*Int. J. Electrochem. Sci.*, 12 **(2017)** 5411 – 5420, doi: 10.20964/2017.06.17

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

# Electrochemical Determination of Catalpol in Rehmannia Glutinosa Based on Polyaniline-Graphene Modified Glassy Carbon Electrode

*Xuemei Wang*[1,2#], *Dike Zhou*[3#] *and Yanchun Wang*[1,2*]

[1] People's Hospital of Zhengzhou University, Zhengzhou, 450003, Henan, P. R. China
[2] Department of Traditional Chinese Medicine,Henan Provincial People's Hospital, Zhengzhou, 450003, Henan, P. R. China
[3] Department of Endocrinology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, Henan, P. R. China
[#]These authors contributed equally to this work
[*]E-mail: wangxuemei211@sohu.com

*Received:* 19 September 2016 / *Accepted:* 28 March 2017 / *Published:* 12 May 2016

In this work, we prepared an electrochemical sensor to quantify the catalpol, where the glassy carbon electrode (GCE) was modified by the polyaniline graphene. Compared with the bare GCE, we found that the GCE modified with polyaniline graphene exhibited a well-defined oxidation of the catalpol. Moreover, a significant enhancement in the current response was observed during the process. Especially, the current response of the oxidation peak of the catalpol exhibited a linear relationship with the concentration of the catalpol in the range of 0.005 to 50 μM, where the limit of the detection was 0.002 μM. The electrochemical sensor was succeeded to be applied in the determination of the catalpol in the root of *Rehmannia glutinosa* the due to the improved voltametric performance.

**Keywords:** *Rehmannia glutinosa*; Catalpol; Polyaniline; Sensing; Electrode

## 1. INTRODUCTION

Rehmannia glutinosa, belonging to the family of Scrophulariaceae, has been widely used to enrich Yin and tonify the kidney in the traditional Chinese medicine (TCM) as the traditional Chinese herb, which has exhibited a remarkable medicinal value. During the past decades, a mass of studies on Rehmannia glutinosa in chemistry and pharmaceutics have been reported. More than 70 compounds such catalpol, iridoids, amino acid and saccharides have been observed in the herb as well as some other microelements. It has been demonstrated that Rehmannia glutinosa and its effective constituents

exhibited a remarkable pharmaceutical effect on the immune system, cardiovascular system, blood system, endocrine system and nervous system. Especially, catalpol ((2S,3R,4S,5S,6R)-2-[[(1aS,1bS,2S,5aR,6S,6aS)-6-hydroxy-1a-(hydroxymethyl)-2,5a,6,6a-tetrahydro-1bH-oxireno[5,6]cyclopenta[1,3-c]pyran-2-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol) has exhibited a remarkable therapeutic and preventive performance to neurodegenerative disorders.

Moreover, catalpol could prevent the primary cultured cortical neurons from apoptosis caused by Aβ1-42 [1] and protect the astrocytes against the oxidative stress induced by hydrogen peroxide ($H_2O_2$) in vitro [2]. Besides, catalpol could also hinder dopaminergic neurons from inflammatory degeneration induced by LPS and prevent the inflammation of the cortical neuron-glia cultures caused by β-amyloid [3, 4]. On the other side, the cognitive damage of the senescent mice induced by d-galactose has been decreased as well as the transient global cerebral ischemia in gerbils in vivo [5, 6]. Nevertheless, so far, only one paper has reported the pharmacokinetics of the catalpol by oral administration with rats [7], where the performance of the catalpol to penetrate the blood-brain barrier (BBB) is still undefined, which is prerequisite to treat neurodegenerative diseases.

For the determination of the catalpol in the insects and the plants [8-10], gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC) [11] and micellar electrokinetic capillary chromatography-mass spectrometry (MECC-MS) have been employed. Nevertheless, low sensitivity (35 μg/mL) and long running time (more than 20 min) have commonly been observed in these techniques. Besides, the complicated procedures to prepare samples are required, whereas this could not work in the pharmacokinetic study [12]. Hence, a new method is needed to determine the catalpol in the herb and drug.

