

Highly Sensitive Determination of Caffeic Acid Using a Multi-walled Carbon Nanotubes Modified Electrode with N-Butylpyridinium Hexafluorophosphate Ionic Liquid and Chitosan as Binders

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Multi-walled carbon nanotubes-N-Butylpyridinium hexafluorophosphate ionic liquid-chitosan composite film modified glassy carbon electrode (MWNTs-[BuPy]PF₆-CS/GCE) was prepared and it was applied to the quantitative analysis of caffeic acid. A pair of redox peak with a well-defined electrochemical response signal of caffeic acid was obtained by using cyclic voltammetry (CV) in 0.04 mol·L⁻¹ BR buffer solution (pH 4.0). A novel electrochemical method to determine the caffeic acid with differential pulse voltammetry (DPV) was developed based on the MWNTs-[BuPy]PF₆-CS/GCE. The DPV oxidation peak current showed a good linear relationship with the concentration of caffeic acid in the range from 2.5×10⁻⁸ to 7.0×10⁻⁶ mol·L⁻¹ and the detection limit can reach 5.7×10⁻⁹ mol·L⁻¹. The proposed method can be used for the determination of caffeic acid with the recovery of 96.9-102.8% and the RSD of 2.2-3.1% in real sample.

Keywords: Caffeic acid; Multi-walled carbon nanotubes; Ionic liquid; N-Butylpyridinium hexafluorophosphate; Chitosan.

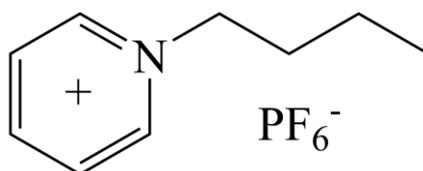
1. INTRODUCTION

As is well known, carbon nanotubes (CNTs) which are a typical one-dimensional nano-material display excellent physical and chemical properties [1], and possess extremely good and wide application prospect in various fields of research and production. Recently, multi-walled carbon

nanotubes (MWNTs) have been widely used in the fabrication of the modified electrode due to their huge specific surface area, strong mechanical performance, stable chemical property, good electrocatalytic effect and charge transfer ability [2,3]. Although MWNTs modified electrodes have good electrochemical properties such as electrocatalysis and chemical sensitization [4], the applications of MWNTs still possess certain limitations such as poor dispersion and film forming properties. Therefore, the development and application of the composite modified electrodes with MWNTs and other advanced materials as the modifiers are necessary and meaningful in the field of electrochemical analysis.

In recent years, several kinds of the MWNTs composite modified electrodes with ionic liquid (IL) [5,6] or chitosan (CS) [7] as binders have been widely used in the electrochemical determination of various active ingredients. In addition, a few studies and reports have shown that the MWNTs-IL-CS composite materials showed a good application prospect in the preparation of modified electrodes [8,9], however, only few reports have focused on research of new type of MWNTs-IL-CS composite materials and determination of active ingredients.

In this paper, the multi-walled carbon nanotubes-N-Butylpyridinium hexafluorophosphate ionic liquid-chitosan/glassy carbon electrode (MWNTs-[BuPy]PF₆-CS/GCE) was fabricated by dropping MWNTs-[BuPy]PF₆-CS suspension onto the GCE. The introduction of ionic liquid (IL) and chitosan (CS) can remedy the shortages of MWNTs and improve the electrochemical properties of composite modified materials [10]. The [BuPy]PF₆ (scheme 1), a hydrophobic ionic liquid, can be used as the modification agent in various electrochemical analysis due to its good solvating properties, high ionic conductivity, wide potential window, good thermal, chemical stability along with strong mechanical properties [11,12], the dispersibility and electroconductivity of MWNTs can be enhanced in IL [13,14]. Another important class of materials for application in the fabrication of the modified electrodes is CS, which is a natural polymer and has biocompatibility and biodegradability [15]. Although the CS has a poor conductivity, it exhibits good dispersion properties and film formation properties [16], which greatly improve the surface properties of the electrode. The MWNTs-IL-CS modified electrodes exhibited excellent electrochemical properties with high sensitivity, selectivity and stability through integrating the advantages of the three kinds of materials.



Scheme 1. The structure of N-Butylpyridinium hexafluorophosphate.

