



Electrochemical Behaviour and Determination of Baicalin on Nano-Al₂O₃ Modified Carbon Paste Electrode

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In this paper, the electrochemical behaviour of baicalin on nano-Al₂O₃ modified carbon paste electrode (NA-CPE) was investigated and further used for determination of baicalin. The nano-Al₂O₃ modified carbon paste electrode showed strong electrocatalytic effects to the oxidation of baicalin. In phosphate buffer solution (PBS, pH 7; 0.2 M) a pair of well-defined cyclic voltammetric oxidation peaks of baicalin appeared with the oxidation peak located at 157 mV (E_{pc}) and 600 mV (E_{pc}) (vs. SCE). The oxidation peak current was increased about 27.5 times more than that on traditional carbon paste electrode. The oxidation peak currents of baicalin in such modified electrode increased linearly with the concentration of baicalin in the range from 1×10^{-7} to 1×10^{-5} mol L⁻¹ with a detection limit of 8×10^{-9} mol L⁻¹. The method was applied in the determination of baicalin in samples with satisfactory results and the spiked recoveries were in the range of 95-105 %. These results suggest that the proposed electrode can be used for sensitive, simple and rapid determination of baicalin.

Keywords: Carbon paste electrode, Baicalin, Cyclic voltammetry, Electrochemistry.

INTRODUCTION

Baicalin(5,6-dihydroxy-4-oxo-2-phenyl-4H-1-benzopyran-7-yl), the corresponding chemical structure shown in Fig. 1), which is one kind of the most bioactive flavonoids compounds, are often used as antitumor¹, anti-inflammatory², antioxidants³ and antibacterial⁴, etc. In clinically, as a flavonoid compound purified from the roots of *Scutellaria baicalensis* that is used as a traditional Chinese herbal medicine with wide range functions, Baicalin can lower blood pressure⁵, spontaneous sleep-wake regulation⁶, reduce capillary permeability. So it is significant to develop simple, economic and sensitive methods for baicalin determination in medicine and natural plant. Meanwhile, a number of different techniques have been exploited for the determination of baicalin, such as high-performance liquid chromatography (HPLC)⁷, ultra-performance liquid chromatographic-tandem mass spectrometric (UPLC-MS-MS)⁸, capillary electrophoresis (CE)⁹⁻¹⁰, chemiluminescence¹¹, UV-visible spectrophotometry¹², electrochemical methods¹³⁻¹⁵. Baicalin can be easily subject to either oxidation or reduction on different working electrodes as an electroactive compound, hence baicalin can be investigated by electrochemical methods. Among these methods, electrochemistry has powerful means to study the electron transfer process in biological systems. With simple, rapid, sensitive

and easily operated features, electrochemical technique has been applied in the related research fields for many years¹⁶. Used 1-butyl 3-methyl-imidazolium hexa fluoride phosphate ([BMIM] PF₆) and nanometer MnO₂ modified glassy carbon electrode for the determination of baicalin and further applied to Chinese medicines samples with satisfactory results. The electrochemical behaviour of baicalin at the gold nanoparticles¹⁷, which was directly deposited to the surface of a single-wall carbon nano tube modified electrode was investigated. The baicalin in Shuanghuanglian oral can be directly determined without pretreatment with a novel Eu-PB composite mercury film electrode (GC/Eu-PB/MFE)¹⁸ based on chemical modified. And an ionic liquid modified carbon paste electrode (IL/CPE)¹⁹ was fabricated with hydrophilic ionic liquid 1-amy-3-methylimidazolium chloride ([AMIM] Cl) as a modifier for the determination of baicalin in urine and serum.