Recently, developing the electrochemical sensors to detect molecules has attracted massive interests as the electrochemical sensors exhibit numberous advantages including short analysis time, high sensitivity and selectivity, relatively inexpensive instrumental requirements and simple experimental procedures which are suitable for various physiological samples [13-16]. The liquiritin could be measured with the common electrodes, but the over-potential electrode contaminants may be induced by the products. Besides, some biological molecules, which undergo reduction or oxidation under a similar potential window as the catalpol, could result in the interference. Thus, the modification of the electrode surface is required to enhance the analytical capacity to detect the catalpol electrochemically.

Polyaniline, which is one of the most remarkable conducting polymers, has promising application in the field of biosensors and electrochemical sensors [17, 18]. PANI, which has exhibited predominant signal amplification and anti-fouling features, could be employed as a proper matrix to immobilize the biomolecules and mediator in the enzymatic and redox reactions [19, 20], due to its excellent biocompatibility and intrinsic electroactivity. In recent researches, it is demonstrated that the electrocatalytic activity and conductivity of the electrode materials generated by integrating carbon nanotubes (CNTs) with PANI have been improved remarkably[21-24]. However, compared with CNTs, various advantages have been observed in graphene, such as high conductivity, easy production and functionalization, massive cheap source materials and excellent biocompatibility [25]. Thus, exploring graphene-polyaniline (GR-PANI) hybrid is significantly necessary for the application in biosensors and electrochemical sensors. Nowadays, several groups have reported the use of the in situ

polymerization approaches to prepare the GR-PANI nanocomposite [26-28]. Moreover, the mechanical strength and conductivity could be significantly enhanced by doping polyaniline into graphene, which has exhibited a predominant capacity for supercapacitors as the electrode material.

Herein, the in situ polymerization method has been employed to prepare the GR-PANI nanocomposite. Then the as-prepared GR-PANI nanocomposite is used to modify the glassy carbon electrode which is fabricated with the electrochemical sensor. The electrochemical performance and the voltammetric measurement of the catalpol on the electrode modified with GR-PANI are studied in detail. However, to our best knowledge, it is the first time to determine the catalpol with the electrochemical method. The results indicate that the GR-PANI composite film is efficient for the catalpol as the electrochemical sense. Besides, the obtained GR-PANI could also be employed to determine the catalpol in the practical Rehmannia glutinosa sample.

## 2. EXPERIMENTS

### *2.1. Chemicals*

Graphite powder (99.95%, 325 mesh), aniline, ammonia solution (28 wt%), ammonium peroxydisulfate (APS) and hydrazine solution (50 wt%) were commercially available (Shanghai Chemical Reagent Co., Ltd., Shanghai, China). Acetic acid, boric acid and phosphoric acid were mixed in the definite weights to prepare the BR buffer solution, where sodium hydroxide (0.2 M) was utilized to adjust and obtain the desired pH. All the reagents in this paper were analytically pure and used as obtained without any process. Besides, the deionized water, which was produced by a Milli-Q water purification system, was used to prepare all the solutions. Noted that the resistivity of the deionized water was below 18 MΩ

### *2.2. Apparatus*

For the electrochemical researches, CHI660A electrochemical workstation (CH Instruments, USA) was employed. Besides, a standard three-electrode cell was utilized in this system, where the modified electrode was used as the working electrode, a saturated calomel was employed as the reference electrode and the platinum wire acted as the auxiliary electrode. Noted that all the potential values described below were related with SCE.

### *2.3. Preparation of GR–PANI nanocomposite*

The graphite powder was used as the starting material to synthesize graphene oxide through the improved Hummers approach [29, 30]. In general, a mixture consisting of 5 g $P_2O_5$, 5 g $K_2S_2O_8$ and 15 mL concentrated $H_2SO_4$ was utilized to pre-oxidize graphite. Then, the mixture was diluted by the deionized water, filtered and naturally dried. Subsequently, the mixture of $KMnO_4$ and concentrated $H_2SO_4$ was employed to re-oxidize the pre-oxidized graphite via Hummers approach. Here, the GR-

PANI nanocomposite was produced according to the literature approach [26]. GR–PANI nanocomposite was prepared by the literature method. In a typical procedure, graphene oxide was under ultrasonic for 1 h to be dispersed in HCl with a concentration of 1 M in the presence of 0.3 M aniline. Subsequently, another solution of APS with a concentration of 0.75 M in 1 M HCl was added into the mixture quickly, where the mixture was stirred vigorously. It was obvious that the colour of the mixture became green after 5 min, which suggested that the aniline was polymerized. Then, the resulting mixture was stirred overnight at room temperature. After that, the mixture was diluted with 100 mL water and filtered. Then, hydrazine was used to reduce the obtained GR-PANI oxide composite at 95 $^{o}$C in water. At last, the reduced composite was dispersed in the mixture of 1 M HCl and APS and stirred overnight at room temperature. After filtration, the resultant GR-PANI nanocomposite was collected and dried in vacuum.