Herein, the MWNTs-[BuPy]PF₆-CS/GCE is applied to the electrochemical analysis of caffeic acid (3-(3,4-Dihydroxy-phenyl)-acrylic acid), which belongs to a phenolic acid compound almost everywhere in many medicinal plants such as honeysuckle, eucommia, oregano and dandelion. As an active ingredient in a variety of drugs, it exhibits an excellent antioxidant properties in pharmacology

research and clinical medicine [17,18], and also has various other medicinal values such as anti-inflammatory [19], anti-virus [20] and anti-cancer [21]. Multiple chromatographic techniques such as HPLC [22], RP-HPLC [23], LC-MS/MS [24] and HPLC-DAD-ESI-MS [25], and capillary electrophoresis (CE) [26] have been used for determination of caffeic acid. And the electrochemical determination of caffeic acid based on the corresponding electrodes [27-30] with cyclic voltammetry [31,32], differential pulse voltammetry [33-35], square wave pulse voltammetry [36], square-wave stripping voltammetric [37] or amperometric [38] have been developed. The electrochemical approaches represent the powerful means to detect trace amounts of active substances with high sensitivity and low cost, and display good application prospect with a simple and fast operation process.

In this study, the electrochemical behavior of caffeic acid was investigated with the MWNTs-[BuPy]PF₆-CS/GCE as the working electrode, and a novel electrochemical method for the determination of caffeic acid was established base on a good electrochemical response signal. The proposed method with the differential pulse voltammetry (DPV) can be used for the determination of caffeic acid in real samples with a high sensitivity.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Caffeic acid, purchased from Shanghai Jin Hui Biotechnology Co., Ltd; the TNM₃ carboxyl functionalized multi-walled carbon nanotubes (MWNTs, purity>95%), purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences; N-Butylpyridinium hexafluorophosphate ([BuPy]PF₆, purity≥99%), purchased from Zhengzhou Four Seasons Chemical Products Co., Ltd; chitosan (deacetylation degree 96%), purchased from Xi'an HP Biotechnology Co., Ltd.

All the other chemicals used were of analytical-reagent grade and all solutions were prepared using twice-distilled water.

2.2 Apparatus

CV and DPV were carried out using the MEC-12B Multi-function Microcomputer Electrochemical Analyzer (Jiangsu Jiangfen Electroanalytical Instrument Co. Ltd., China). The scanning electron microscope (SEM) image was obtained by the Quanta 200 FEG SEM (FEI company, USA). EIS measurement was performed with the Zahner Zennium IM6 Electrochemical Workstation (ZAHNER-elektrik GmbH & Co. KG, Kronach, Germany). A three-electrode system was used, including a multi-walled carbon nanotubes-N-Butylpyridinium hexafluorophosphate-chitosan composite film modified glassy carbon electrode (MWNTs-[BuPy]PF₆-CS/GCE) as working electrode, a platinum counter electrode (213 type) and a saturated calomel reference electrode (SCE).

2.3 Preparation of the modified electrodes

10 mg [BuPy]PF₆ was firstly dissolved in 2 mL of 0.15% chitosan solution (with 1% acetic acid as solvent) by ultrasound, and then MWNTs-[BuPy]PF₆-CS homogeneous suspension was achieved through dispersing 1 mg functionalized MWNTs in above solution under ultrasound for 180 min. Prior to modification, a bare glassy carbon electrode (GCE) was firstly polished using fine sandpaper and 0.5 μm Al₂O₃ slurry, and was then cleaned successively in 50% nitric acid, ethanol and doubly distilled water with ultrasound for 5 min. Finally, the multi-walled carbon nanotubes-N-Butylpyridinium hexafluorophosphate-chitosan composite film modified glassy carbon electrode (MWNTs-[BuPy]PF₆-CS/GCE) was fabricated by casting the MWNTs-[BuPy]PF₆-CS homogeneous suspension (5.0 μL) onto the surface of pretreated GCE, and then dried under an infrared lamp.

For a comparison, a MWNTs modified GCE (MWNTs/GCE) was fabricated using the same approach.

2.4 Analytical procedure

The sample tested was firstly diluted to 10 mL with 0.04 mol·L⁻¹ BR buffer solution (pH 4.0). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) analysis were carried out with MWNTs-[BuPy]PF₆-CS/GCE as working electrode in the potential range from 0.1 to 0.7 V at a scan rate of 0.08 V·s⁻¹.

