Int. J. Electrochem. Sci., 9 **(2014)** 238 - 248

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

The Electrochemical Behavior and Determination of Quercetin in Choline Chloride/Urea Deep Eutectic Solvent Electrolyte Based on Abrasively Immobilized Multi-Wall Carbon Nanotubes Modified Electrode

Yan Zheng[1,2,*], *Liqing Ye*[1,2], *Lili Yan*[1,2], *Yuntao Gao*[1,2,*]

[1]The Engineering Laboratory of Polylactic Acid-Based Functional Materials of Yunnan, Yunnan University of Nationalities, Kunming, 650500, China
[2]Key Laboratory of Ethnic Medicine Resource Chemistry, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities, Kunming, 650500, China
[*]E-mail: 29817206@qq.com; 2314972096@qq.com

Received: 11 September 2013 / *Accepted:* 27 October 2013 / *Published:* 15 November 2013

A new electrochemical analysis method, using choline chloride/urea deep eutectic solvents electrolyte and MWNTs modified electrode system has been developed. The electrochemical behavior of quercetin was investigated in the mixed choline chloride/urea deep eutectic solvent electrolyte and acetate buffer solution supporting electrolyte. A pair of redox peak of quercetin was obtained by using cyclic voltammetry in the mixed supporting electrolyte with 0.407 V of E_{pa} and 0.386 V of E_{pc}. A differential pulse voltammeter method (DPV) for the determination of trace quercetin was proposed. A good linear relationship was obtained between the oxidation peak current and the concentration of quercetin within the range from 9.95×10^{-7} mol·L^{-1} to 4.76×10^{-5} mol·L^{-1}, was obtained with the linear regression equation of $i_{pa}=526.49-5\times10^{8}$ c (r=0.9991), and the detection limit reach of to 3.6×10^{-8} mol·L^{-1}. The proposed method was used to determine quercetin in practical samples with the RSD of 1.1 to 1.6% and the percentage recovery of 9.3 to 102.2%, and the result was agreed well that by HPLC.

Keywords: Quercetin, Choline chloride/urea deep eutectic solvent, Electrolyte, Multi-wall carbon nanotubes, Electrode

1. INTRODUCTION

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one; Scheme1, R=OH) belongs to an extensive class of polyphenolic flavonoid compounds almost ubiquitous in plants and

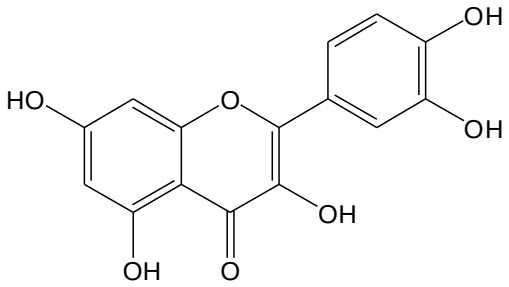
plant food sources[1]. As a major bioflavonoid in the human diet, it can inhibit the oxidation process of cell membrane lipid and protect cells from oxidation damage[2], inhibits inflammation[3] and human cancer growth[4]

Hitherto, different methods have been reported for the determination of quercetin, including spectrophotometry[5], high performance liquid chromatography[6-7] luminol electrogenerated chemiluminescence (ECL)[8] and capillary electrophoresis[9], et al. Electrochemical analysis methods were introduced to determine quercetin with the advantages of simplicity, cheapness, fast analysis along with high sensitivity[10-12]. Conventional electrochemical methods with simple modification of the electrode surface can enable the determination of quercetin[13-14]. Recently, carbon nanotubes (CNTs) and graphene modified electrodes have been found to be excellent electrodes for the determination of quercetin at trace levels due to their strong surface adsorption and sensitization effect[15–18].

Over the past 10 years, room temperature ionic liquid (RTIL) modified electrode has been widely used in the electrochemical analysis owing to its unique electrochemical properties, including higher ionic conductivity, wider electrochemical windows, low cost and environment-friendly[19-20]. RTIL/CNTs composite modified electrode has gained increasing attention[21-22] recently due to their potential applications to develop high performance electrochemical sensors. RTIL/CNTs composite electrodes have several advantages over traditional RTIL modified electrodes, including improved sensitivity, compatibility and stability. For example, an Ag nanoparticles/1-butyl- 3-methylimidazolium bis (trifluoromethylsulfonyl) imide based β-cyclodextrin/ epichlorohydrin (β-CDEpi) biosensor was developed for quercetin determination in real samples with a low detection limit (0.037±0.004 μM)[23]. RTIL/CNTs composite electrode has a good prospect in electrochemical analysis, however, its preparation involving a complex procedure to the synthesis of ionic liquids and electrode modification, is cost, tedious and time consuming, which restricts its application in practical wok.