### *2.4. Preparation of modified electrode*

The resultant GR-PANI described above was dispersed in DMF under ultrasonic for 1 h to generate homogenous suspension with a concentration of 1 mg/mL. Subsequently, 5 μL of the obtained suspension was taken out and deposited on the surface of the GCE which was freshly polished. Then it was dried at room temperature to obtain the GCE modified with GR-PANI (GR-PANI/GCE). Besides, the homogenous suspensions (5 μL) of graphene and PANI in DMF (1 mg/mL) were deposited on the bare GCE to prepare the GCE modified with GR and PANI (GR/GCE and PANI/GCE), respectively.

### *2.5. Real sample analysis*

First, the purchased liquorice Rehmannia glutinosa was grinded into powder. Then, around 1g powder was dispersed in 70% ethanol solution (50 mL) and refluxed for 1 h at 80 $^{o}$C. After that, the mixture was cooled and filtered by a paper filter. Then the obtained solution was evaporated to 50 mL under vacuum. At last, 2.0 mL of the above solution was diluted to 50 mL with the BR buffer.

## 3. RESULTS AND DISCUSSION

The in situ polymerization was used to prepare the GR-PANI nanocomposite. For this method, the exfoliated graphene oxide sheets were first mixed with the aniline monomer to generate a homogenous suspension. The APS was added to initiate the in situ polymerization to prepare the homogenous polyaniline-graphene oxide. Subsequently, the obtained composite was reduced by hydrazine and then re-oxidized and re-protonated to prepare the GR-PANI nanocomposite. The mass ratio of graphene oxide to aniline monomer in the original materials was kept at 4:1 to make graphene as the prime constituent. Scanning electron microscopy (SEM) was used to characterize the morphology of the obtained GR-PANI composite and graphene. In Figure 1, it was obvious that the layered and wrinkled form was obtained in GR-PANI, where the PANI nanofibers distributed among

and on the surface of the graphene sheets. Figure 2 showed the Nyquist plots recorded at the bare GCE, GR/GCE and GR-PANI/GCE. It was obvious that GR-PANI exhibited slightly higher interfacial charge-transfer resistance than GR. Nevertheless, they were both relatively low because of the excellent conductivity of GR. Besides, the Warburg region in the Nyquist plot of GR-PANI was negligible, which indicated that the length of the diffusion path of the ions in the electrolyte was short and equal. This might be ascribed to that the morphology of the GR-PANI composite was homogenous so that the ions in the electrolyte could only access to the surface of the nanocomposite.