The DPV parameter setting were: pulse width of 250 ms, pulse amplitude of 20 mV and pulse interval of 250 ms.

3. RESULTS AND DISCUSSION

3.1 Morphology characterization of the modified materials

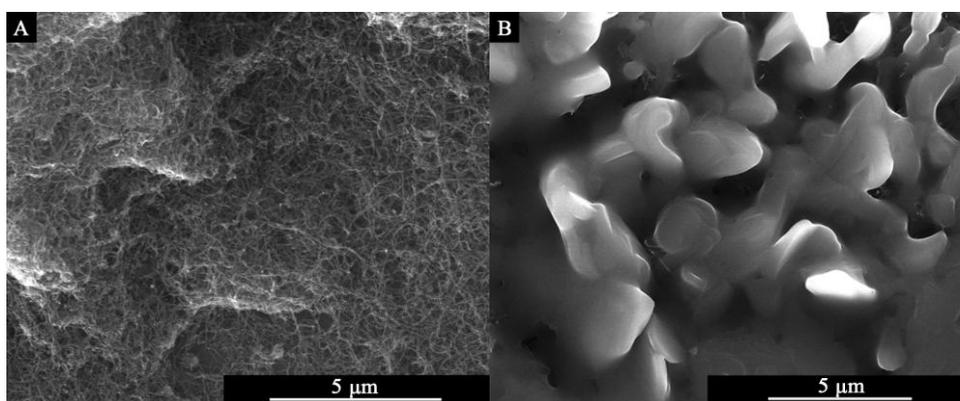


Figure 1. SEM images of the surfaces of the MWNTs film (A) and the MWNTs-[BuPy]PF₆-CS composite film (B).

To obtain information about the morphology of the modified materials, scanning electron microscope (SEM) was performed for the surface of MWNTs film and MWNTs-[BuPy]PF₆-CS

composite film as shown in Fig. 1. It can be seen that MWNTs are distributed irregularly and intertwined with each other for MWNTs film (A), however, the MWNTs-[BuPy]PF₆-CS composite material (B) forms a structure of network and film. which means that the certain interaction force among them changed the structure of the film and the combination of them improved film forming ability of the composite material.

3.2 The influence of scan rate

Fig. 2 A exhibits the influence of scan rate (from 0.04 to 0.45 V·s⁻¹) on the CV peak potential and current of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE. It is found that the oxidation peak potential (E_{pa}) moves to positive direction and the reduction peak potential (E_{pc}) moves toward the negative direction with the increase of scan rate. Both the oxidation peak current (I_{pa}) and reduction peak current (I_{pc}) increase as the increase of scan rate, and showed a good linear relationship with the square root of the scan rate in the range from 40 to 450 mV·s⁻¹ (Fig. 2 B), with the linear regression equation as I_{pa} (μA)= 0.65531 $v^{1/2}$ -3.0579 (r=0.9965) and I_{pc} (μA)= -0.6182 $v^{1/2}$ +2.3638 (r=0.9971), respectively, which means that the electrode reaction process of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE was diffusion-controlled. The maximum available peak signal-to-noise ratio with a good redox peak symmetry for caffeic acid was achieved at the scan rate of 0.08 V·s⁻¹, the scan rate of 0.08 V·s⁻¹ was identified in this study.