Recently, ionic liquids analogous, known as deep eutectic solvents (DESs), have been recognized as a low-cost alternative to traditional ionic liquids[24-26]. Term “deep eutectic solvent” is used to a type of ionic solvent comprising a mixture which forms a eutectic with a melting point significantly lower than that of its individual components. EDSs are much cheaper and easier to make, much less toxic and sometimes more biodegradable than those of traditional RTIL. Choline chloride based DESs have been developed as electrolyte for the electrodeposition of grey selenium[27], Cd and Cd-Te films[28]. But to the best of our knowledge, EDSs/CNTs based analysis system has not been reported.

In this paper, we applied a simple and fast way to develop a high sensitive voltammetric analysis method of quercetin by using choline chloride/urea EDSs electrolyte and abrasively immobilized MWCNTs electrode[29-30]. The proposed method is very easier, faster and cheaper than that by RTIL/CNTs composite modified electrodes. Furthermore, EDSs supporting electrolyte can improve the sensitivity of MWCNTs modified electrode effectively.



Scheme 1. Structure formula of quercetin

2. EXPERIMENTAL

2.1. Apparatus and Materials

Electrochemical measurement was carried out by ZAHNER Zennium IM6 Electrochemical Workstation (ZAHNER-Elektrik GmbH&Co. KG, Kronach, Germany). A three-electrode system including a abrasively immobilized MWCNT graphite working electrode (diameter: 5 mm), a saturated calomel regerence electrode (SCE) and a platinum auxiliary electrode was employed.

Multi-walled carbon nanotubes (MWNTs, 95%) was purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu, China. Ouercetin (98.9%) was purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. Stock quercetin solution of 1.0×10^{-3} mol·L^{-1} was prepared in ethanol. Quercetin capsule were obtained from Source Naturals Company. 0.1 mol·L^{-1} sodium acetate-acetic acid buffer solution (pH 4.0).

Choline chloride [$Me_3NC_2H_4OH$]Cl, (ChCl) (Sigma-Aldrich, 99.5%) was recrystallized in anhydrous ethanol and dried for 24 h in vacuum at 80 °C. Urea (Sinopharm Chemical Reagent Co., Ltd) was pre-dried under vacuum prior to use.

All the chemicals used were of analytical-reagent grade. Twice-distilled water was used throughout the experiments.

2.2. Experimental methods

2.2.1. Preparation of choline chloride/urea deep eutectic solvent

Choline chloride/urea deep eutectic solvent was prepared by stirring the mixture of choline chloride and urea (mole ratio 2:1) at 90 °C until a homogeneous colorless liquid was formed[9], stored in vacuum dryer.

2.2.2. Preparation of the abrasively immobilized MWCNTs electrode

A graphite electrode (5 mm in diameter) was polished to a mirror-like surface with metallographic sand paper and 0.05 μm Al_2O_3 suspension, respectively. After rinsed thoroughly with

doubly distilled water between each polishing step, the electrode was subjected successively with 50% nitric acid, ethanol and doubly distilled water in ultrasonic bath, and dried in air. The abrasively immobilized MWCNTs electrode was prepared by gently rubbing the electrode surface on a filter paper supporting two milligrams of MWCNTs for two minutes[29-30]. The prepared electrode was cleaned with distilled water before use.

2.2.3. The voltammetric analysis of quercetin

Cyclic voltammetry (CV) and differential pulse voltammeter (DPV) were performed in the three-electrode cell in 5% (v/v) choline chloride/urea DES, 0.175 ml 1.0×10^{-3} mol·L^{-1} quercetin stock solution and 0.1 mol·L^{-1} pH 4.0 acetate buffer solution between the potential range of 0.0 V and 0.8 V at a scan rate of 0.05 V·s^{-1}. The DPV conditions were pulse width 250 ms, pulse amplitude 60 mV and pulse interval 250 ms.

3. RESULTS AND DISCUSSION

3.1. Influence of supporting electrolyte and pH

The CV determination for 1.64×10^{-5} mol·L^{-1} quercetin was performed in a mixed choline chloride/urea choline chloride/urea deep eutectic solvent buffer solution supporting electrolyte.