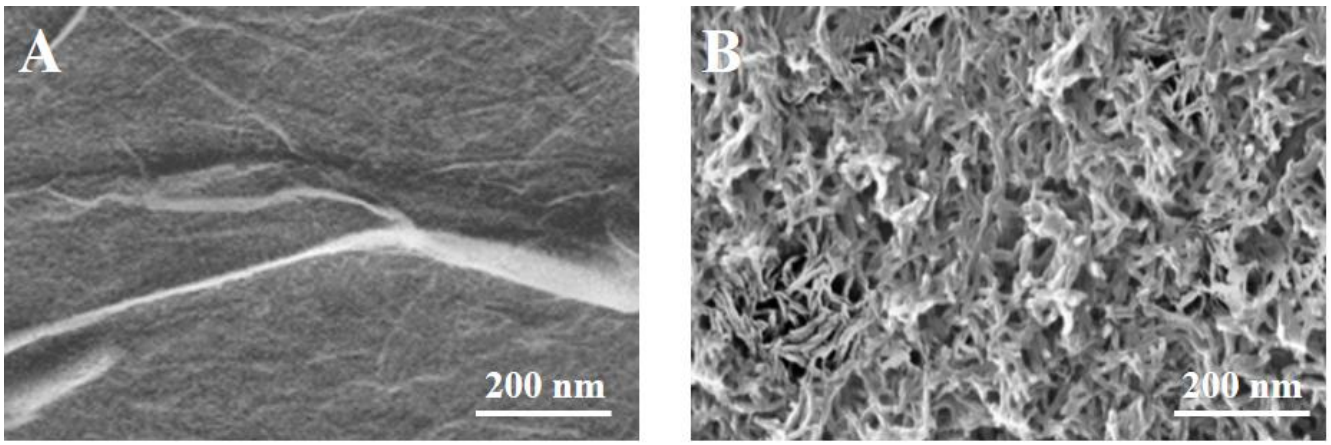


**Figure 1.** SEM images of GR (A) and GR–PANI nanocomposite (B).

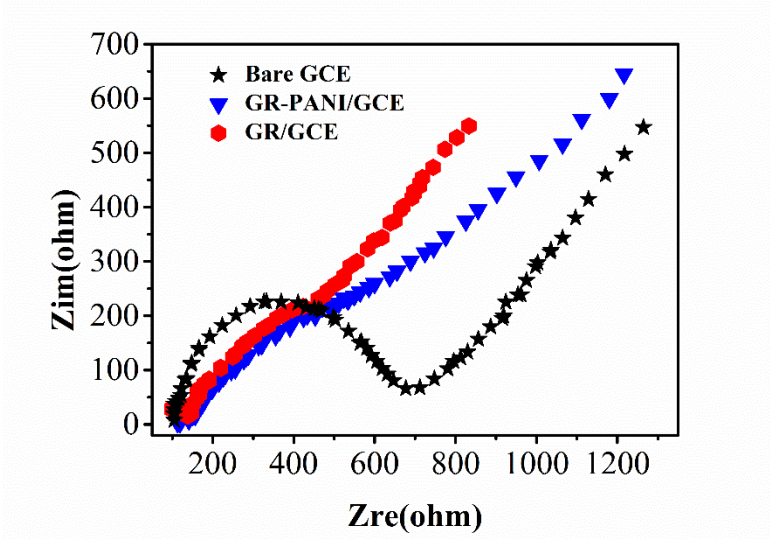


**Figure 2.** The Nyquist plots of bare GCE (A), GR/GCE (B) and GR–PANI/GCE (C) in $Fe(CN)_6^{3-/4-}$ with a concentration of 5 mM in the presence of 0.1 M KCl. The frequency range is 0.01 Hz to 100 kHz.

Furthermore, cyclic voltammetry was employed to evaluate the electrochemical performance of the GR/GCE and GR-PANI/GCE. Figure 3A, 3B and 3C illustrated the cyclic voltammograms of catalpol with a concentration of 0.01 mM dissolved in 0.1 M BR buffer solution with a pH of 7.0 at the

bare GCE, GR/GCE and GR-PANI/GCE, respectively. For all the electrodes described above in BR buffer solution with a concentration of 0.1 M when pH was 7.0, no responses were observed without adding catalpol. However, it was obvious in the cyclic voltammograms that the background current at GR/GCE and GR-PANI/GCE was larger than that of GCE, which indicated that both GR and GR-PANI had been coated on the bare GCE efficiently. Besides, the electroactive surface area of these two materials were more effective compared with the bare GCE. After adding catalpol, the oxidation peak observed at the bare GCE and GR/GCE in 0.1 M BR buffer solution with a pH of 7.0 was around 0.70 V and 0.61 V, respectively. However, for the GR-PANI/GCE in 0.1 M BR buffer with a pH of 7.0, a pair of definite redox peaks was obtained after adding catalpol, where the potentials of the reduction and oxidation peak were 0.34 and 0.54 V, respectively. Especially, the oxidation peak current observed at the GR-PANI/GCE was 80 times higher than that of the bare GCE, which indicated that the electroactive surface area and the electrocatalytic capacity of the GR-PANI to catalpol were relatively higher. However, compared with the bare GCE and GR/GCE, the oxidation peak potential of catalpol at the GR-PANI/GCE was relatively lower, which indicated that the GR-PANI/GCE exhibited an efficiently electrocatalysis performance for the redox reaction of catalpol. The observed enhancement in the anodic peak current as well as lowering of the overpotential can be attributed not only to the enlargement of microscopic surface area of the electrode, but also to the contribution of GR as electron conductor that promoted the conductivity of the modifier film [31]. In order to testify these assumptions, the microscopic surface areas of GR-PANI/GCE was calculated by CV method using $K_3Fe(CN)_6$ as a redox probe at different potential scan rates. Randles-Sevick equation is used to obtain the microscopic electrode surface area [32]. The electrode surface area of the bare and modified GCE was 0.23 and 0.67 $cm^2$, respectively. This indicates that the microscopic area of the 3 GR-PANI/GCE increased significantly and is almost 3 times larger than the microscopic area of the bare GCE.

