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pH-controlled voltammetric behaviors and detection of phytohormone 6-benzylaminopurine using MWCNT/GCE

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ABSTRACT

The electrochemical detection of phytohormones is extremely difficult owing to their poor electrochemical activities, strong electrode passivation (fouling), and complicated electrochemical behaviors and reaction mechanism. In this paper, an interesting electrochemical phenomenon on voltammetric behaviors for the electrochemical oxidation of 6-benzylaminopurine (6-BAP) which was one of the well-known phytohormones under the different pH conditions was investigated, and the content of 6-BAP in real samples was detected by the adsorptive stripping voltammetry using MWCNT/GCE. The pH-controlled voltammetric behaviors like peak potentials, peak currents, peak shapes and peak number of 6-BAP were discussed by the protonation–dissociation equilibria of 6-BAP in combination with electrocatalytic oxidation mechanism of 6-BAP. The electrochemical sensing performance such as the linear range, limit of detection (LOD), limit of quantification (LOQ), sensitivity, selectivity, precision and applicability for the pH-controlled stripping voltammetric detection of 6-BAP was also evaluated. There was an observable difference in the number of peaks for the electrochemical oxidation of 6-BAP under strong acidic (three peaks), near neutral (two peaks), and strong alkaline (one peak) pH conditions. The electrochemical oxidation reaction of 6-BAP was adsorption-controlled irreversible processes, which were not affected by three different pH values. Oxidation peaks with relatively well-defined peak shapes and high peak currents were selected for the adsorptive stripping voltammetric detection of 6-BAP, and peak currents were proportional to 6-BAP concentrations in linear ranges of 0.3–50 μM with LOD of 0.098 μM and LOQ of 0.326 μM at pH 3.0, 0.5–60 μM with LOD of 0.165 μM and LOQ of 0.545 μM at pH 7.0, and 0.7–60 μM with LOD of 0.235 μM and LOQ of 0.776 μM at pH 10.0. The adsorptive stripping voltammetric determination of 6-BAP content with real samples in acid solution was more feasible and applicable.

1. Introduction

It is well-known that phytohormones (plant hormones) play an important role in the development and growth, aging, dormancy, and other physiological functions of plants. If phytohormones are synthesized by plants themselves, they are known as natural phytohormones or endogenous phytohormones, these are natural compounds with physiological active organic substances produced in minute quantities in one part of a plant and exert a physiological effect when transported to another part of the plants [1–3]. Whereas these natural compounds and their functional analogs can be artificially synthesized or extracted from plants, they are usually considered to be phytoregulators (plant growth regulators) or exogenous phytohormones, which are normally regarded as one of important chemical classes of agricultural chemicals and can be employed to modulate the development and growth, aging, dormancy, and other physiological functions of crops at a site. Traditionally, there are five major classical phytohormones which are auxins, cytokinins (CK), absicisic acid, ethylene, and gibberellin [2,4,5].

Phytohormone levels in crops are very low, even ultra-trace, which require a simple, rapid, accurate, and low-cost method for the trace determination of phytohormones. The existing methods such as capillary electrophoresis, chromatography, spectroscopy, enzyme-linked immunosorbent assay, solid and liquid phase microextraction, and their combination have been used for the determination of phytohormones, and these methods displayed

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unique advantages in accuracy and selectivity in mixtures with real samples [6–9]. Nevertheless, these methods had several drawbacks such as complex and time-consuming sample pretreatment, tedious sample purification steps, strong interference resulting from colored substances or impurities present in real samples, low sensitivity, and the high cost of equipment, reagent, and operator training. Owning these disadvantages, they are difficult to meet the increasingly urgent requirements, which include ultratrace, high sensitivity and rapid analysis. In recent years, researchers continually developed new analytical methods and manufactured novel analytical apparatuses to detect phytohormones.

Sensor is a novel, practical, inexpensive, efficient, and sensitive technology, and electrochemical methods have been proved to be a powerful tool due to their advantages of high sensitivity, low cost, rapid response, low reagent consumption, simple operation and convenient apparatus [10–12]. However, there have been relatively few reports regarding phytohormone electrochemical chemosensors owing to no or very low electrochemical activities of phytohormones, no or bad electrocatalytic abilities of most of electrode materials for the oxidation or reduction of phytohormones, complex electrochemical behaviors and dynamics caused by biotic and abiotic factors, and the strong electrode passivation (fouling) resulted from the electrooxidation and electropolymerization of phytohormones. Moreover, carbon materials, especially carbon nanocomposite materials are superior sensing materials for the electrochemical of phytohormones [13–17].