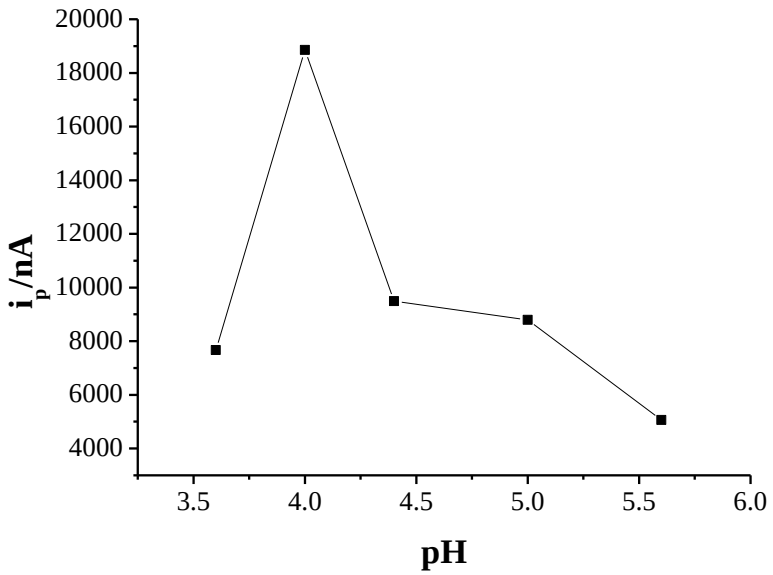


Figure 1. The effect of buffer system pH on peak

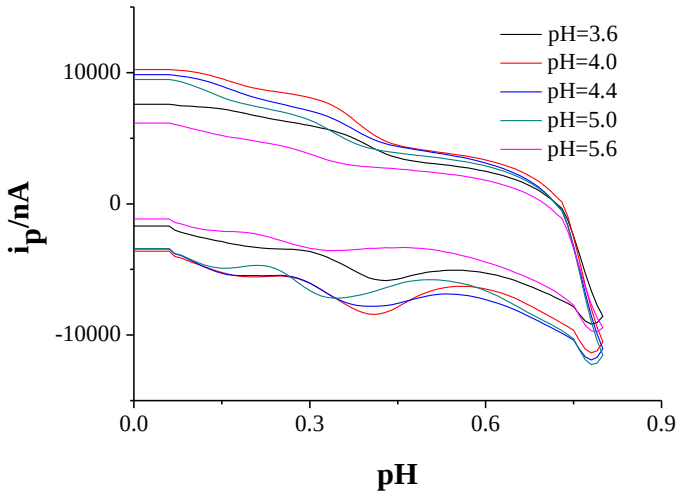


Figure 2. The curves of quercetin at different buffer system pH at MWCNT GE

Different buffer solutions such as 0.04 mol·L^{-1} B-R, 0.10 mol·L^{-1} acetate or 0.10 mol·L^{-1} citric acid-disodium hydrogen phosphate were tested in 5.0% (v/v) of choline chloride/urea DES. A pair of redox peak was observed in three mixed supporting electrolyte. Well-defined CV response with high redox peak of quercetin was obtained in mixed choline chloride/urea DES acetate buffer solution supporting electrolyte. The influence of pH was then investigated in 0.10 mol·L^{-1} acetate buffer and 5.0% (v/v) of choline chloride/urea DES. As Figure 1 and Figure 2 show, the best current response of quercetin was obtained at pH 4.0.

The influence of choline chloride/urea DES concentration is shown in Figure 3, the current response of quercetin increases first with the increase of the concentration of choline chloride/urea DES until it reaches to 5.0% (v/v), and then decreases as the concentration of choline chloride/urea DES above 5.0% (v/v). In present work, 0.1 mol·L^{-1} acetate buffer (pH 4.0) and 5.0% (v/v) of choline chloride/urea DES concentration were therefore chosen for subsequent experiments.

