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Determination of oleuropein using multiwalled carbon nanotube modified glassy carbon electrode by adsorptive stripping square wave voltammetry

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ABSTRACT

A multi-walled carbon nanotube modified glassy carbon electrode was used to prepare an electrochemical sensing platform for the determination of oleuropein. Results showed that, the accumulation of oleuropein on the prepared electrode takes place with the adsorption process. Electrochemical behavior of oleuropein was studied by using cyclic voltammetry. Compared to the bare GCE, the oxidation peak current of oleuropein increased about 340 times at MWCNT/GCE. Voltammetric determination of oleuropein on the surface of prepared electrode was studied using square wave voltammetry where the oxidation peak current of oleuropein was measured as an analytical signal. A calibration curve of oleuropein was performed between 0.01 and 0.70 μM and a good linearity was obtained with a correlation coefficient of 0.9984. Detection and quantification limits of the method were obtained as 2.73 and 9.09 nM, respectively. In addition, intra-day and inter-day precision studies indicated that the voltammetric method was sufficiently repeatable. Finally, the proposed electrochemical sensor was successfully applied to the determination of oleuropein in an olive leaf extract. Microwave-assisted extraction of oleuropein had good recovery values between 92% and 98%. The results obtained with the proposed electrochemical sensor were compared with liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

1. Introduction

Oleuropein belongs to a specific group of coumarin-like compounds, the secoiridoids. They are found only in plants belonging to the family of Oleaceae, which includes olive tree (*Olea europaea* L). The secoiridoids are formed from a phenyl ethyl alcohol (hydroxytyrosol and tyrosol), elenolic acid and, eventually, a glucosidic residue [1]. Oleuropein is an ester of hydroxytyrosol and the elenolic acid glucoside (an oleosidic skeleton common to the secoiridoid glucosides of Oleaceae) [2–4].

O. europaea L is one of the most important fruit trees in Mediterranean countries [5]. The leaves of olive tree are rich in olive biophenols and there is a rising interest in natural antioxidants as bioactive components of natural raw materials. Oleuropein has several pharmacological effects including antioxidant, anti-inflammatory, anticancer, antiviral, antimicrobial and anti-atherogenic [6] and olive leaves are wealth and cheap sources of oleuropein. The oleuropein level in olive leaves varies with olive variety, time of collection, climate, etc. [7]. Therefore, the development of simple and effective extraction methods and accurate determination of oleuropein is important.

Different techniques have been used to extract oleuropein from olive leaves. Superheated liquid extraction using aqueous or organic solvents at a high pressure and temperature without reaching the critical point [8], supercritical fluid extraction [9], liquid-liquid extraction [10], derivatized polar extraction [11], fractionation by solid phase extraction [12], dynamic acidified pressurized hot water extraction [13], dynamic ultrasound-assisted extraction [14] and microwave-assisted extraction [15] techniques have all been performed for this purpose.

The determination of oleuropein in olive leaf extract is usually carried out using chromatographic techniques such as high performance liquid chromatography (HPLC) equipped with ultraviolet (UV) and photodiode array detector (DAD) [16–18] and liquid chromatography–mass spectrometry (LC-MS) [19,20]. On the other hand, several electrochemical techniques [21–24] have also been proposed for the determination of oleuropein in olive oil and olive leaf extract. The electrochemical signal was associated with the

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oxidation of catechol group of oleuropein [22]. Electrochemical techniques provide simple and sensitive alternatives in the analysis of bioactive materials. Here an electrochemical sensor is proposed to determine oleuropein in olive leaf extract.

The physical and catalytic properties of carbon nanotubes (CNTs) such as large accessible surface area, low electrical resistance, extremely high mechanical strength and stiffness, outstanding charge-transport characteristics, and high chemical stability make them ideal electrode materials for use in electrochemical sensor applications [25]. CNT-based electrochemical sensors in general exhibit low detection limits and fast response due to the signal enhancement provided by high surface area and rapid electrode kinetics [26,27]. Multi-walled carbon nanotubes (MWCNTs) are hydrophobic [28]. However, acid treatment forms -OH functional groups at the surface and MWCNTs become hydrophilic.

Porous materials with carbon nanotube were prepared to enrich oleuropein from olive leaves extracts and the adsorption behavior of oleuropein on prepared porous materials with carbon nanotube was described in the literature [29]. In this work, a MWCNT modified glassy carbon electrode (GCE) is proposed for the determination of oleuropein by accumulation on the prepared MWCNT/GCE. Quantitative determination of oleuropein was carried out with square wave voltammetric method. The proposed method was applied for the determination of oleuropein in olive leaf extract and the obtained result was compared with those obtained by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The developed electrochemical technique would be a simple, cheap and sensitive alternative to the existing chromatographic methods.

2. Experimental

2.1. Apparatus

Autolab PGSTAT128N voltammetric analyzer with a three electrode system involves a working electrode (bare GCE with a diameter of 3 mm and a geometric area of 0.0707 cm^2), a platinum wire counter electrode and an Ag/AgCl (sat. KCl) reference electrode for voltammetric measurements. Cyclic voltammetry (CV) was used at a scan rate 50 mV s^{-1} between 100 and 1200 mV. For square wave voltammetry (SWV), the parameters were: amplitude 50 mV and frequency 10 Hz, corresponding to an effective scan rate of 50 mV s^{-1} between 100 and 1200 mV.