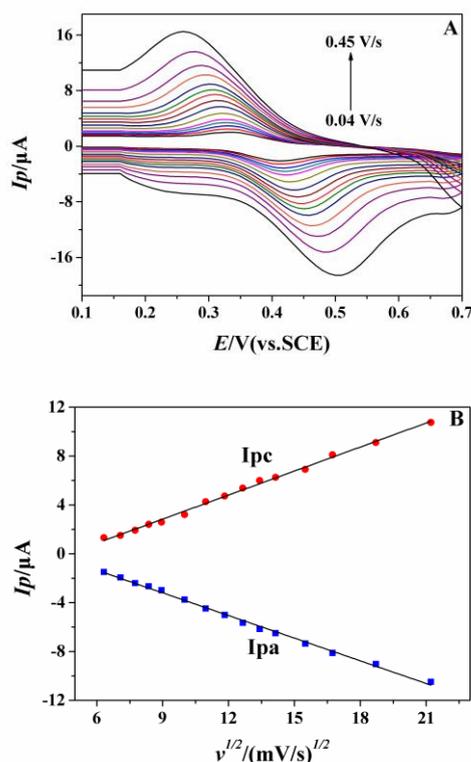


Figure 2. (A) Cyclic voltammograms of caffeic acid at different scan rates (from inner to outer curves: 0.04, 0.05, 0.06, 0.07, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.24, 0.28, 0.35, 0.45 V·s⁻¹). (B) The linear relationship between the peak currents (I_{pa} and I_{pc}) and the square root of the scan rate.

3.3 Influence of pH

The effect of pH (from 2.0 to 6.0) on the CV behavior of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE was tested in 0.04 mol·L⁻¹ BR buffer solution. The redox peak potentials move to the negative direction with the increase of pH as shown in Fig. 3 A, and displayed good linear relationship with the pH from 2.0 to 6.0 (Fig. 3 B), with the linear regression equation as E_{pa} (V)= -0.0503pH+0.6341 (r=0.9960) and E_{pc} (V)= -0.0513pH+0.5310 (r=0.9931), respectively, the slopes which close to 0.059 indicate that the equal number of electrons and protons are involved in the electrochemical reaction of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE.

In addition, the slightest changes in redox peak current are found when pH is in the range of 2.0 to 3.5 and the redox peak currents decrease with the increase of pH in the range from 4.0 to 6.0, however, the maximum redox peak current of caffeic acid is obtained at a pH of 4.0. Therefore, the 0.04 mol·L⁻¹ BR buffer solution (pH 4.0) was selected as supporting electrolyte with a high sensitivity in this study.

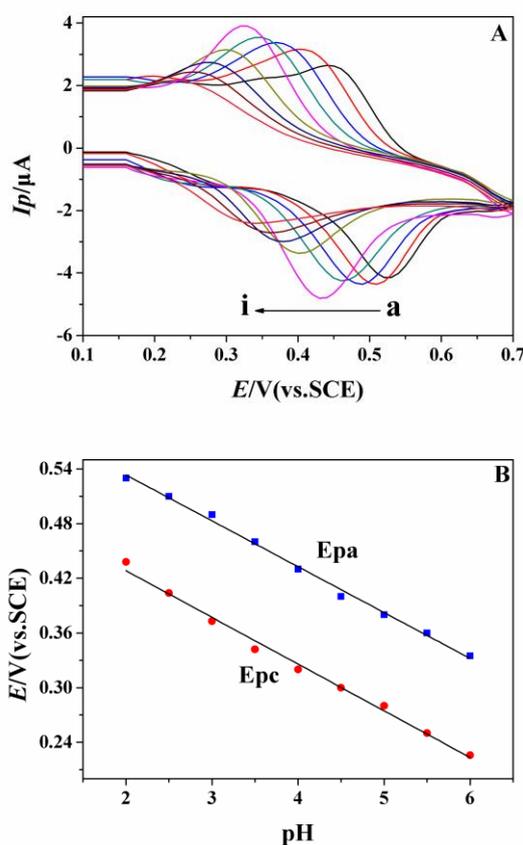


Figure 3. (A) Cyclic voltammetry curves of caffeic acid in 0.04 mol·L⁻¹ BR buffer solution at different pHs: 2.0 (a), 2.5 (b), 3.0 (c), 3.5 (d), 4.0 (e), 4.5 (f), 5.0 (g), 5.5 (h), 6.0 (i). (B) The linear relationship between the peak potentials (E_{pa} and E_{pc}) and pH.

3.4 Cyclic voltammetry behaviors of caffeic acid

The CV behaviors of 1.0×10^{-5} mol·L⁻¹ caffeic acid were investigated at bare GCE (a), MWNTs/GCE (b) and MWNTs-[BuPy]PF₆-CS/GCE (c) in 0.04 mol·L⁻¹ BR buffer solution (pH 4.0),

as shown in Fig. 4. A pair of redox peak was observed at MWNTs/GCE (b) and MWNTs-[BuPy]PF₆-CS/GCE (c), while the oxidation and reduction peak currents of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE is much higher than that at MWNTs/GCE, which means that the MWNTs has a synergistic sensitizing effect, while the introduction of [BuPy]PF₆ and CS can effectively improve its electrochemical performance on the surface of the electrode, and the MWNTs-[BuPy]PF₆-CS composite film exhibited an excellent analytical performance for the determination of caffeic acid.

