Electrochemical Oxidation of Luteolin at a Glassy Carbon Electrode and Its Application in Pharmaceutical Analysis

Ailin LIU,* Shaobo ZHANG, Liying HUANG, Yangyuan CAO, Hong YAO, Wei CHEN, and Xinhua LIN*

Department of Pharmaceutical Analysis of the Fujian Meidical University; Fuzhou 350004, China.
Received June 23, 2007; accepted March 2, 2008; published online March 25, 2008

Luteolin is a flavonoid reported to occur widely in many medicinal plants. The electrochemical behavior of luteolin was studied in phosphate buffer solution (PBS) of pH 4.0 at a glassy carbon electrode (GCE) by cyclic voltammetry (CV) and differential pulse voltammetric method (DPV). The results indicated the well-defined redox peak of luteolin which was involving two electrons and two protons was observed and the electrode process is adsorption-controlled. The charge transfer coefficient (α) was calculated as 0.66. The relationships between oxidation peak current and the concentration of luteolin are linear in the range of 1.0×10^{-8}—1.0×10^{-6} M by DPV method. The detection limit had been estimated as 5.0×10^{-9} M. The facile and rapid method has been successfully applied to the detection of luteolin in tablets.

Key words luteolin; electroanalysis; pharmaceutical analysis; flavonoid; tablets

Luteolin (3′,4′,5,7-tetrahydroxy-flavone) is one of the most bioactive flavonoids (structure is shown in Fig. 1), and found in high amounts in parsley, thyme, and peppermint, luteolin appears to cause many beneficial effects on human health, including cardiovascular protection, anticancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity, anti-inflammatory effects and anti-allergic properties.[1—3] Now, Various analytical methods have been reported for the determination of luteolin in flavonoids thin-layer chromatography,[4] gas chromatography (GC),[5] high-performance liquid chromatography (HPLC),[6—13] and capillary electrophoresis (CE),[14—16] coupled with various detection techniques, such as UV spectrophotometry, *etc.* The coupling of these techniques may provide high selectivity of the assay, but brings also some disadvantages of operating complexity, time and reagent consuming, high cost, *etc.*

Flavonoids are characterized by the number of hydroxyl groups on the B and A ring. Therefore, flavonoids including luteolin are electroactive, easily subject to either oxidation or reduction electron transfer reactions, hence they can be investigated by electrochemical methods. The oxidation reaction of flavonoids is strongly related to their structure, which contains several free phenolic hydroxyl groups. It has been shown that the antioxidant activity of flavonoids resides in their aromatic OH groups.[17] However, there are a few literatures on the electrochemical behavior of luteolin and directly electrochemical methods to determine luteolin. Howard[18] and Filipiak[19] reported to investigate the electrochemical property of luteolin. Jorgensen *et al.*[20,21] studied electrochemical property and measured luteolin by electrochemical method in acetonitrile and dimethylformamide (DMF).

In the present paper, the objective of this study was to investigate the mechanism of oxidation of luteolin by cyclic voltammetry (CV) and differential pulse voltammetric method (DPV) at glassy carbon electrode (GCE), and finally a DPV method was developed for the measurement of luteolin.

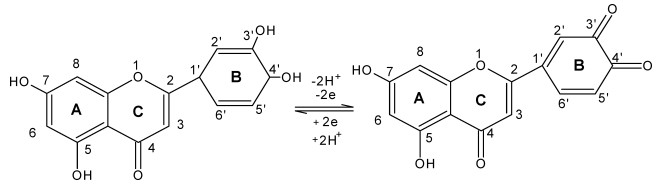

Fig. 1. The Electrochemical Behavior of Luteolin on a Glassy Carbon Electrode

Experimental

Reagents and Solutions Luteolin was purchased form National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The standard solution of 1.0×10^{-3} M luteolin was prepared by dissolving luteolin in 5 mM NaOH, and then it was stored in the dark. All reagents were of analytical grade and used without any further purification. Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.05 M NaCl and 0.05 M NaH_2PO_4–Na_2HPO_4, and then adjusting the pH with 0.05 M H_3PO_4 or 0.05 M NaOH. All solutions were prepared with double-distilled water. Luteolin tablets (20 mg luteolin per tablet, Shanghai Usea Biotech Co., Ltd., Shanghai, China) were purchased from local drug store.

