Lee, M. Tracy, S.E. Lukas, *J. Chromatogr. B Analyst. Technol. Biomed. Life Sci*., 823 (2005) 108.
14. I. Baranowska, S. Magiera, J. Baranowski, *J. Chromatogr. B Analyst. Technol. Biomed. Life Sci*., 879 (2011) 615.
15. J.K. Prasain, K. Jones, N. Brissie, R. Moore, J.M. Wyss, S. Barnes, *J. Agric. Food Chem*., 52 (2004) 3708.
16. N. Li, Y. Deng, D. Wang, Y. Qiao, F. Li, *Talanta*, 104 (2013) 109.
17. J.K. Prasain, K. Jones, M. Kirk, L. Wilson, M. Smith-Johnson, C. Weaver, S. Barnes, *J. Agric. Food Chem*., 51 (2003) 4213.
18. H.R. Jung, S.J. Kim, S.H. Ham, J.H. Cho, Y.B. Lee, H.Y. Cho, *J. Chromatogr. B*., 971 (2014) 64.

19. Q.Q. Wang, X.S. Li, S.J. Dai, L. Ou, X. Sun, B.Z. Zhu, F. Chen, M.M. Shang, H.F. Song, *J Chromatogr B.*, 863 (2008) 55.
20. Y. Wang, Y.M. Yao, R. An, L.S. You, X.H. Wang, *J Chromatogr B.*, 877 (2009) 1820.
21. C.F. Luo, M. Yuan, M.S. Chen, S.M. Liu, B.Y. Huang, X.W. Liu, L. Zhu, *J Chromatogr B.*, 879 (2011) 1497.
22. S.Q. Han, *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, 877 (2009) 1591.
23. Z. Cai, X. Zhang, D.F. Lu, *Chin J Chem.*, 29 (2011) 1261.
24. Z.Y. Fan, S.Q. Han, J.M. Zhang, *J. Chin. Chem. Soc.*, 64 (2017) 993.
25. W. Xiao, F.Q. Wang, C.H. Li, Q. Zhang, Z.N. Xia, F.Q. Yang, *Anal. Methods.*, 7 (2015) 1098.
26. Y. Ji, G. Wang, C. Zhang, B. Fang, *Chin. J. Chem.*, 29 (2011) 1017.
27. J. Wang, J. Mu, J. Ma, Y. Yang, M. Wang, L. Zhu, X. Du, *J. Food Drug Anal.*, 20 (2012) 611.
28. W.L. Zhang, P.P. Zhang, H. Zhang, J.Y. Qin, Q.L. Wang, *Int. J. Electrochem. Sci.*, 12 (2017) 841.
29. Y.L. Ji, G.F. Wang, C.H. Zhang, B. Fang, *Chin J Chem.*, 29 (2011) 1017.
30. S.S Jing, H.J. Zheng, L. Zhao, L.B. Qu, L.L. Yu, *Talanta*, 174 (2017) 477.
31. H.C. Zhang, Y.Y. Shang, T. Zhang, K.L. Zhuo, J.N. Wang, *Sens. Actuators B.*, 242 (2017) 492.
32. R. Yang, D. Miao, Y. Liang, L. Qu, J. Li, P.B. Harrington, *Electrochim. Acta*, 173 (2015) 839.
33. Y. Li, Y. Li, J. Gao, L. Wang, L. Zou, B. Ye, *Electroanalysis*, 27 (2015) 1719.
34. V. Toutam, P. Jain, R. Sharma, S. Bathula, A. Dhar, *Appl. Surf. Sci.*, 349 (2015) 196.
35. B.N. Chandrashekar, B.E. Kumara Swamy, M. Pandurangachar, T.V. Sathisha, B.S. Sherigara, *Colloids Surf., B*, 88 (2011) 413.
36. B. Unnikrishnan, V. Mani, S.M. Chen, *Sens. Actuators B.*, 173 (2012) 274.
37. GB/T22251-2008, Determination of puerarin in health foods.