LC analyses were performed with an Agilent Technologies 1560 Infinity liquid chromatography system hyphenated to a 6420 Triple Quad mass spectrometer. Microwave-assisted extraction of the samples was performed with Cem Mars 6.

2.2. Reagents

All reagents used were of analytical grade. Oleuropein, magnesium sulfate heptahydrate, calcium nitrate tetrahydrate, sodium chloride, acetic acid, perchloric acid, hydrochloric acid, chloroplatinic acid, methanol and acetonitrile were purchased from Sigma-Aldrich. A stock solution of oleuropein at 500 mg L^{-1} was prepared by dissolving 0.005 g solid in 10 mL methanol. Standard solutions of oleuropein were prepared using appropriate dilutions of the stock solution with methanol. L(+)-ascorbic acid, iron(II) sulfate heptahydrate, nickel(II) sulfate hexahydrate, lead(II) nitrate, copper(II) nitrate trihydrate, sodium hydroxide, nitric acid, sulfuric acid, trifluoroacetic acid and N,N-dimethylformamide were obtained from Merck. Multi-walled carbon nanotubes (MWCNTs) were purchased from Aldrich (purity > 95%, diameter 7–15 nm, length 10 μm) and taurine was obtained from Alfa Aesar. 1.0 mM $HAuCl_4$ was prepared by dissolving Au wire (99.999% in purity) in 1:3 nitric acid: hydrochloric acid. Ammonium acetate and o-phosphoric acid were obtained from J.T. Baker and D(+) glucose monohydrate was purchased from Carlo Erba.

2.3. LC-MS/MS method

A Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 μm) column was used for the separation of oleuropein. The mobile phase was made up from solvent A (5 mM ammonium acetate solution) and solvent B (acetonitrile containing 0.1% acetic acid). The gradient profile was set as follows: 0.00 min 5% B eluent, 1.00 min 25% B eluent, 2.00 min 50% B eluent, 4.00 min 95% B eluent, 6.00 min 95% B eluent and 7.00 min 5% B eluent. The column temperature was maintained at 25 °C. The flow rate was 0.4 mL min^{-1} and the injection volume was 5.0 μL.

The tandem mass spectrometer was interfaced to the LC system via an electrospray ionization (ESI) source. The electrospray source of the MS was operated in negative mode and the interface conditions were as follows: capillary voltage of −3.5 kV, gas temperature of 300 °C and gas flow of 11 L min^{-1}. The nebulizer pressure was 40 psi.

Infusion of the oleuropein standard was performed in negative scan mode to investigate the $[M-H]^-$ ion of oleuropein (m/z 539.2 amu). Product ion scan mode was used to determine the most abundant product ions, for oleuropein. Among the products ions the most abundant were the ion at m/z 275.1 amu and m/z 377.1 amu for oleuropein, respectively. Consequently, the transition m/z 539.2 > 275.1 amu was chosen for the multiple reaction monitoring (MRM) analytical mode while the transition m/z 539.2 > 377.1 amu was selected qualitatively control of oleuropein. The retention time was 5.328 min

2.4. Pre-treatment of MWCNT and preparation of MWCNT/GCE, poly (taurine)/MWCNT/GCE, AuNPs/MWCNT/GCE and PtNPs/MWCNT/GCE

40 mg MWCNT was boiled in an adequate amount of HNO_3 and then acid-treated MWCNT was rinsed with ultrapure water for multiple times [30]. Then a suspension was obtained by dispersing 37 mg of purified MWCNT in 3.7 mL dimethylformamide (DMF). GCE was cleaned by polishing on a synthetic cloth with Al_2O_3 slurry, followed by ultrasonication of the electrode for 5 min in pure water. Finally, for MWCNT/GCE, 10 μL of the suspension were deposited on the GCE surface and dried under a 150 W infrared lamp for 20 min

Electropolymerization of taurine monomers (1.0 mM) on the MWCNT/GCE was carried out in the potential range of −1.5 V to 2.0 V at 50 mV s^{-1} for 20 cycles [31]. The formation of Au and Pt nanoparticles on the MWCNT/GCE were provided by cyclic voltammetry at a scan rate of 100 mV s^{-1} for 20 cycles with scanning in the potential range of 1.0 V to −1.5 V in 0.1 M HCl solution containing 1 mM $HAuCl_4$ and 0.2 V to −1.0 V in 0.1 M HCl solution containing 1 mM H_2PtCl_6, respectively.

2.5. Microwave-assisted extraction (MAE) of oleuropein

Microwave-assisted extraction was proposed for the first time in order to accelerate the extraction of biophenols from olive leaves by Japón-Luján et al. [15]. A modified form of this technique was used in the present study. Olive leaf sample of 10 mg was transferred into the microwave extraction vessel and suspended in 10 mL of a mixture of methanol and water (80:20, v:v). The temperature of the system was raised to 80 °C in approximately 8 min, and it remained at 80 °C for 6 min. The extraction vessels were allowed to cool for 30 min at room temperature after extraction. The extracts were filtered through a 0.45 μm syringe filter prior to analysis.