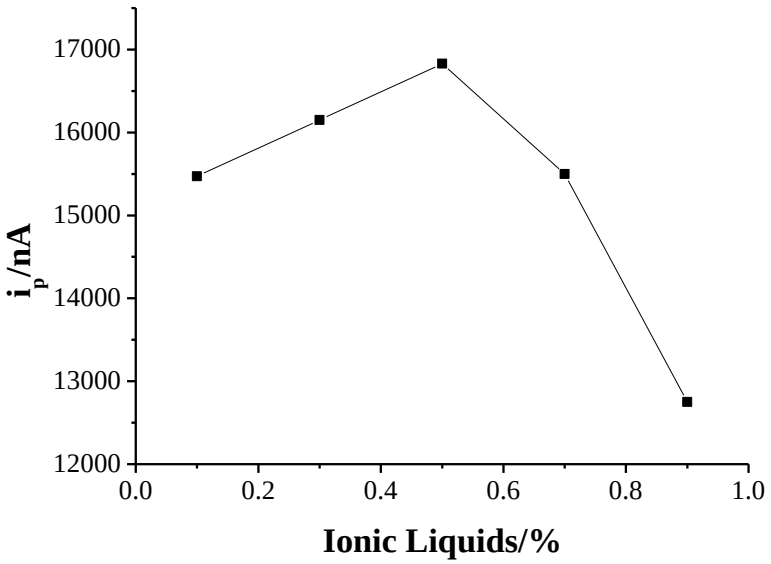


Figure 3. The effect of choline chloride DES concentration on peak current

3.2. The electrochemical behavior of quercetin in mixed choline chloride/urea DES acetate buffer solution supporting electrolyte

Figure 4 displays the CV curves of 1.64×10^{-5} mol·L^{-1} quercetin in 0.1 mol·L^{-1} acetate buffer solution (pH 4.0) supporting electrolyte (a) and mixed 5.0% choline chloride/urea DES + 0.1 mol·L^{-1} acetate buffer solution (pH 4.0) supporting electrolyte (b) at MWCNT GE. A pair of quasi-reversible redox peak was observed in two supporting electrolyte, the oxidation peak potential (E_{pa}) and reduction peak potential (E_{pc}) of quercetin for mixed choline chloride/urea DES acetate buffer solution supporting electrolyte were 0.407 V and 0.386 V (vs. SCE), respectively, $\triangle E = 0.21$ V. The ratio of oxidation peak current (I_{pa}) and reduction peak (I_{pc}) was 1.47. While, the oxidation and reduction peak currents of quercetin in mixed choline chloride/urea DES acetate buffer solution supporting electrolyte were higher than those in acetate buffer solution, which means that choline chloride/urea DES can effectively improve the sensibility of MWCNT GE to detect trace quercetin.

RTIL/CNTs composite electrodes have been reported to have the advantages over traditional modified electrodes, including improved sensitivity, compatibility and stability [23]. The major problem on the promising applications of RTIL/CNTs composite electrodes in practical wok is that their preparation involving a complex procedure to the synthesis of ionic liquids and electrode modification is cost, tedious and time consuming. We applied a simple and fast way to develop a EDSs supporting electrolyte abrasively immobilized MWCNTs electrode system in this work, the proposed electrode system is very easy, fast and cheap to preparation as compared with RTIL/CNTs composite electrode system.

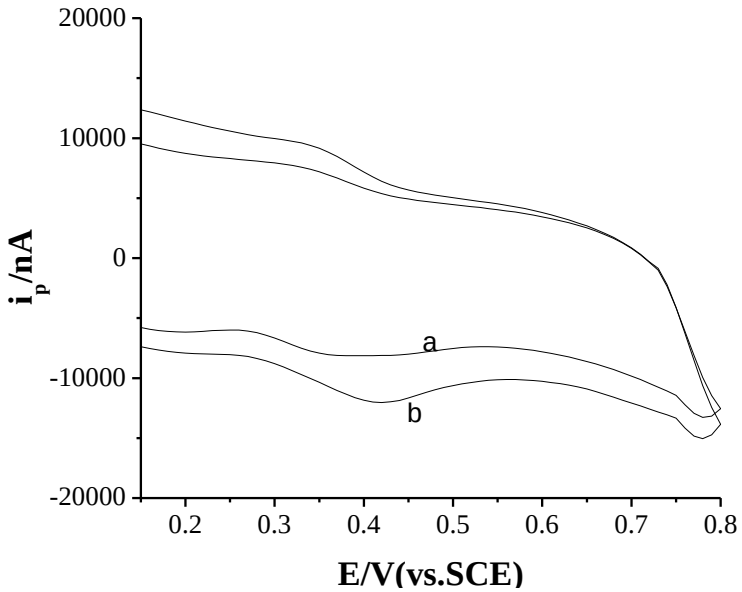


Figure 4. The cycle voltammetric analysis curve of quercetin on MWCNT GE, (a) acetate buffer solution supporting electrolyte, (b) reline ionic liquid/acetate buffer solution mixed supporting electrolyte

The result above indicates that EDSs supporting electrolyte MWCNTs electrode system can improve the sensitivity of MWCNTs modified electrode effectively. But, to the best of our knowledge,

EDSs supporting electrolyte MWCNTs electrode electrochemical analysis system has not been reported in the literature.

