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Analytical Methods

Voltammetric method using a lead film electrode for the determination of caffeic acid in a plant material

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ABSTRACT

This paper deals with development of an adsorptive stripping voltammetric method for determination of caffeic acid (CA) at a lead film electrode (PbFE). The working electrode was prepared in situ on a glassy carbon substrate as a "mercury-free" electrochemical sensor. The method is based on the accumulation by adsorption of caffeic acid on PbFE and then the oxidation of CA during the stripping step. In a acetate buffer based supporting medium the oxidation signal for caffeic acid was found to be proportional to the CA concentration in the range from 1×10^{-8} to 5×10^{-7} mol/L with the limit of detection equal to 4×10^{-9} mol/L (with preconcentration for 30 s). The method, operated in the square-wave voltammetric mode, was successfully applied to the determination of CA in a plant material (herbs of *Plantago lanceolata*). The content of caffeic acid received by the proposed method was in close agreement with that obtained by high performance thin-layer chromatography (HPTLC) combined with densitometry. This appears to be the first application of a lead film electrode to the determination of an organic compound in such complicated matrix.

1. Introduction

Some naturally occurring phenolic acids and analogues, namely caffeic and gallic acids, are known to exhibit a wide variety of physiological and pharmacological properties. These compounds demonstrate antioxidant activity and are able to prevent auto-oxidation via the radical formation inhibition (Alanko et al., 1999). Because of the importance of antioxidant activities of these compounds and potential health benefits in human dietary (consumption of fruits, vegetables, coffees and spices) associated with coronary heart diseases and cancers, interest in this field has increased (Da Silva, Stradiotto, & Oliveira, 2008; Nardini & Ghiselli, 2004; Riahi, Ganjali, Khajehsharifi, Norouzi, & Taghipoor, 2009; Robbins, 2003). Amongst cinnamic acid derivatives is one of the most investigated not only because of its protective antioxidant behaviour but also due to its effectiveness against immunoregulation diseases, asthma and allergic reactions.

Caffeic acid (CA) is a kind of polyphenol that is widely distributed in higher plants as glycosides, esters and the free form. CA (3,4-dihydroxycinnamic acid) esters display selective antiproliferative activity against some types of cancer cell (Nagaoka, Banskota, Tezuka, Saiki, & Kadota, 2002). Furthermore, caffeic acid phenethyl ester, which is a component of propolis, a honeybee hive product, has shown anticarcinogenic and immunomodulatory properties (Papathoma, Zoumpourlis, Balmain, & Pintzas, 2001). Many biological activities have been reported for free caffeic acid. In bioassay experiments, it inhibited the growth of plants (Baghestani, Lemieux, Leroux, Baziramakenga, & Simard, 1999) fungi (Bostock, Wilcox, Wang, & Adaskaveg, 1999) bacteria (Reinders, Biesterveld, & Bijker, 2001) and insects (Summers & Felton, 1994). Caffeic acid is one of many phenolics considered to be an important part of the general plant defence mechanism against infection predation (Faulds & Williamson, 1999). Additionally caffeic acid derivatives exhibited potential activity against HIV integrase and can inhibit HIV replication with moderate anti-HIV activity in cell culture (Robinson, Reinecke, Abdel-Malek, Jia, & Chow, 1996).

Several analytical methods including liquid and gas chromatography (Robbins, 2003; Romani et al., 2000) and capillary electrophoresis (Peng, Liu, & Ye, 2005) have been employed for the determination of phenolic acids in food samples and plant materials. Concerning the electrochemical measurements, most of them are focused on the study of the mechanism of caffeic acid oxidation (Makhotkina & Kilmartin, 2009; Sousa, da Rocha, Cardoso, Silva, & Zanoni, 2004; Zeng, Liu, Zeng, & Zhong, 2007). In the literature just a few studies, in which electrochemical techniques are used for a quantitative analysis of caffeic acid in different matrices, are proposed (Da Silva et al., 2008; Nardini & Ghiselli, 2004; Sousa et al., 2004). For example, in article (Sousa et al., 2004) the determinations

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of the relative contribution of phenolic antioxidants in orange juice by voltammetric methods were presented. The obtained calibration graph for caffeic acid was linear from 1×10^{-4} to 1×10^{-3} mol/L.

For the reasons given above, studies involving the development of analytical methods for detection and measurements of caffeic acid and its derivatives from plant sources, human fluids and several beverage products should be elaborated. So, the aim of this study was to develop and optimise a sensitive, rapid, inexpensive and accurate adsorptive stripping voltammetric method (AdSV) for the determination of caffeic acid in plant materials. The proposed method is based on CA adsorption on the lead film electrode in the accumulation step and then the oxidation of CA during the stripping step.

The voltammetric method proposed in this paper was successfully applied to the determination of CA in the presence of other components in herbs of *Plantago lanceolata*. The received results were compared to those obtained by high performance thin-layer chromatography (HPTLC) combined with densitometry. The HPTLC method was previously applied by co-authors for separation and quantitative analysis of triterpenoids in the leaves of *Jovibarba sobolifera* (Szewczyk, Komsta, & Skalska-Kamińska, 2009) and synthetic drugs (Wójciak-Kosior, Skalska, & Matysik, 2006a; Wójciak-Kosior, Skalska, Matysik, & Kryska, 2006b).