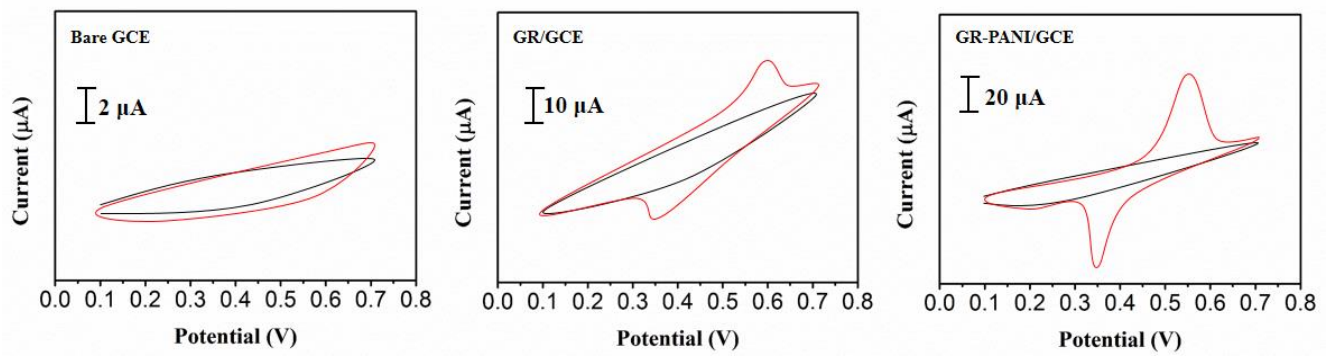


**Figure 3.** Cyclic voltammograms at the bare GCE, GR/GCE and GR-PANI/GCE in BR buffer solution with a concentration of 0.01 mM (pH 7.0) in the absence and presence of 0.01 mM catalpol. Scan rate: 50 mV/s.

In theory, the pH of the solution should influence the redox system as catalpol may involve in the protons during the whole of the electrode reaction. Thus, the effect of the solution pH ranging from 3 to 11 on the redox reaction of catalpol at the GR-PANI/GCE was investigated through measuring the cyclic voltammetry of catalpol with a concentration of 0.01 mM in BR buffer solution with a

concentration of 0.1 M with various pH at the GR-PANI/GCE. The negative shift was observed in the potentials of both the oxidation and reduction peak of the GR-PANI/GCE when the solution pH increased. In Figure 4A, a linear variation with a slope of −59.6 mV/pH, which was approximate to the theoretical value of −58 mV/pH, was observed in the oxidation peak potential ($E_{pa}$) when varying pH from 3 to 11. Besides, this result was in accordance with the Nernst equation of the two-proton and two-electron reaction. In Figure 4B, the anodic peak currents of the liquiritin was enhanced when the pH values of the solution were increased from 3 to 11. However, a decreased response was obtained with the sensor when the pH value of the solution was increased to beyond 8. Thus, the pH employed in this work was determined to be 8. On the other hand, it was observed that buffer solution composition had no significant influence on stripping current [33].