Up to now, various modifiers have been developed to determine biological material on modified carbon paste electrode²⁰. Fabricated an ionic liquid of octyl pyridinium hexafluorophosphate (OPFP) modified carbon paste electrode and investigated the electrochemical oxidation of some phenolic compounds²¹. The electrochemical behaviour of rutin²² on a pyridinium-typed ionic liquid modified carbon paste electrode (IL-CPE) was investigated and further used for rutin sample determination. Sun *et al.*^{23,24} also fabricated a ionic liquid modified carbon

paste electrode and used it as the basal electrode for protein electrochemistry. A LTG selective molecular imprinted polymer (MIP) and a non-imprinted polymer (NIP) were synthesized and then incorporated in the carbon paste (CP) electrodes²⁵ as a recognition element, construction of a high selective voltammetric sensor for lamotrigine (LTG) was performed. A carbon-paste electrode modified with multiwall carbon nanotubes (MWCNTs) was also used for the sensitive and selective voltammetric determination of ascorbic acid (AA) in the presence of 3,4-dihydroxycinnamic acid (3,4-DHCA) as mediator^{26,27}. Studies the Al_2O_3 nanometer powder preparation technology, by doping rare earth material so that the gas sensor detection element surface area was greatly improved; its sensitivity and stability were also improved. Research and development of rapid detection of gas sensor can avoid gas explosion accident.

However, the electrochemical determination of baicalin using nano- Al_2O_3 modified carbon paste electrode (NA-CPE) has not been reported yet. In this paper a nano- Al_2O_3 modified carbon paste electrode (NA-CPE) was used for the investigation of electrochemical behaviour of baicalin. The results suggest that NA-CPE shows good electrocatalytic activity for the oxidation of baicalin with the increase of the redox peak current in the phosphate buffer solution. The electrochemical behaviours of baicalin on the NA-CPE were carefully investigated and a sensitive voltammetric method for baicalin determination was further established, which showed no interferences from the coexisting ascorbic acid.

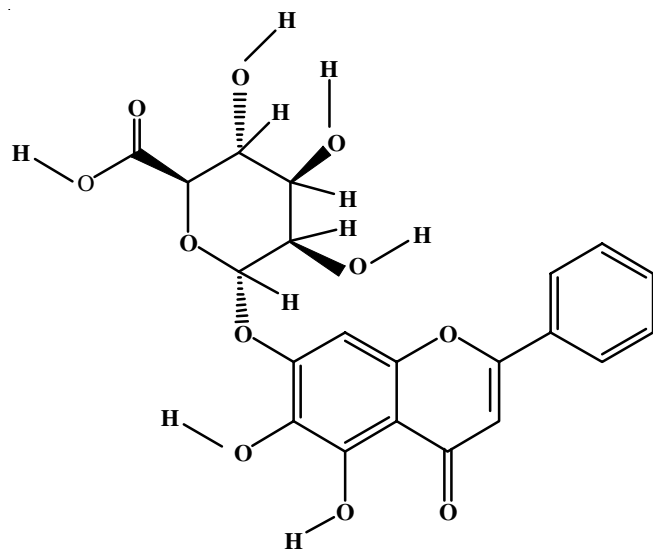


Fig. 1. Baicalin structure (m.f. $\text{C}_{21}\text{H}_{18}\text{O}_{11}$, m.w.: 466.37)

EXPERIMENTAL

All the electrochemical experiments were performed on a CHI660E electrochemical workstation (Shanghai Chenhua Co., China) controlled by a microcomputer with CHI660E software. A three-electrode system was used, where a Carbon paste electrode (4.6 mm diameter) or a modified carbon paste electrode served as the working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. All the measurements were carried out at 25 ± 1 °C.

Baicalin (Sigma), graphite powder (Average particle size 30 m, Shanghai Colloid Chemical Plant, China), Nano- Al_2O_3 , (Gaoss Union, Uk); paraffin liquid (Tianjin fuyu Chemical Limited Company, China); ascorbic acid and ethanol (Tianjin Basifu Chemical Limited Company, China) were used as received. 0.2 M phosphate buffer solution (PBS) of various pH values were used as supporting electrolyte. Citric acid and disodium hydrogen phosphate dodecahydrate (Tianjin Chemical Reagent Factory, Tianjin, China), potassium ferricyanide and potassium chloride (Xi'an Chemical Reagent Factory, Xi'an, China) were of analytical reagent grade. All other chemicals were of analytical reagent grade and doubly distilled water was used in the experiments.