Apparatus CHI 660C electrochemical workstation (Shanghai CH Instruments, China) was used for electrochemical measurements. A conventional three-electrode system was used throughout the experiments, including a bare GCE as the working electrode, a platinum wire as a counter electrode, and an Ag/AgCl (saturated) electrode as a reference. They were all used in a conjunction with an electrochemical cell of 10 ml. All potentials mentioned in this paper were referred to this reference electrode. The experiments were conducted in PBS (0.05 M, pH 4.0) at room temperature (25±1 °C). All cyclic voltammetric experiments were carried out with a scan rate of 100 mV s^{-1} unless otherwise stated. The pH measurements were carried out with a pHS-3B pH-meter (Shanghai Precision & Scientific Instruments, China) at room temperature.

GCE Pretreatment The bare GCE was polished successively with 0.3 and 0.05 μm Al_2O_3 slurry on silk. Then it was rinsed with doubly distilled water, and sonicated in 1 : 1 HNO_3, acetone and doubly distilled water for 10 min, respectively. After being cleaned, the electrode was immersed in 0.05 M H_2SO_4 and was conditioned by cyclic sweeping from −0.4 to 1.6 V at 100 mV s^{-1} for 20 scan times. Then the pre-treated GCE was obtained.

Tablet Sample Preparation Ten tablets (20 mg luteolin per tablet, Shanghai Usea Biotech Co., Ltd., Shanghai, China) were finely pulverized, then weighted a average mass of ten tablets and dispersed in a 50 ml volumetric flask, and was dissolve and diluted to required volume with ethanol. After sonication, it was filtered. After that, a suitable aliquot of the clear filtrate was diluted with pH 4.0 PBS to prepare appropriate sample solutions. The sample was then added with appropriate amount of luteolin for recovery experiments.

Analytical Procedure The required volume of standard solution and sample solution of luteolin were added with a micropipette to the electrochemical cell which was placed 10 ml of pH 4.0 PBS, and underwent a preset adsorption potential and an adsorption time for the analyte accumulation, before a perturbation program was applied for measurement. Then the CV or DPV was recorded. The CV was recorded from −0.2 to 0.8 V at a scan rate of 100 mV s^{-1}, sample interval of 0.001 and quiet time of 2 s while the

* To whom correspondence should be addressed. e-mail: ailinliu@mail.fjmu.edu.cn; xinhua63@163.com

DPV was recorded from 0.0 to 0.8 V with amplitude of 0.05 V, pulse width of 0.05 s, pulse period of 0.2 s and quiet time of 2 s. CV and DPV were recorded in quiescent solutions at room temperature.

Results and Discussion

Electrochemical Behaviors of Luteolin The cyclic voltammograms of luteolin on the bare GCE in PBS (pH 4.0) were shown in Fig. 2 at 100 mV s^{-1}. The cyclic voltammograms of luteolin shows that luteolin on the bare GCE had a chemically reversible redox couple (peak 1 and peak 3) at lower potentials (E_{pa}=0.410 V, E_{pc}=0.379 V), and an irreversible oxidation peak 2 at higher positive potentials (E_{pa}=1.05 V) in pH 4.0 PBS.