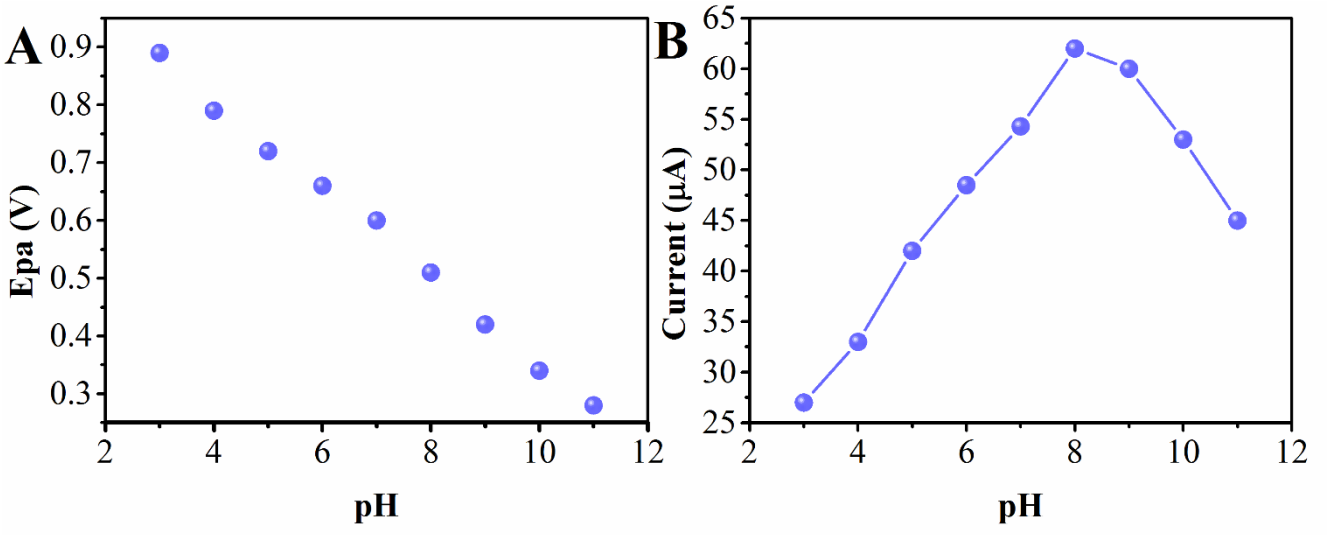


**Figure 4.** The relationship between $E_{pa}$ and the pH value of the solution (A); The relationship between the anodic peak current response and the pH value of the solution collected in cyclic voltammograms at the GR–PANI/GCE in 0.1 M BR buffer solution with 0.01 mM catalpol (B).

In addition, to improve the sensitivity and reduce the limitation of the catalpol detection, the square-wave voltammetry of catalpol was carried out, where catalpol was dispersed in BR buffer solution with a concentration of 0.1 M, of which the pH is 8.0. Figure 5 illustrated the responses of the square-wave voltammetry at GR-PANI/GCE during the successive addition of catapol. In Figure 5, a positive relationship was observed between the anodic peak current and the concentration of catapol in the range of 0.005 to 50 μM. The equation of the linear regression for the GR-PANI/GCE was $I_{pa}$ (A) = $23.4C_{catalpol}$ (mM) + $1.47 \times 10^{-3}$ (mA) ($r$ = 0.998), where the limit of detection was calculated to be 2 nM. Thus, it indicated that the GR-PANI/GCE was suitable for the potential detection of catalpol in the practical samples as the proposed electrochemical sensor exhibited a high sensitivity. Electrode modification is a modern and versatile concept, attracting much attention in the field of electrochemistry. Modified electrodes can exhibit high selectivity and sensitivity toward catapol determination. A number of studies are reported in the literature. Some of them are summarized in the Table 1.