The oxidation peak potential (E_{pa}) and reduction peak potential (E_{pc}) of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE were 0.43 V and 0.32 V (vs. SCE), respectively, $\Delta E=0.11$ V (vs. SCE). The oxidation peak current and reduction peak current were $I_{pa}=3.34$ μ A, $I_{pc}=2.83$ μ A, respectively, $I_{pa}/I_{pc}=1.18$, indicating that the electrode reaction process of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE is quasi-reversible.

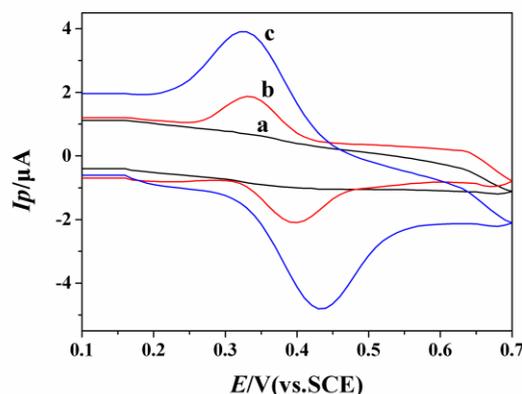


Figure 4. Cyclic voltammograms of 1.0×10^{-5} mol·L⁻¹ caffeic acid at bare GCE(a), MWNTs/GCE (b) and MWNTs-[BuPy]PF₆-CS/GCE (c) in 0.04 mol·L⁻¹ BR buffer solution (pH 4.0).

3.5 Analytical performance of proposed method

Fig. 5 A shows the differential pulse voltammetry (DPV) curves of the different concentrations of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE in 0.04 mol·L⁻¹ BR buffer solution (pH 4.0). The DPV oxidation peak current showed a good linear relationship with the concentration of caffeic acid in the range from 2.5×10^{-8} to 7.0×10^{-6} mol·L⁻¹ (Fig. 5 B), the linear regression equation was $I_{pa}(\mu\text{A}) = 0.001c$ (nM)+1.2304 ($r=0.9962$). According to the approach proposed by IUPAC, the detection limit of caffeic acid can be calculated by equation: $CL=3S_b/S_x$, where, 3 is confidence factor, S_b is blank standard deviation ($n=20$), S_x corresponds to the slope of calibration curve (sensitivity), as the result, the detection limit for caffeic acid can reach 5.7×10^{-9} mol·L⁻¹.

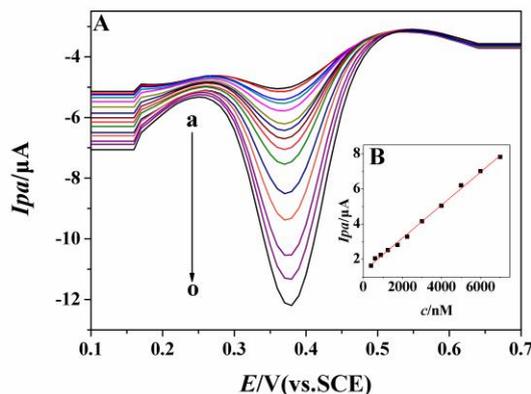


Figure 5. (A) Differential pulse voltammetry curves of different concentrations of caffeic acid at MWNTs-[BuPy]PF₆-CS/GCE: 25 (a), 50 (b), 100 (c), 200 (d), 400 (e), 600 (f), 900 (g), 1250 (h), 1750 (i), 2250 (j), 3000 (k), 4000 (l), 5000 (m), 6000 (n), 7000 (o) nM. (B) The linear relationship between the oxidation peak current and the concentration of caffeic acid.

The analytical performance of the MWNTs-[BuPy]PF₆-CS/GCE for caffeic acid has been compared with that of the other reported electrochemical methods based on electrodes, as shown in table 1. It is indicated that the analytical performance of the proposed method was better with an excellent linear range and a low detection limit than most electrochemical methods.