The reversible oxidation peak 1 of luteolin occurs at E_{pa}=+0.410 V is corresponded to the oxidation of the 3′,4′-dihydroxy substituent on the ring-B of luteolin. The corresponding reduction peak 3 of the 3′,4′-diquinone formed occurs at E_{pc}=+0.379 V. The second oxidation, peak 2, occurred at E_{pa}=+1.05 V, corresponding to an irreversible reaction which involves the 5,7-dihydroxy group on the ring-A of luteolin.[22—25)]

Separation of the reversible redox peak potentials, ΔE_p ($=E_{pa}-E_{pc}$), was 31 mV, ΔE_p is close to 2.3 RT/nF (or 59/n mV at 25 °C), so that the number of electrons involved in the reaction was n=1.9≈2, and the ratio of the anodic peak current to the cathodic peak current is almost equal to unity ($I_{pa}:I_{pc}$=1.28 : 1≈1 : 1). The transfer coefficient (α) can be deduced from the peak width at half-height by adopting the method developed by Laviron.[26)] According to this method, the width at half of the anodic peak was 62.5/(1−α) mV. We found that the width at half of the anodic peak was 92 mV at a scan rate of 100 mV s^{-1}, so α=0.66 can be obtained.

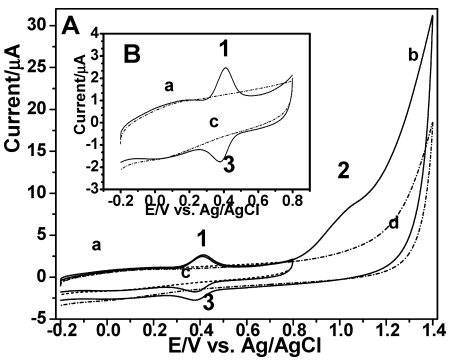

Fig. 2. Cyclic Voltammograms of 0.5 μM Luteolin in PBS (pH 4.0)

(A) a, b: cyclic voltammograms in the presence of 0.5 μM luteolin in pH 4.0 PBS; c, d: the absence of luteolin in pH 4.0 PBS; (B) inset is enlargement of (A). Scan rate: 100 mV s^{-1}.

The cyclic voltammograms (CVs) of the luteolin on glassy carbon eletctrode at different scan rates are shown in Fig. 3A. There appear a well defined redox couple and the peak current increases with increasing the scan rate from a to f (20, 40, 80, 100, 120, 140, 200, 300, 400, 500 mV s^{-1}). Plots of reversible redox couple currents *versus* scan rate (shown in Fig. 3B) yielded straight lines in the range 20—500 mV s^{-1} ($I_{pa\ luteolin}$ (μA)=0.36921+0.0123v, r=0.9989; $I_{pc\ luteolin}$ (μA)=−0.15219−0.01113v, r=−0.9998). Therefore, the adsorption-controlled surface adsorption kinetics played a more important role in the electrode process. Luteolin was also adsorbed strongly on the electrode surface and this adsorption process was observed with all the voltammetric methods used.

What is more, anodic peak potentials for the oxidation of luteolin shifted towards negative direction with an increase in pH and the relationship between E_{pa} and pH is well linear (E_{pa}=−0.05814 pH+0.62597, r=0.998) (in Fig. 3C). The linear segment was found with slope values of −58.1 mV/pH in the pH ranges of 2—9, following the Nernst equation slope. It can therefore be concluded that equal number of electron and proton is involved in the electrode reactions. Based on the discussion mentioned above, the mechanism for oxidation of luteolin at a bare GCE can be illustrated (shown in Fig. 1). The mechanism of electrooxidation of luteolin involves in losing a proton to give the monoanionic species followed by a one electron, one proton oxidation of the monoanionic species to form a radical anion, and then yield the final product of 3′4′-diquinone.[27)]

DPV Determination of Luteolin at GCE. Preconcentration of Luteolin For consideration of the electrode process of luteolin at the GCE surface being an adsorption-controlled surface adsorption kinetics. DPV technique coupled with preconcentration procedure was used for study. Both potential (E_{ads}) and time (t_{ads}) of the preconcentration were investigated for obtaining optimal DPV signals.

