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# Voltammetric Sensors for the Simultaneous Quantification of Natural Phenolics in Coffee and Citrus Juices †

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**Abstract:** Phenolic antioxidants of various groups are important nutrients in human diet providing positive health effects. Nevertheless, these effects are dose-dependent that requires the control of natural phenolics contents in their sources. Coffee and citrus juices containing significant amounts of hydroxycinnamic acids and flavanones, respectively, are among most widely consumed beverages all over the world. Electroactivity of phenolics allows application of voltammetric sensors for quantification purposes. Highly sensitive and selective voltammetric sensors for the simultaneous quantification of hydroxycinnamic (caffeic (CA), ferulic (FA), and *p*-coumaric(*p*-CA)) acids and flavanones (hesperidin and naringin) has been developed for the first time using glassy carbon electrodes modified with single-walled carbon nanotubes functionalized by polyaminobenzene sulfonic acid (f-SWCNTs) and polymeric coverages from triphenylmethane dyes (phenol red (PR) or aluminon). Polymeric layers have been obtained in potentiodynamic mode. Conditions of dye's electropolymerization have been optimized using voltammetric response of hydroxycinnamic acids or flavanone mixtures. Three separated oxidation peaks of CA, FA, and *p*-CA at the electrode with polyPR as well as hesperidin and naringin at the polyaluaminon-modified electrode have been observed. The oxidation currents are significantly increased vs. bare glassy carbon (GCE) and carbon nanotube-modified electrodes. Both sensors provide highly sensitive response to target analytes in differential pulse voltammetric mode. Other natural phenolics of various classes do not affect the response of the sensors developed to the target analyte. Quantification of hydroxycinnamic acids in coffee and flavanones in orange and grapefruit juices has been successfully realized.

**Keywords:** food analysis; natural phenolics; electrochemical sensors; electropolymerization; triphenylmethane dyes; simultaneous quantification

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## 1. Introduction

Phenolic antioxidants of various groups are important nutrients in human diet providing positive health effects [1]. Nevertheless, these effects are dose-dependent that requires the control of natural phenolics contents in their sources [2]. Coffee and citrus juices containing significant amounts of hydroxycinnamic acids [3] and flavanones [4], respectively, are among most widely consumed beverages all over the world.

Electroactivity of phenolics allows application of voltammetric sensors for quantification purposes. The possibility of simultaneous determination of structurally related phenolic of the same class is one of the most important problems in modern electroanalysis that can be partially solved by using sensors based on the chemically modified electrodes [5]. Hydroxycinnamic acids and flavanones are less studied analytes from this point of view. There are several voltammetric sensors for the simultaneous determination of hydroxycinnamic acids (Table 1), while flavanones are out of consideration. Among various types of electrode surface modifiers, electropolymerized coatings based on the compounds containing phenol fragments in the structure are of interest as far as have been shown as an effective sensing layer for the natural phenolics [9].

**Table 1.** Voltammetric sensors for the simultaneous quantification of hydroxycinnamic acids.

| Sensor | Method | Hudroxycinnamic acid | Detection limit (μM) | Linear Dynamic Range (μM) | Ref. |
|---|---|---|---|---|---|
| Carbon nanofiber-based screen-printed electrode | CV * | CA | 0.239 | 10–1000 | 6 |
| | | FA | 0.233 | 10–1000 | |
| Poly(Sunset Yellow)/Multi-walled carbon nanotubes/GCE | DPV ** | Chlorogenic acid | 0.076 | 0.10–4.0 | 7 |
| | | FA | 0.098 | 0.5–4.0 | |
| $WS_2$ flakes decorated with catechin-capped gold nanoparticles and carbon black/screen-printed electrode | DPV | CA | 0.09 | 0.4–112.5 | 8 |
| | | Sinapic acid | 0.36 | 0.7–125.0 | |
| | | *p*-CA | 0.39 | 1.4–93.7 | |

* Cyclic voltammetry. ** Differential pulse voltammetry.