Fabrication of electrodes: The traditional carbon paste electrode (CPE) was prepared by hand-mixing of graphite powder with paraffin oil at a ratio of 70/30 (w/w). The NA-CPE was fabricated with the following procedures: 3.0 g of graphite powder and 1.0 g of nano- Al_2O_3 were mixed thoroughly in a mortar to form a uniform carbon paste and further heated at 80 °C for about 1 h. A portion of the resulted carbon paste was filled into one end of a glass tube ($\phi = 4.6$ mm) and a copper wire was inserted through the opposite end to establish an electrical contact. The NA-CPE surface was smoothed on a piece of polishing paper just before use²⁸.

All the cyclic voltammetric experiments were carried out at a quiescent PBS with the scan rate of 100 mVs^{-1} unless otherwise stated. The buffer solution (pH 7) with certain concentration bacalin was transferred into a cell and then the three-electrode system was set up. The voltammograms were recorded between 1.2 and -0.4 V. All experiments were carried out at room temperature.

RESULTS AND DISCUSSION

Electrochemical behaviour of bacalin on NA-CPE: The cyclic voltammograms of bacalin were recorded at a bare carbon paste electrode and NA-CPE in phosphate buffer (pH 7) (Fig. 2). In the absence of bacalin, no redox peaks was observed at both bare and modified carbon paste electrodes during the cyclic voltammetry within the potential window from -0.4 to 1.20 V (Fig. 2, curve a). A larger background current was observed at the NA-CPE (Fig. 2, curve c) compared with the bare carbon paste electrode (Fig. 2, curve b) during the cyclic voltammetric scan, which is probable due to the electrocatalytic behaviour of nano- Al_2O_3 powder. Cyclic voltammogram of bacalin at bare GCE demonstrates a weak and broad irreversible oxidation peak at 127 mV (Epc) (Fig. 2, curve b) due to slow electron transfer, while the response considerably improved at the NA-CPE. Peak potential at NA-CPE was observed at less positive potential to be 154 mV (Epc) (Fig. 2, curve c), the peak current increases compared with bare carbon paste electrode.

It can be seen from the experimental results, the NA-CPE for baicalin redox catalyst has good electricity. According to the polymer membrane electrode model²⁹, nano- Al_2O_3 particles disperse in bare carbon electrode surface to form porous layer, the porous layer is composed by nano- Al_2O_3 and carbon powder, the double layer capacitance increases. Put NA-CPE in solution, which molecules and ions diffuse into layers of

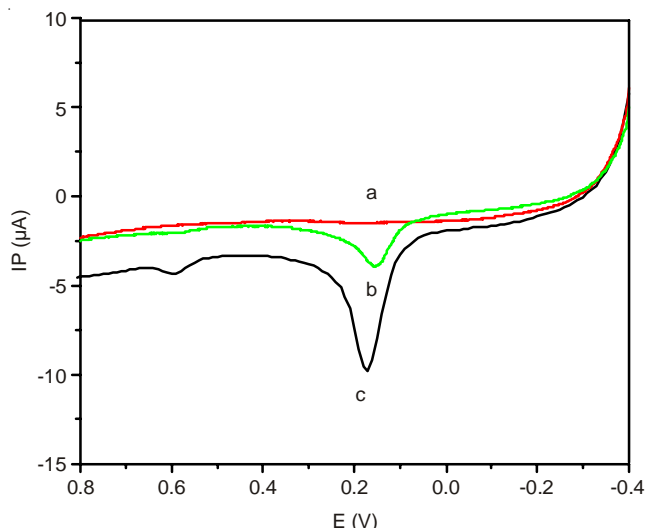


Fig. 2. Cyclic voltammograms of (a) NA-CPE in the buffer solution, (b) carbon paste electrode and (c) NA-CPE in pH 7 PBS containing 5×10^{-5} M baicalin, Scan rate: 100 mVs^{-1}

microporous, just as the polymer membrane electrode, when the solvent expands to form many pores and channels, the molecules, ions inserted or removed. The porous film of the conductor area increases by the presence of carbon powder. The increasing current as well as positive shift of anodic peak demonstrates an efficient catalytic oxidation of baicalin on the NA-CPE.