Effect of preconcentration time on DPV peak current of

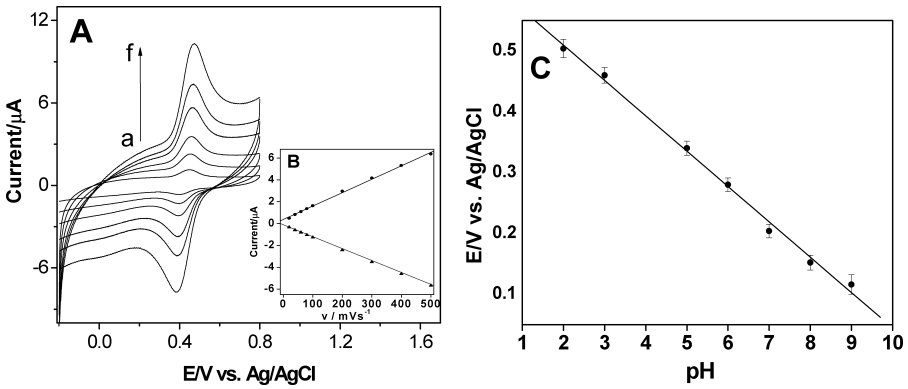

Fig. 3. (A) CV of 0.5 μM Luteolin on Glassy Carbon Eletctrode at Different Scan Rates

(B) The Relationship between the Scan Rate and Peak Current

Scan rates: 20, 40, 80, 100, 120, 140, 200, 300, 400, 500 mV s^{-1}.

(C) The Effect of pH on the Potential of Luteolin Oxidized Peak in Phosphate Buffer Solutions with Different pH

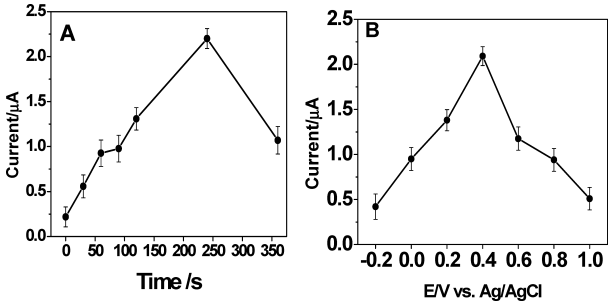

Fig. 4. (A) Effect of Preconcentration Time on Peak Current of 0.5 μM Luteolin by DPV in pH 4.0 PBS

E_{ads}=0.4 V.

(B) Effect of Preconcentration Potential on Peak Current of 0.5 μM Luteolin by DPV in pH 4.0 PBS, t_{ads}=240 s

Scan rate: 100 mV s^{-1}.

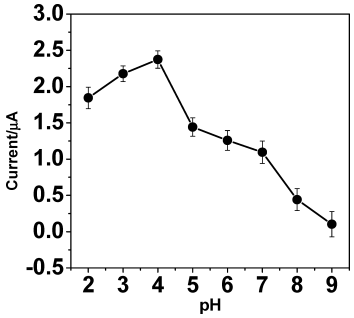

Fig. 5. Effect of pH of PBS on Peak Current of 0.5 μM Luteolin by DPV, E_{ads}=0.4 V, t_{ads}=240 s

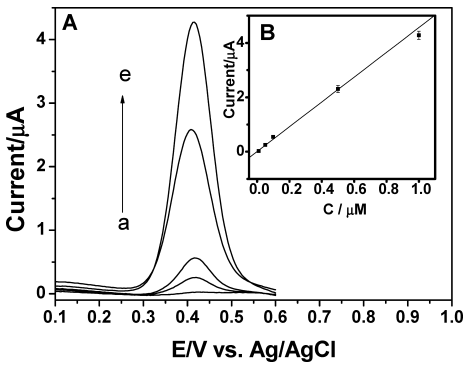

Fig. 6. (A) DPVs of Different Luteolin Concentrations at a Glassy Carbon Electrode, Luteolin Concentrations (from a to e, μM): 0.01, 0.1, 0.2, 0.3, 0.4, 1

(B) The Relationship between Luteolin Concentrations and Peak Current

Scan rate: 100 mV s^{-1}.