Novel voltammetric sensors with high sensitivity and selectivity have been developed using f-SWCNTs and poly(triphenylmethane dyes) as sensing layer for the simultaneous quantification of hydroxycinnamic acids (CA, FA, and *p*-CA) and flavanones (hesperidin and naringin) in coffee and citrus juices.

## 2. Materials and Methods

Hydroxycinnamic acids (98% CA, 98% *p*-CA from Sigma (Steinheim, Germany), 99% FA from Aldrich (Steinheim, Germany)) and flavanones (94% hesperidin and 95% naringin from Sigma-Aldrich (Germany)) were used as analytical standards. Stock solutions of analytes (10 mmol $L^{-1}$ (0.40 mmol $L^{-1}$ for hesperidin)) were prepared in ethanol (rectificate) for hydroxycinnamic acids and in methanol (c.p.) for flavanones. The exact dilution was used to obtained a less concentrated solution prior to the measurements.

Homogeneous 1.0 mg $mL^{-1}$ suspension of polyaminobenzene sulfonic acid functionalized single-walled carbon nanotubes (f-SWCNTs) (ø × *l* was 1.1 nm × 0.5–1.0 µm) from Sigma-Aldrich (Steinheim, Germany) was prepared in dimethylformamide using 30 min sonication in the ultrasonic bath (WiseClean WUC-A03H) (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea).

Standard 10 mM solutions of monomers were prepared in methanol for aluminon (Sigma-Aldrich, Steinheim, Germany) and in ethanol for the phenol red (PR) (Sigma-Aldrich (St. Louis, MO, USA).

Potentiostat/galvanostat Autolab PGSTAT 302N with the FRA 32M module (Metrohm B.V., Utrecht, The Netherlands) and NOVA 1.10.1.9 software was used. All measurements were carried out in the 10 mL glassy electrochemical cell. The working bare GCE (ø 3 mm from CH Instruments, Inc., Bee Cave, TX, USA) or a modified electrode and a counter platinum electrode were used. The potentials were measured vs. Ag/AgCl reference electrode.

"Expert-001" pH meter (Econix-Expert Ltd., Moscow, Russia) supplied with the glassy electrode was applied.

## 3. Results and Discussion

### 3.1. Effect of Electropolymerization Conditions on the Voltammetric Response of Target Analyte Mixtures at the Modified Electrode

The insulating nature of electropolymerized triphenylmethane dyes requires increase in the electrode conductivity that is successfully provided by carbon nanomaterials used a support for further polymeric coverage deposition. f-SWCNTs were drop casted at the GCE surface (2.0 µL) and evaporation of the solvent to dryness at the ambient conditions were performed. Then, electropolymerization of aluminon or PR was done in potentiodynamic mode. Conditions of dye's electropolymerization have been optimized using voltammetric response of hydroxycinnamic acids or flavanone mixtures and summarized in Table 2.

**Table 2.** Triphenylmethane dyes electropolymerization conditions providing the best response of natural phenolics mixture.

| Electropolymerization Conditions | Monomer | |
|---|---|---|
| | Aluminon | PR |
| Supporting electrolyte | 0.1 M NaOH | 0.1 M NaOH |
| pH | 13 | 13 |
| Monomer concentration (μM) | 100 | 75 |
| Number of cycles | 10 | 10 |
| Electrochemical window (mV) | +100 to +800 | −300 to +1200 |
| Potential scan rate (mV $s^{-1}$) | 100 | 75 |

Clear separated oxidation peaks of CA, FA, and *p*-CA at the electrode with polyPR electrode in Britton-Robinson buffer (BRB) at pH 2.0 as well as hesperidin and naringin at the polyaluaminon-modified electrode in phosphate buffer (PB) at pH 7.0 have been observed under conditions of cyclic voltammetry. The oxidation currents are significantly increased indicating high sensitivity of the electrode towards hydroxycinnamic acids and flavanones (Table 3). Only first oxidation peak parameters are presented for the hesperidin and naringin.