The immobilization of activated CNTs on the electrode surface because CNTs generally exist as highly tangled ropes and are insoluble in almost all solvents, which greatly hinder their capacity of forming uniform and stable films. To overcome this deficiency, CNTs are firstly dispersed or dissolved in various solutions or suspensions and immobilized on the surfaces of various substrates by physical or chemical methods. This section focuses on the introduction of some typical immobilization methods of CNTs on electrode surfaces that are widely used in constructing CNT-based electrochemical sensors. There is no comparison the results with other similar results published by other authors.

3.3. Influence of scan rate

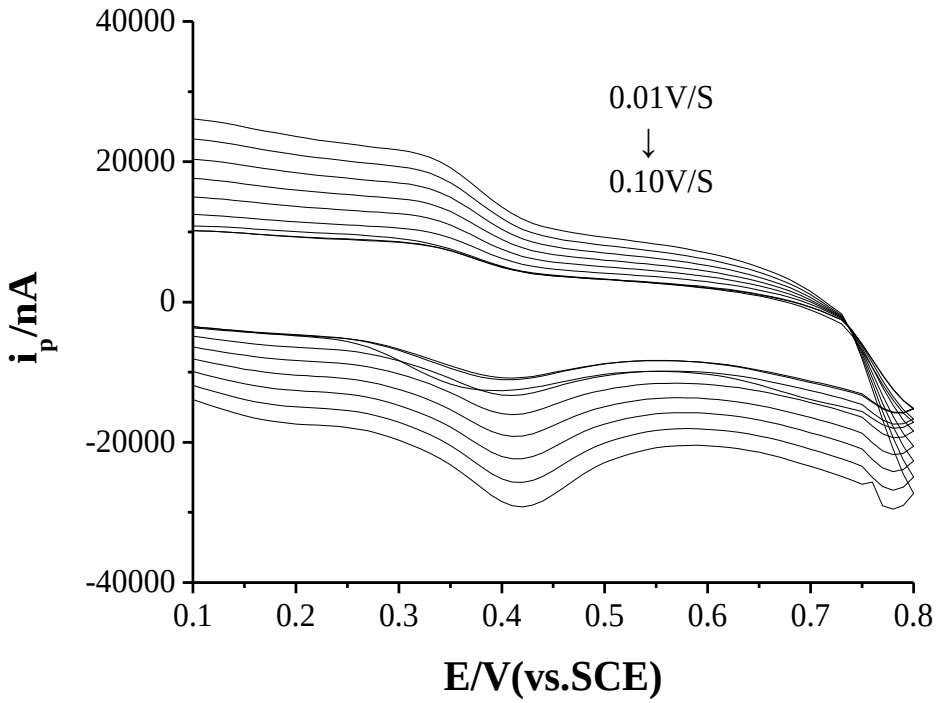


Figure 5. The curves of quercetin at different scan rate at MWCNT GE

Figure 5 shows the effect of scan rate on the CV response of quercetin in mixed choline chloride/urea DES acetate buffer solution supporting electrolyte. Both the oxidation peak current (I_{pa}) and reduction peak current (I_{pc}) showed a well linear relationship with the scan rate (υ) in the potential range of 0.01 to 0.1 $V \cdot s^{-1}$, the regression equation for I_{pa} and I_{pc} is I_{pa}＝55.851υ + 499.74 (r＝0.9992) and I_{pc}＝16.495υ+699.85 (r＝0.9989), respectively. The results indicate that electrode process in the choline chloride/urea DES electrolyte and MWNTs modified electrode system is governed by the surface adsorption-controlled electrochemical process [10].

The number of electrons transferred (n) was calculated according to the following equation[11]:

$$i_p = \frac{nFQ\upsilon}{4RT}$$

Where i_p represents the anodic or cathodic peak current, Q is the quantity of charge measured from the oxidation or reduction peak area of voltammogram. $Q = nFA\Gamma_T$, A is the surface area of the electrode, *v* is the scan rate and Γ_T is the surface coverage of the electroactive quercetin. In this work, the electron transfer number (n) was calculated to be 1.8 at 0.05 $V \cdot s^{-1}$ of scan rate.