0.5 μM luteolin is shown in Fig. 4A. The peak current of luteolin increases slightly to the preconcentration time within a range of 0—240 s. The preconcentration time around 240 s was obviously favorable for obtaining the maximal peak currents. With the preconcentration time for more than 240 s, the peak current for luteolin decreases significantly. The results demonstrate that 240 s of the preconcentration time is selected. But further enhancing the adsorption time will lead the peak current to become decreasing. The response sensitivity is significantly improved about 30 times by increasing the preconcentration time at 240 s (in Fig. 4A).

Figure 4B shows the influence of the peak current of 0.5 μM luteolin on preconcentration potential. The peak current changes when the preconcentration potential is varied in the range of −0.2 to +1.0 V, indicating that the adsorption potential has influence on the oxidation peak height of luteolin. It can be seen from Fig. 4A that the DPV peak current of luteolin increases slightly until the preconcentration potential reaches 0.4 V, and then it decreases when the preconcentration potential increases further. Thus, an optimal preconcentration potential was performed under 0.4 V.

The Effect of pH of Luteolin on the Peak Current by DPV The effect of the pH value of the supporting solution on the electrochemical response of the GCE towards the determination of 0.5 μM luteolin was studied, and variations of peak current with respect to the change in pH of the electrolyte in the pH range from 2.0 to 9.0 are shown in Fig. 5. It can be seen from Fig. 5 that the DPV peak current of luteolin increases slightly with an increase in the solution pH until it reaches 4.0, and then it decreases when the pH increases further; therefore, pH 4.0 was selected as the optimum pH value in the subsequent process.

Linearity, Detection Limit and Reproducibility Under the optimum conditions (0.4 V preconcentration potential and 240 s preconcentration time and pH 4.0), the peak current of luteolin was measured by DPV; a series of DPVs are shown in Fig. 6A. When the concentration of luteolin increased, the peak current increased. The peak current increased linearly with the concentration of luteolin within a range of 1.0×10^{-8}—1.0×10^{-6} M (I_{pa} (μA)=0.055+4.584C (μM), r=0.9933), the detection limit is 5.0×10^{-9} M (S/N=3). Nine repetitive measurements of standard solution containing 0.5 μM luteolin resulted in a R.S.D. of 2.54% for the current response of luteolin, showing good reproducibility.

Table 1. Determination of Luteolin in Tablet (n=6)

Analyst	Labeled (mg)	Added (mg)	Found (mg)	R.S.D. (%)	Recovery (%)
Luteolin	20	0	19.8	2.5	
	20	5	25.2	2.6	108
	20	10	29.6	2.4	98
	20	15	35.4	3.2	104

Interferences To evaluate the interferences of some foreign species on the determination of the current response of luteolin. Other possible interferents, such as 100 μM lysine, 100 μM cysteine, 100 μM citric acid, 100 μM tartaric acid, 100 μM glucose, 100 μM cyclodextrin, 100 μM lactose, 100 μM saturated starch, were individually and simultaneously added into a standard solution containing 0.5 μM luteolin. The results indicated that on interference effect (signal change <5%) on the determination of luteolin was observed.

Samples Analysis Luteolin in tablets was determined by above DPV method. The results are listed in Table 1. From the Table 1, it can be seen that the recovery and RSD (<3.0%) were acceptable, showing that the proposed methods could be used efficiently for the determination of luteolin in tablet.

Conclusion

The electrochemical behavior of luteolin was studied at a glassy carbon electrode by CV and DPV. The experiments

showed a reversible process corresponding to oxidation of the catechol 3′,4′-hydroxyl group and another irreversible at high potentials corresponding to the oxidation of the 5,7-dihydroxyl groups. And novel and rapid electrochemical method to determine the luteolin was established. In conclusion, the results obtained from the determination of luteolin showed a good stability, sensitivity and the possibility of determination of pharmaceuticals.

Acknowledgements We gratefully acknowledge the financial support of the National High Technology Investigation Project Foundation of China (2006AA02Z4Z1), the National Natural Science Foundation of China (20675015), the Fujian Provincial Natural Science Foundation (200610016), the Fujian Provincial Young Talent Foundation (2006F3058) and the Natural Science Foundation of Fujian Province of China (C0710024).