Effect of the supporting electrolyte: The electrochemical behaviours of baicalin in the different supporting electrolyte were investigated, such as sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium acetate and acetic acid, sodium carbonate and sodium bicarbonate, a hydrogen phosphate and citric acid, sodium chloride and hydrochloric acid solution. The electrochemical results showed that the sodium dihydrogen phosphate and disodium hydrogen phosphate buffer system current shape were better, no impurity peak, so sodium dihydrogen phosphate and disodium hydrogen phosphate buffer system were chosen for the supporting electrolyte of the experiment. Further investigation of the effect of the concentration of supporting electrolyte peak current, peak current suggest that the most stable in $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ buffer concentration of 0.20 mol L^{-1} , Hence the 0.20 mol L^{-1} buffer concentration was used throughout this work.

Effect of pH: The effect of the pH value of PBS on the current response of NA-CPE to 0.2 M BPS in the pH range from 5 to 8.5 (Fig. 3) was investigated. As can be seen, the oxidation peak current gradually increased with the increasing pH value from 5 to 8.5. However, when the pH further increased to 8.5, the oxidation peak current conversely decreased. The relationship between the oxidation peak potential and pH was also shown in Fig. 3. Therefore, considering the sensitivity of the determination of BPS, the pH value of 7 was chosen for the subsequent analytical experiments.

Effect of the scan rate: Fig. 4 showed the cyclic voltammograms of 5×10^{-5} M baicalin at NA-CPE with different scan rate. It could be seen that with the increase of the scan rate, the oxidation peak currents increased gradually. As shown in the inset of Fig. 4, the peak current increased linearly with

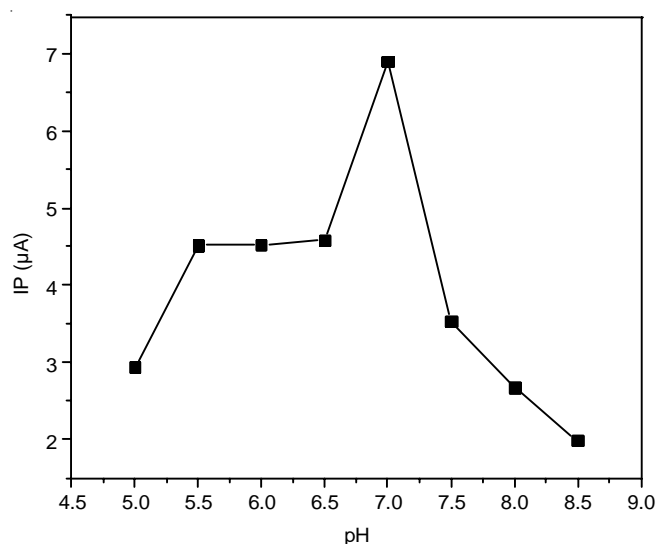


Fig. 3. pH dependence of reduction peak currents with 5×10^{-5} M baicalin, scan rate: 100 mVs^{-1}