3.4. The electrochemical impedance

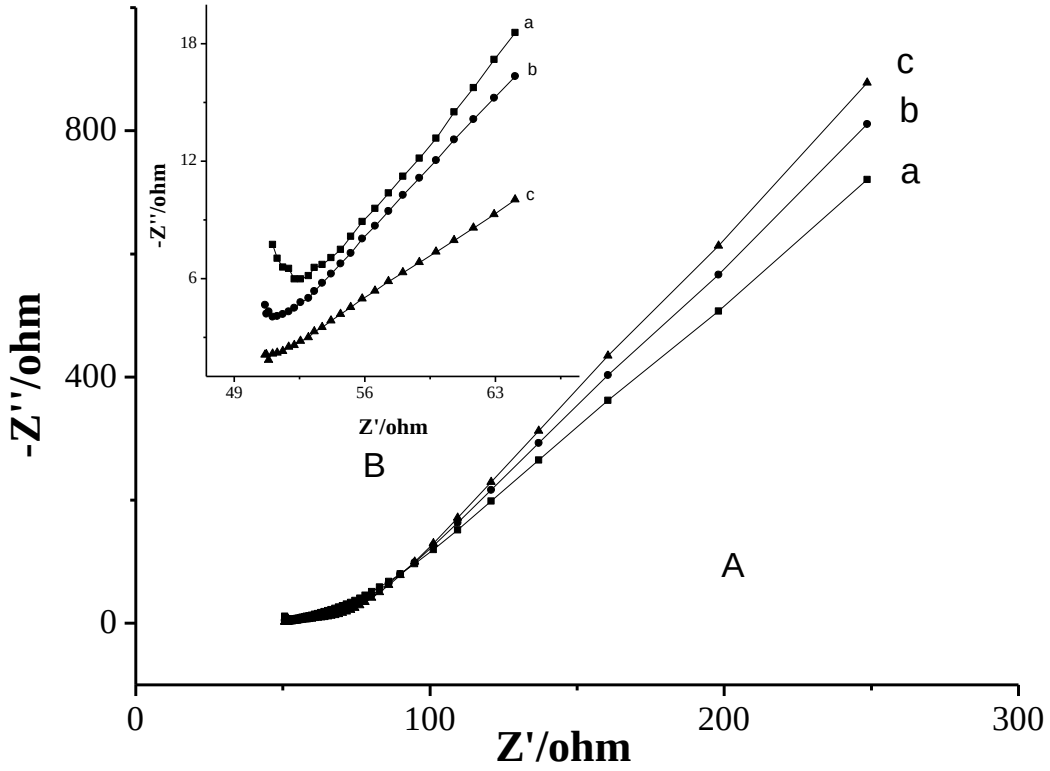


Figure 6. The Nyquist graph of quercetin at MWCNTs GE, (a) acetate buffer solution, (b) acetate buffer solution + 1%(v/v) reline ionic liquids, (c) acetate buffer solution + 5%(v/v) reline ionic liquids

The electrochemical impedance of quercetin in the choline chloride/urea DES electrolyte and MWNTs modified electrode system was investigated. Figure 6 shows the Nyquist graph of quercetin at MWCNTs GE in 0.1 $mol \cdot L^{-1}$ acetate buffer solution (a), mixed 0.1 $mol \cdot L^{-1}$ acetate buffer solution + 1% choline chloride/urea DES electrolyte (b) and mixed 0.1 $mol \cdot L^{-1}$ acetate buffer solution + 5% choline chloride/urea DES electrolyte (c). The radius in high frequency region for mixed acetate buffer solution/choline chloride/urea DES electrolyte (b and c) was smaller obviously than that for acetate buffer solution (a) and the slope of curves b and c was larger than that of curve a, indicating that mixed acetate buffer solution/choline chloride/urea DES electrolyte can effectively reduce the charge transfer resistance and, reaction resistance of quercetin, and increase the electronic exchange rate, which can result in a sensitization effect for quercetin at MWCNTs GE.

3.5. Analytical performance characteristics of the proposed method

Well-defined oxidation peak DPV response with a high peak current of quercetin was observed in the choline chloride/urea DES electrolyte and MWNTs modified electrode system as Figure 7 shown.