2. Experimental

2.1. Apparatus

All voltammetric measurements were performed using a μAutolab analyser made by Eco Chemie, the Netherlands. A classical three-electrode quartz cell of volume 10 mL was used. A glassy carbon electrode (GCE) of diameter 1 mm was polished daily using 0.3 μm alumina slurry on a Buehler polishing pad. Pt wire and Ag/AgCl were used as auxiliary and reference electrodes, respectively. The pH measurements were made on an Elmetron pH meter CI-316 equipped with a combined glass pH-sensor (Metron, Czekanów, Poland). The measuring cell was calibrated with buffer solutions of the conventional activity scale.

Chromatography experiments were performed on 200×100 mm glass plates percolated with a 0.25 mm layer of silica-HPTLC Kieselgel Si 60 with a fluorescent indicator (Merck, Darmstad, Germany). For application standards and sample on plates an AS 30 automatic applicator (Desaga, Heidelberg, Germany) was used. The plates were developed "face down" in horizontal Teflon DS chambers (Chromdes, Lublin, Poland). Zone area measurements were performed at 320 nm in the absorbance mode using a Desaga CD 60 densitometer controlled with a Pentium computer.

For sample preparation exhaustive extraction in an ultrasonic bath Sonorex Type RK 102 HB (Bandelin, Berlin, Germany) was conducted. Extracts were evaporated to dryness in a rotary evaporator HB Basic RV 05-ST (IKA, Łódź, Poland) under reduced pressure. In the acid and alkaline hydrolysis process water solutions were heated in a water bath EkoTerm TW12 (Julabo, Sellbach, Germany).

2.2. Reagents

An acetate buffer, used as a supporting electrolyte for the proposed voltammetric method, was prepared from CH_3COOH and NaOH obtained from Merck. The standard of CA was obtained from Fluka (purity equal 99%). A stock standard solution of CA was prepared by a dissolving 0.01 g reagent in 10 mL of methanol and stored at 4 °C in the dark until used. The working solutions were prepared by appropriate dilution of a stock standard solution in methanol. All solvents used in the chromatographic study and sample preparation experiments were the pro analysis grade from Polish Reagents (POCh, Gliwice, Poland).

2.3. Procedure of the voltammetric measurements at a lead film electrode

In the optimised conditions of measurements an acetate buffer of pH = 4.0 (0.05 mol/L $CH_3COONa + CH_3COOH$) was used as a supporting electrolyte. The concentration of $Pb(NO_3)_2$ added to the electrolyte was 1×10^{-5} mol/L. The electrode was cleaned from the lead remaining after the preceding measurement by a 10-time repeated cycle of the potential from −0.55 to 0.5 V with the scan rate of 0.1 V/s. The electrochemical activation was followed by the deposition of the lead layer at −1.3 V for 7 s, and by the adsorption of caffeic acid on the electrode at −0.65 V for 30 s. During all three steps the solution was stirred using a magnetic stirring bar. After a rest period of 5 s the square-wave voltammogram was recorded at a frequency of 100 Hz, while the potential was scanned from −0.65 to 1.5 V. The other experimental parameters were as follows: step potential 2 mV, amplitude 25 mV. The measurements were carried out in undeaerated solutions. The oxidation peak current of lead appearing at −0.5 V was much larger than the oxidation peak current of CA appearing at +0.4 V, so the recorded voltammograms were cut in the potential range from 0.1 to 1.1 V.

2.4. Procedure of the chromatographic measurements

In the chromatographic experiments glass plates were washed before use with methanol and acetone and dried for five minutes in 105 °C for activation. Standards, in the concentrations range of caffeic acid from 1.1×10^{-3} to 5.5×10^{-3} mol/L, and sample (3 μL) were spotted under nitrogen at 2533 hPa for 5 s as 6 mm streaks. The application of five concentrations of standards and one sample was repeated three times on one plate. During the experiments three plates were developed "face down" in horizontal Teflon DS chambers. Chromatograms were obtained by the multiple development method in a three-step elution programme. The optimal compositions of eluents were determined experimentally. Before development the plates were conditioned for 15 min above the mobile phase from the first step of development containing hexane, diizoprophyl ether, and formic acid (90%) (6.0:4.0:0.5) v/v. In the second and third steps a mixture of hexane, diizoprophyl ether, dichlorometan, formic acid (90%), propan-2-ol (6.0:4.0:2.0:1.0:0.1) v/v was used. Then, the plates were dried at room temperature for approximately 20 min in a stream of air after each step of development. Zone area measurements were performed at 320 nm in the absorbance mode.

2.5. Plant material and sample preparation

The plant material analysed was herbs of *Plantago lanceolata* available in trade (Herbapol, Lublin, Poland). The way of extraction of phenolic acids from the plant material and hydrolysis of the glycosides and esters to free phenolic acids was based on literature data (Häkkien, 2000; Krygier, Sosulski, & Hogge, 1982; Robbins, 2003). For sample preparation exhaustive extraction in an ultrasonic bath was conducted. 20 g of dried and pulverised plant material was extracted three times at 55 °C: 45 min with 200 mL of methanol, 30 min with a new portion of the extractant and 30 min with 80% solution of methanol in water. Extracts were put together and evaporated to dryness in a rotary evaporator under reduced pressure. Dry residue was washed with portions of hot distilled water (about 100 mL in total) put at 4 °C for 24 h. Then the solution was filtrated with the use of a paper filter, grade 390 (84 g/m^2). The filtrate was defatted by shaking out twice in a separator with petroleum ether (30 mL each time). Then, the water