Table 1. Comparison of the analytical performance for proposed method with other reported electrochemical methods.

Methods	Linear range (M)	LOD (M)	References
PG [a]/GCE[b]	9.0×10^{-6} - 4.0×10^{-5}	3.9×10^{-6}	31
Plant-peroxidase-chitin/CPE [c]	2.0×10^{-5} - 2.0×10^{-4}	2.0×10^{-6}	36
Glassy polymeric carbon	9.7×10^{-7} - 1.1×10^{-5}	2.9×10^{-7}	33
PbFE [d]	1.0×10^{-8} - 5.0×10^{-7}	4.0×10^{-9}	27
AuNPs [e]-chitosan/GE [f]	5.0×10^{-8} - 2.0×10^{-3}	2.5×10^{-8}	34
AuNP[e]/GN[g]/GCE[b]	5.0×10^{-7} - 5.0×10^{-5}	5.0×10^{-8}	28
Nafion/ER-GO [h]/GCE [b]	1.0×10^{-7} - 1.5×10^{-6}	9.1×10^{-8}	37
PEDOT [i]/Pt [j]	1.0×10^{-8} - 6.5×10^{-3}	3.0×10^{-9}	32
MIS [k]/GE [f]	5.0×10^{-7} - 6.0×10^{-5}	1.5×10^{-7}	29
CRGO [l]/GCE [b]	1.0×10^{-8} - 8.0×10^{-4}	2.0×10^{-9}	38
LDHf [m]/GCE [b]	7.0×10^{-6} - 1.8×10^{-4}	2.6×10^{-6}	30
PGE [n]	1.0×10^{-7} - 3.0×10^{-3}	8.8×10^{-8}	35
MWNTs-[BuPy]PF ₆ -CS/GCE	2.5×10^{-8} - 7.0×10^{-6}	5.7×10^{-9}	This work

[a] Poly(glutamic acid); [b] glassy carbon electrode; [c] carbon paste electrode; [d] lead film electrode; [e] colloidal gold nanoparticles; [f] gold electrode; [g] graphene Nanosheets; [h]

electrochemically reduced graphene oxide; [i] poly(3,4-ethylenedioxy) thiophene; [j] platinum electrode; [k] molecularly imprinted siloxanes; [l] chemically reduced graphene oxide; [m] layered double hydroxide film; [n] pencil graphite electrode.

3.6 Application analysis

The method using the MWNTs-[BuPy]PF₆-CS/GCE was employed for the determination of caffeic acid in tablets through standard addition experiment as shown in Table 2. A satisfactory result was obtained with the recovery of 96.9-102.8% and the RSD of 2.2-3.1%, which means that the developed method to measure the caffeic acid in tablets is useful and feasible with a high sensitivity. Moreover, the MWNTs-[BuPy]PF₆-CS/GCE displayed an excellent analytical performance with a high selectivity and strong anti-interference, indicating that it can be used for the determination of caffeic acid in real samples and has the certain practical application value.

Table 2. Determination results of caffeic acid in tablets (n=5).

Samples	Detected (μM)	RSD (%)	Added (μM)	Recovery (%)
1	1.88	3.1	1.0	96.9
2	1.76	2.6	2.0	102.8
3	1.84	2.2	3.0	98.5

4. CONCLUSIONS

A novel electrochemical method with a high sensitivity for the determination of caffeic acid was developed by using a multi-walled carbon nanotubes-N-Butylpyridinium hexafluorophosphate ionic liquid-chitosan composite film modified glassy carbon electrode (MWNTs-[BuPy]PF₆-CS/GCE). The MWNTs-[BuPy]PF₆-CS composite material not only has excellent film forming ability but also can facilitate electron transfer of electrode surface, which effectively improve the surface properties and analytical performances of the electrode. A well-defined electrochemical response signal with a pair of cyclic voltammetric (CV) redox peak of caffeic acid was obtained at MWNTs-[BuPy]PF₆-CS/GCE. The proposed method with differential pulse voltammetry (DPV) exhibited a good linear relationship and high sensitivity, and has the feasibility to detect caffeic acid in tablets. The MWNTs-[BuPy]PF₆-CS/GCE is a development for CNTs-IL-CS modified electrodes and has a potential application value for establishing an electrochemical analysis approach to determine the active ingredients in real samples.

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