A good linear relationship between the oxidation peak current and the concentration of quercetin within the range from 9.95×10^{-7} mol·L^{-1} to 4.76×10^{-5} mol·L^{-1}, was obtained with the linear regression equation of $I_{pa}=526.49-5\times10^{8}$ c (r=0.9991), where, I_{pa} is the oxidation peak current (μA), c is the concentration of quercetin (mol·L^{-1}). According to the method recommended by IUPAC, detection limit (CL) = $3S_b/S_x$, where, 3 is confidence factor, S_b is background noise standard deviation, S_x is the measurement sensitivity (The slope of the standard curve), as the result, the detection limit for quercetin was 3.6×10^{-8} mol·L^{-1}. The repeatability of the method was evaluated by analyzing seven replicates of 2.0×10^{-6} mol·L^{-1} quercetin, RSD was found to be 1.82%.

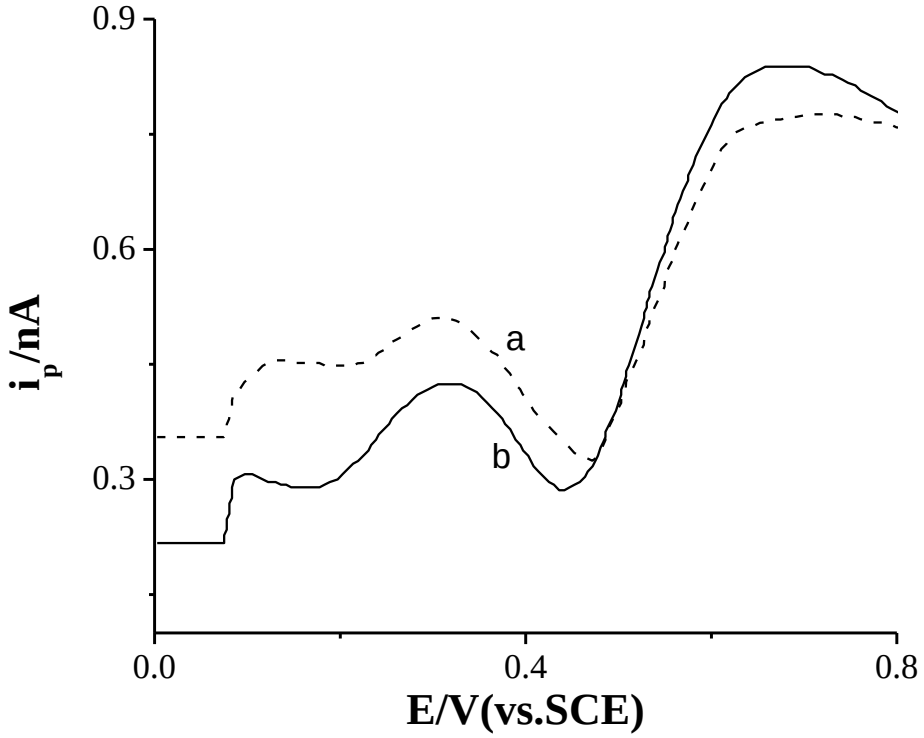


Figure 7. The differential pulse voltammetric analysis curve of quercetin at MWCNT GE

3.6. Sample analysis

Three quercetin capsule samples were weighted and were prepared in ethanol. A certain volume of solution was added respectively to electrolysis cell for DPV analysis, the content of quercetin was calculated by standard addition method. The average content for the five replicates were calculated to be 501.2 to 507.3 mg·g^{-1}, the relative deviations for accuracy ranged from 1.1 to 1.6%, the percentage recovery ranged from 99.3 to 102.2%.

Table 1. Sample analysis results (n=5)

	Sample 1	Sample 2	Sample 3
Marked Value* ($mg \cdot g^{-1}$)	513.2	513.1	513.1
Measured Value ($mg \cdot g^{-1}$)	507.3	501.2	507.2
RSD (%)	1.3	1.1	1.6
Added ($mg \cdot g^{-1}$)	99.1	102.2	297.2
Found ($mg \cdot g^{-1}$)	608.0	605.6	802.3
Recovery (%)	101.6	102.2	99.3

*Determined by HPLC

4. CONCLUSION

A new electrochemical analysis method, using choline chloride/urea DES electrolyte and MWNTs modified electrode system has been developed. The proposed system has the advantages such as rapid and easy to preparation, low cost over the RTIL/CNTs composite modified electrodes system. A pair of quasi-reversible redox peak of quercetin with two electron-transfer reaction mechanism was obtained in the system. An improved sensitivity for quercetin was observed by cyclic voltammetry. The electrochemical impedance result indicates that choline chloride/urea DES can effectively reduce the charge transfer resistance and reaction resistance of quercetin. A sensitive differential pulse voltammetric method was proposed for the determination of trace quercetin.