6-Benzylaminopurine (6-BAP), one of the naturally occurring CK, is a kind of well-known phytohormones and plays an important regulatory role in many stages of the development and growth of plants. Because of its significant role in plants, 6-BAP had been artificially synthesized or extracted from plants and was widely used in agriculture and horticulture for plants. In addition, 6-BAP is derived from adenine which can be electrochemically oxidized or reduced under proper conditions [17–19]. Voltammetric methods, especially stripping voltammetric methods were used for the trace determination of 6-BAP [20–22]. In stripping voltammetric analysis, the working electrode which is a variety of mercury electrodes is the core part of voltammetric analysis and the "heart" of detecting system. However, because of toxicity of mercury, it is highly desired to explore other "green" electrode materials. In the stripping voltammetric detection of 6-BAP, different carbon materials such as pencil graphite [23], carbon nanotubes [20], acetylene black-dihexadecyl hydrogen phosphate [21], and ordered mesoporous carbons [17] had been used for the fabrication of modified electrodes. However, to our best knowledge, there is no report on the voltammetric behaviors for the electrochemical oxidation of 6-BAP under the different pH conditions.

In this work, the pH-controlled voltammetric behaviors like peak potentials, peak currents, peak shapes and peak number for the electrochemical oxidation of 6-BAP were discussed by protonation–dissociation equilibria of 6-BAP in combination with electrocatalytic oxidation mechanism of 6-BAP using MWCNT/GCE. The electrochemical sensing performance such as the linear range, limit of detection (LOD), limit of quantification (LOQ), sensitivity, selectivity, precision and applicability for the pH-controlled stripping voltammetric detection of 6-BAP was also evaluated.

2. Experimental

2.1. Chemicals

6-BAP was obtained from J&K Scientific Ltd. 4.0 wt% MWCNT suspension was purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences. 0.1 M phosphate buffer solution (PBS) of different pH values was prepared from a stock solution of 0.1 M H_3PO_4, 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4. The stock solution of 0.05 M 6-BAP were prepared in ethanol and kept in the dark. All used reagents were analytical grade and used without further purification. All other solutions were prepared using doubly distilled water.

2.2. Apparatus

Cyclic voltammetry (CV), Cyclic Voltammetric Stripping (CVS) and linear sweep stripping voltammetry (LSSV) were performed using a CHI660E electrochemical cell workstation (Shanghai Chenhua Instrument Co., Ltd, China) in a conventional electrochemical cell containing a three-electrode system, which are glassy carbon electrode (GCE, 3 mm in diameter), platinum (Pt, 1 mm in diameter) wire and saturated calomel electrode (SCE), respectively. The modified GCE was used as the working electrode, and the Pt wire was served as a counter electrode, as well as SCE acted as a reference electrode. The pH values of PBS were measured with a CT-6023 portable pH meter (Shanghai Precision Instruments Co., Ltd, China).

2.3. Preparation of modified electrode

Before modifying the electrode, the GCE was carefully polished using 0.05 μM alumina on the chamois leather until a mirror-shine surface appeared, and rinsed thoroughly with doubly distilled water between each polishing step, then sonicated with doubly-distilled water, ethanol and doubly-distilled water for 5 min, respectively, and dried in air afterwards. 0.2% MWCNT suspension was obtained by diluting 4.0 wt% MWCNT with doubly distilled water. The MWCNT/GCE was prepared by drop-coating 5 μL MWCNT suspension on the surface of bare GCE, and dried in infrared lamp.

2.4. Electrochemical measurements

All stripping voltammetries were coupled with adsorptive voltammetry for electrochemical measurements of 6-BAP. 5 mL of 0.1 M PBS (pH 3.0, 7.0, 10.0) with a specific amount (50 μM) of 6-BAP solution was added into the electrochemical cell by the pipette. The modified electrode was immersed into a stirring 0.1 M PBS (pH 3.0, 7.0, 10.0) containing the desired concentration of 6-BAP for 20 s. The detection of 6-BAP was using linear sweep adsorptive stripping voltammetry (LSAdSV). All adsorptive stripping voltammetric conditions were as follows: scan rate: 50 mV s^{-1}, equilibration time: 20 s, deposition potential: 0 V, deposition time: 30 s, quite potential: 0 V.

2.5. Determination of 6-BAP in real sample

The tap water was obtained from the drinking water in the campus of Jiangxi Agricultural University. Farmland soil was selected from the experimental field in Jiangxi Agricultural University, and the lettuce and small vegetables were purchased from a local supermarket. The pretreatment of typical samples was as follows: 10 g farmland soil, lettuce, and small vegetable were accurately weighed, respectively. The lettuce and small vegetable were ground finely with a mortar and pestle, and then both the pretreated samples and farmland soil were dissolved in 100 mL doubly distilled water. The mixtures containing the pretreated samples solutions or farmland soil sample were sonicated and centrifuged, and then 500 μL supernatant liquids or tap water sample were transferred and blended with 500 μL 0.01 M 6-BAP standard solutions. Finally, sample solutions were obtained and stored at 4 °C prior to use.