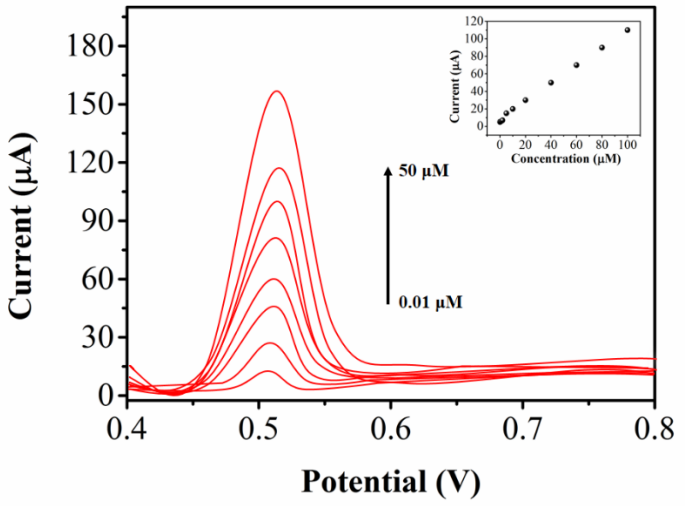


**Figure 5.** Square-wave voltammetric responses to the successive additions of catalpol with a concentration ranging from 0.005 to 50 μM in 0.1 M BR buffer solution (pH 7.0) at the GR–PANI/GCE. The inset is the corresponding calibration curve.

Four extracting samples of Rehmannia glutinosa were investigated to evaluate the applicability of the proposed GR-PANI/GCE for the determination of catalpol. Besides, the Spike and recovery process was employed to confirm the validation of the proposed approach. Table 2 illustrated the results of the proposed electrochemical sensor. It was obvious that the proposed GR-PANI/GCE exhibited a remarkable capacity to the detection of catalpol in the extracting samples of Rehmannia glutinosa root, which indicated that the proposed approach was effective in the detection of catalpol in the practical herb samples.

**Table 1.** Comparison of analytic performance for catalpol determination..

| Method | Detection range | Limit of detection | Reference |
|---|---|---|---|
| HPLC–APCI–MS/MS | 10–50 000 ng/mL | — | [34] |
| HPLC | 0.5 to 7 μM | — | [35] |
| Sidiming Capsules by HPLC | 0.03 to 5 50 μM | 0.01 μM | [36] |
| GR–PANI/GCE | 0.005 to 50 μM | 2 nM | This work |

**Table 2.** The determination of the catalpol content in the extracting samples of Rehmannia glutinosa root with GR–PANI/GCE.

| Sample | Addition (μM) | Found (μM) | Recovery (%) |
|---|---|---|---|
| 1 | 0 | 12.31 | — |
| | 5 | 17.56 | 101.44 |
| 2 | 0 | 13.65 | — |
| | 20 | 34.51 | 99.60 |
| 3 | 0 | 12.54 | — |
| | 30 | 41.12 | 99.01 |

## 4. CONCLUSION

In conclusion, GR-PANI was used to modify the surface of the electrode. A selective, sensitive and reliable method to determine the catalpol in the extracts of the Rehmannia glutinosa root was firstly elaborated by the obtained GR-PANI/GCE. The results indicated that the GR-PANI/GCE exhibited a remarkable capacity to the electrochemical oxidation and reduction of the catalpol. Moreover, a linear relationship was observed between the response and the concentration in the range of 0.002 to 50 μM.