References

1) Mann C., Staba E. J., "Herbs, Spices and Medicinal Plants: Recent Advances in Botany, Horticulture and Pharmacology," Vol. 1, ed. by Craker L. E., Simon J. E., Oryx Press, Phoenix, 1984, pp. 235—280.
2) Peters N. K., Frost J. W., Long S. R., *Science*, **233**, 977—980 (1986).
3) Park Y. J., Kim H. J., Lee S. J., Choi H. Y., Jin C., Lee Y. S., *Chem. Pharm. Bull.*, **55**, 1065—1066 (2007).
4) Fecka I., Cisowski W., *J. Planar Chromatogr.*, **15**, 429—432 (2002).
5) Liu C. S., Song Y. S., Zhang K. J., Ryu J. C., Kim M., Zhou T. U., *J. Pharm. Biomed. Anal.*, **13**, 1409—1414 (1995).
6) Ku Y. R., Chen C. Y., Ho L. K., Lini J. H., Chang Y. S., *J. Food Drug Anal.*, **10**, 139—142 (2002).
7) Areias F. M., Valentao P., Andrade P. B., Ferreres F., Seabra R. M., *Food Chem.*, **73**, 307—311 (2001).
8) Liu F. F., Catharina Y. W. A., Heinze T. M., Rankin J. D., Berger R. D., Freeman J. P., Lay J. O., Jr., *J. Chromatogr. A*, **888**, 85—92 (2000).
9) Srinivasa H., Bagul M. S., Padh H., Rajani M., *Chromatographia*, **60**, 131—134 (2004).
10) Jin D. R., Hakamata H., Takahashi K., Kotani A., Kusu F., *Bull. Chem. Soc. Jpn.*, **77**, 1147—1152 (2004).
11) Qian Z. M., Li H. J., Li P., Ren M. T., Tang D., *Chem. Pharm. Bull.*, **55**, 1073—1076 (2007).
12) Zhou Y. Z., Liu X. X., Zheng X. H., Zheng J. B., *Chromatographia*, **66**, 635—637 (2007).
13) Wittemer S. M., Veit M., *J. Chromatogr. B*, **793**, 367—375 (2003).
14) Lü Y. Q., Wu C. H., Yuan Z. B., *Chinese Analytical Chemistry*, **6**, 805—808 (2005).
15) Xu X., Yu L., Chen G., *J. Pharm. Biomed. Anal.*, **41**, 493—499 (2006).
16) Chu Q. C., Wu T., Fu L., Ye J. N., *J. Pharm. Biomed. Anal.*, **37**, 535—541 (2005).
17) Heijnen C. G. M., Haenen G. R. M. M., Vekemans J. A. J. M., Bast A., *Environ. Toxicol. Pharmacol.*, **10**, 199—206 (2001).
18) Hendrickson H. P., Kaufman A. D., Lunte C. E., *J. Pharm. Biomed. Anal.*, **12**, 325—334 (1994).
19) Filipiak M., *Anal. Sci.*, **17**, 1667—1670 (2001).
20) Jorgensen L.V., Cornett C., Justesen U., Skibsted L. H., Dragsted L. O., *Free Radic. Res.*, **29**, 339—350 (1998).
21) Jorgensen L. V., Madsen H. L., Thomsen M. K., Dragsted L. O., Skibsted L. H., *Free Radic. Res.*, **30**, 207—220 (1999).
22) Brett A. M. O., Ghica M. E., *Electroanalysis*, **15**, 1745—1750 (2003).
23) Ghica M. E., Brett A. M. O., *Electroanalysis*, **17**, 313—318 (2005).
24) Janeiro P., Brett A. M. O., *Electroanalysis*, **17**, 733—738 (2005).
25) Janeiro P., Corduneanu O., Brett A. M. O., *Electroanalysis*, **17**, 1059—1064 (2005).
26) Laviron E. J., *Electroanal. Chem.*, **101**, 19—28 (1979).
27) Kang J., Lu X., Zeng H., Liu H., Lu B., *Anal. Lett.*, **35**, 677—686 (2002).