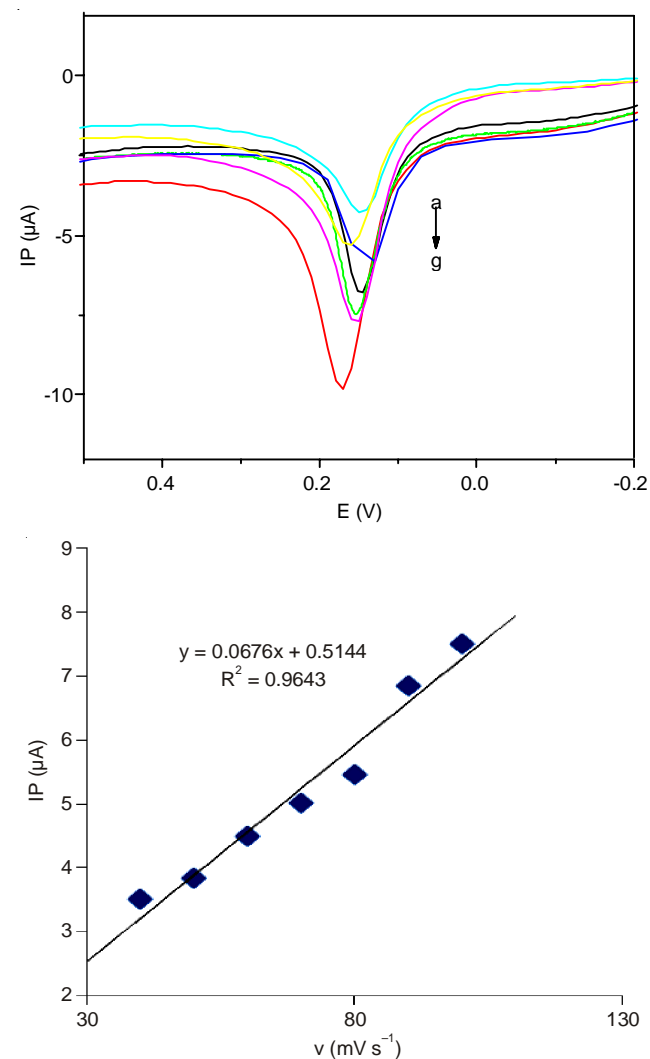


Fig. 4. Cyclic voltammograms of 5×10^{-5} M baicalin on NA-CPE with different scan rates (a-g: 40, 50, 60, 70, 80, 90, 100 mVs^{-1}) in pH 7 PBS

the scan rate in the range of $40\text{-}100 \text{ mVs}^{-1}$ Fig. 4. (A) cyclic voltammograms of 5×10^{-5} M baicalin at NA-CPE with different scan rates. Curve a-g is obtained at 40, 50, 60, 70,

80, 90 and 100 mVs⁻¹, respectively. 100 mV s⁻¹ was chosen for the subsequent analytical experiments.

Reproducibility, linear range and stability: Besides, the voltammetric determination under the selected optimum conditions were investigated, the results showed in Fig. 5, the baicalin concentration range in 1×10^{-7} to 1×10^{-5} mol L⁻¹ had good linear relationship with the peak current, linear regression equation: I_p (uA) = 0.841C (umol L⁻¹) + 0.6017, the correlation coefficient $r = 0.9946$. Method detection limit ($S \times N = 3$) 8×10^{-9} mol L⁻¹. The NA-CPE was applied to the 10 parallel determinations of 5×10^{-5} M baicalin and the relative standard deviation (RSD) was calculated as 4.2 %. The results indicated that NA-CPE showed good reproducibility. The stability of NA-CPE was also studied. After the NA-CPE was stored for 15 days, no apparent decrease of the electrochemical response to baicalin was observed, which indicated the good stability of the NA-CPE.