## References

1. H.L. Jian, D. Jing, D.X. Lian, J. Tao, H. Shuang, B. Jing and J. Bo, *Neurochemistry International*, 55 (2009) 741
2. J. Bi, B. Jiang, J.H. Liu, C. Lei, X.L. Zhang and L.-J. An, *Neuroscience Letters*, 442 (2008) 224
3. Y.Y. Tian, L.J. An, L. Jiang, Y.L. Duan, J. Chen and B. Jiang, *Life Sciences*, 80 (2006) 193
4. B. Jiang, J. Du, Jh, Y. Bao and L. An, *Brain Research*, 1188 (2008) 139
5. D.Q. Li, Y.M. Bao, J.J. Zhao, C.P. Liu, Y. Liu and L.J. An, *Brain Research*, 1029 (2004) 179
6. L.Z. Xiu, J. Bo, B.L. Zhi, H. Shuang and J.A. Li, *Pharmacology Biochemistry & Behavior*, 88 (2007) 64
7. R. Lu, Y. Gu, D. Si and C. Liu, *Journal of Chromatography B Analytical Technologies in the Biomedical & Life Sciences*, 877 (2009) 3589
8. E.C. Lampert and M.D. Bowers, *Journal of Chemical Ecology*, 37 (2011) 496
9. J. Suomi, H. Sirén, M. Jussila, S.K. Wiedmer and M.L. Riekkola, *Analytical & Bioanalytical Chemistry*, 376 (2003) 884
10. J. Suomi, H. Sirén, K. Hartonen and M.L. Riekkola, *Journal of Chromatography A*, 868 (2000) 73
11. H.K. Wu, W.C. Chuang and S.J. Sheu, *Journal of Chromatography A*, 803 (1998) 179
12. J. Suomi, H. Sirén, S.K. Wiedmer and M.L. Riekkola, *Anal. Chim. Acta.*, 429 (2001) 91
13. S.A. Wring and J.P. Hart, *The Analyst*, 117 (1992) 1215
14. L. Zhang, S. Wu, Y. Tai, C. Lv and X. Zhang, *Fullerenes, Nanotubes and Carbon Nanostructures*, 24 (2016) 116
15. A.E. Awadallah, A.A. Aboul-Enein, N.A. Aboul-Gheit and O.M. El-Ahwany, *Fullerenes, Nanotubes and Carbon Nanostructures*, 23 (2015) 591
16. E.D. Rivera-Tapia, C.A. Fajardo, Á.J. Ávila-Vega, C.F. Ávila, F.M. Sánchez-Arévalo, I. Chango-Villacís, F.J. Quiroz-Chávez, J. Santoyo-Salazar and R.C. Dante, *Fullerenes, Nanotubes and Carbon Nanostructures*, 24 (2016) 8
17. C. Dhand, M. Das, M. Datta and B.D. Malhotra, *Biosensors & bioelectronics*, 26 (2011) 2811
18. D.W. Hatchett and M. Josowicz, *Chemical Reviews*, 39 (2008) 746
19. Z. Wang, S. Liu, P. Wu and C. Cai, *Anal. Chem.*, 81 (2009) 1638
20. H. Chang, Y. Yuan, N. Shi and Y. Guan, *Anal. Chem.*, 79 (2007) 5111
21. E. Granot, B. Basnar, Z. Cheglakov, E. Katz and I. Willner, *Electroanalysis*, 18 (2006) 26
22. Y. Tao, Z. Na, Y. Zhang, Z. Wei, K. Jiao and G. Li, *Biosensors & bioelectronics*, 24 (2009) 2165
23. Y. Li, Y. Umasankar and S.M. Chen, *Talanta*, 79 (2009) 486
24. M. Li and L. Jing, *Electrochimica Acta*, 52 (2007) 3250
25. M. Pumera, A. Ambrosi, A. Bonanni, E.L.K. Chng and H.L. Poh, *Trac Trends in Analytical Chemistry*, 29 (2010) 954
26. K. Zhang, L.L. Zhang, X.S. Zhao and J. Wu, *Chemistry of Materials*, 22 (2010) 1392
27. W. DW, L. F, Z. J, R. W, C. ZG, T. J, W. ZS, G. I, L. GQ and C. HM, *Acs Nano*, 3 (2009) 1745

28. X. Yan, J. Chen, J. Yang, Q. Xue and P. Miele, *ACS applied materials & interfaces*, 2 (2010) 2521
29. W.S. Hummers, R.E. Offeman, W.S. Hummers and R.E. Offeman, *Journal of the American Chemical Society*, 80 (1958)
30. N.I. Kovtyukhova, P.J. Ollivier, B.R. Martin, T.E. Mallouk, S.A. Chizhik, E.V. Buzaneva and A.D. Gorchinskiy, *Chemistry of Materials*, 11 (1999) 771
31. E. Asadian, S. Shahrokhian, A. Iraji Zad and F. Ghorbani-Bidkorbeh, *Sensors and Actuators B: Chemical*, 239 (2017) 617
32. K. Ngamchuea, S. Eloul, K. Tschulik and R.G. Compton, *Journal of Solid State Electrochemistry*, 18 (2014) 3251
33. S. Akbar, A. Anwar and Q. Kanwal, *Analytical Biochemistry*, 510 (2016) 98
34. Q. Wang, M. Xing, W. Chen, J. Zhang, H. Qi and X. Xu, *Journal of pharmaceutical and biomedical analysis*, 70 (2012) 337
35. Y. Luo, S. Zhang, J. Suo, D. Sun and X. Cui, *Chinese Pharmaceutical Journal*, 29 (1994) 38
36. J.-c. HUANG, Z. YANG, Y. LI and R.-b. HUANG, *Lishizhen Medicine and Materia Medica Research*, 3 (2010) 029