ACKNOWLEDGMENTS
This work was supported by the National Natural Science Fundation of China (51062018), the Natural Science Fundation of Yunnan (2010FXW004), Program for Innovative Research Team (in Science and Technology) in University of Yunnan Province (2010UY08, 2011UYN09) and Program for Yunnan Provincial Innovation Team (2011HC008).

References

1. S.S. Baghel, N.Shrivastava, R. S. Baghel, Preeti. Agrawal, S. Rajput, *J PHARM PHARMl SCI*, (2012) 146-160
2. C. Gupta, A. Vikram, D.N. Tripathi, P. Ramarao, G. B. Jena, *Phytother Res*, 28 (2009) 119
3. R. Gonzalez-Segovia, J. L. Quintanar, E. Salinas, R. CeballosSalazar, F. Aviles-Jimenez, J. Torres-Lopez, *J. Gastroenterol*, 43 (2008) 441-447
4. B. E. Shan, M. X. Wang, R. Q. Li, *Cancer Inves*, 27 (2009) 604-12
5. R. V. Swann, *J PHARM PHARM*, 1 (2011) 323-329
6. K. Ishii, T. Furuta, Y. Kasuya, *Chromatog. B*, 794 (2003) 49-56
7. L. Biasutto, E. Marotta, S. Garbisa, M. Zoratti, C. Paradisi, *Molecules*, 15 (2010) 6570-6579
8. R. Lei, X. Xu, F. Yu, N. Li, H. W. Liu, K. Li, *Talanta*, 75 (2008) 1068–1074
9. Y, Sun, T. Guo, Y. Sui, F. M. Li, *J. Sep. Sci*, 26 (2003) 1203–1206
10. H. R. Zare, M. Namazian, N. Nasirizadeh, *J. Electroanal. Chem*, 584 (2005) 77–83

11. D. Nematollahi, M. Malakzadeh, *J. Electroanal. Chem*, 547 (2003) 191–195
12. G. J. Volikakis, C. E. Efstathiou, *Talanta*, 51 (2000) 775–785
13. M. Y. Wang, D. E. Zhang, Z. W. Tong, X. Y. Xu, X. J. Yang, *J. Appl. Electrochem*, 41 (2011) 189–196
14. A. C. Franzoi, I. C. Vieira, C. W. Scheeren, J. Dupont, *Electroanal*, 22 (2010) 1376–1385
15. F. Gutiérrez, G. Ortega, J. L. Cabrera, M. D. Rubianes, G. A. Rivas, *Electroanal*, 22 (2010) 2650–2657
16. P. Xiao, F. Q. Zhao, B. Z. Zeng, *Microchem. J*, 85 (2007) 244–249
17. X. F. Yang, D. Sun, X. F. Xie, H. J. Zhang, *Nanoscience and Nanotechnology Letters*, 5 (2013) 413-417
18. S. Sun, M. Q. Zhang, Y. J. Li, X. W. He, *Sensors*, 13 (2013) 5493-5506
19. J. F. Zhong, D. L. He, Z. Zhou, *CHINESE CHEM LETT*, 19 (2008) 319 –323
20. D. L. Compton, J. A. Laszlo, *Electroanal.Chem*, 520 (2002) 71-78
21. J. Cao, P. Li, L. Yi, *J. Chromatogr. A*, 1281 (2011) 9428-9434
22. X. H. Liu, L. Li, X. P. Zhao, *Colloids Surf., B:Biointerfaces*, 81 (2010) 344-349
23. A. C. Franzoi, I. C. Vieira, C. W. Scheeren, J. Dupont, *Electroanalysis*, 12 (2010) 1376-1385
24. A. P. Abbott, G . Capper, D. L. Davies, R. K. Rasheed, V. Tambyrajah, *Chem. Commun*, 1 (2003) 70-71
25. H. Y. Wang, Y. Jing, X. H. Wang, Y. Yao, Y. Z. Ji, *J. Mol. Liq*, 163 (2011), 77–81
26. J. Chil, *Chem. Soc*, 57 (2012) 1208-1212
27. M. Bougouma, A. V. Elewyck, M. Steichen, C. Buess-Herman, T. Doneux, *J SOLID STATE ELECTR* , 17 (2013) 527-536
28. G. Florentina, V. Teodor, *Chalcogenide Letters*, 9 (2012) 165 - 174
29. A. Salimi, S. Lasghari, A. Noorbakhash, *Electroanal*, 22 (2010) 1707 – 1716
30. A. Salimi, R. Hallaj, *Talanta*, 66 (2005) 967-975