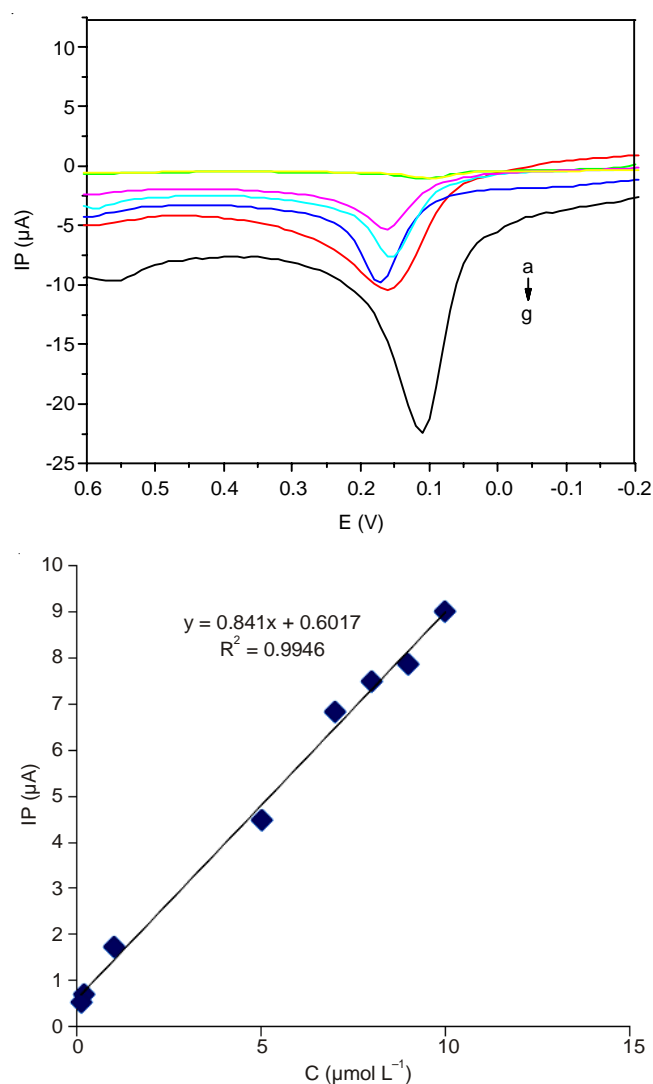


Fig. 5. Cyclic voltammograms of baicalin on NA-CPE with different concentration (a-g: 10, 9, 7, 5, 1, 0.2, 0.1 $\mu\text{mol L}^{-1}$) in pH 7 PBS. Scan rate: 100 mVs⁻¹

Interferences: The influences of some foreign substances on the determination of 5×10^{-5} M baicalin were tested according to the general procedure and the results shown that most of them did not interfere with the determination.

Practical application: Took 10 pieces of baicalin tablets (one of the traditional Chinese medicine tablets), removed the skin, weighted 0.500 g after crushing, then added 40 mL 95 % ethanol reflux extraction steeped for 3 h, filtering, washing residue with 5 mL 95 % ethanol three times, merged the filtrate, diluted with water to 100 mL and shake well. Precision measured 10 mL in 10 mL volumetric flask soluted with anhydrous ethanol and diluted to scale, shoke well and set aside¹⁶. Used the standard addition method to measure the content of baicalin finally. The content of baicalin in the traditional Chinese medicine tablets is 6.98 %. The recovery experiment results are shown in Table-1.

TABLE-1
DETERMINATION OF THE CONTENT OF BAICALIN IN
TRADITIONAL CHINESE MEDICINE TABLETS AND
THE RECOVERY DATA (n = 6). cA($\times 10^{-5}$)

No.	Added cA($\times 10^{-5}$ mol/L)	Found cF($\times 10^{-7}$ mol/L)	Recovery R (%)
1	1.00	1.05	105
2	2.00	1.94	97
3	3.00	2.89	96
4	4.00	4.19	105
5	5.00	5.17	103
6	6.00	5.87	98

It can be seen the current method was sensitive for baicalin determination with a good recovery in the range of 95-105 %

Conclusion

In this work, nano-Al₂O₃ was successfully applied to modified carbon paste electrode to study the electrochemical oxidation of baicalin. Owing to the regular and specific mesoporous structure of nano-Al₂O₃, NA-CPE possessed large surface area and highly efficient accumulation ability. The results indicates that after electrode modification, the oxidation signal of baicalin is remarkably improved. Moreover, the NA-CPE has preferable stability and the modified process is simple and easily compared. Therefore, a novel electrochemical method is proposed for the determination of baicalin, which is successfully demonstrated with determination of baicalin samples. Moreover, the preparation of the electrode is simple and also has lower cost.

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