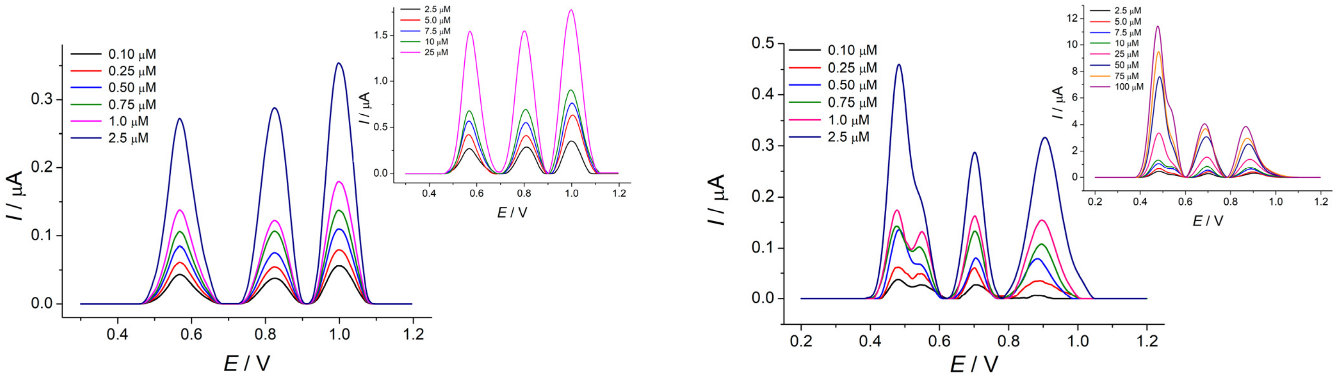
**Table 3.** Voltammetric characteristics of hydroxycinnamic acids and flavanones at the polymer-based electrodes ($n = 5$; $P = 0.95$).

| Sensor | Analyte | $E_{ox}$ (mV) | $I_{ox}$ (μA) |
|---|---|---|---|
| GCE | Hesperidin | 544 | 0.025 ± 0.001 |
| | Naringin | 745 | 0.0070 ± 0.0003 |
| f-SWCNTs/GCE | Hesperidin | 505 | 0.123 ± 0.005 |
| | Naringin | 705 | 0.104±0.003 |
| Polyaluminon/f-SWCNTs/GCE | Hesperidin | 498 | 0.25 ± 0.01 |
| | Naringin | 705 | 0.19 ± 0.01 |
| GCE | CA | 530 | 0.150 ± 0.008 |
| | FA | 760 | 0.110 ± 0.006 |
| | *p*-CA | 940 | 0.080 ± 0.004 |
| f-SWCNTs/GCE | CA | 523 | 0.200 ± 0.007 |
| | FA | 753 | 0.180 ± 0.005 |
| | *p*-CA | 954 | 0.110 ± 0.003 |
| PolyPR/f-SWCNTs/GCE | CA | 524 | 0.61 ± 0.02 |
| | FA | 715 | 0.54 ± 0.01 |
| | *p*-CA | 906 | 0.53 ± 0.01 |

### *3.2. Quantification of Analytes in Differential Pulse Mode*

Both sensors provided highly sensitive response to target analytes in differential pulse voltammetric mode (Figure 1). Taking into account the shape of voltammograms for the mixture of hydroxycinnamic acids, the peak area was used for calibration plot fitting. The analytical ranges of 0.10–2.5 μmol $L^{-1}$ and 2.5–100 μmol $L^{-1}$ for CA, 0.10–2.5 μmol $L^{-1}$ and 2.5–50 μmol $L^{-1}$ for both FA and *p*-CA s using polyPR-modified electrode were obtained. The detection limits of 48, 22, and 38 nmol $L^{-1}$ were achieved for the CA, FA, and *p*-CA, respectively. Polyaluminon-based sensor allowed determination of 0.10–2.5 and 2.5–25 μmol $L^{-1}$ of both flavanones. The detection limits were calculated as 29 and 20 nmol

$L^{-1}$, respectively. The analytical characteristics obtained using polymer-based sensors are meaningful and improved compared to those presented in Table 1.

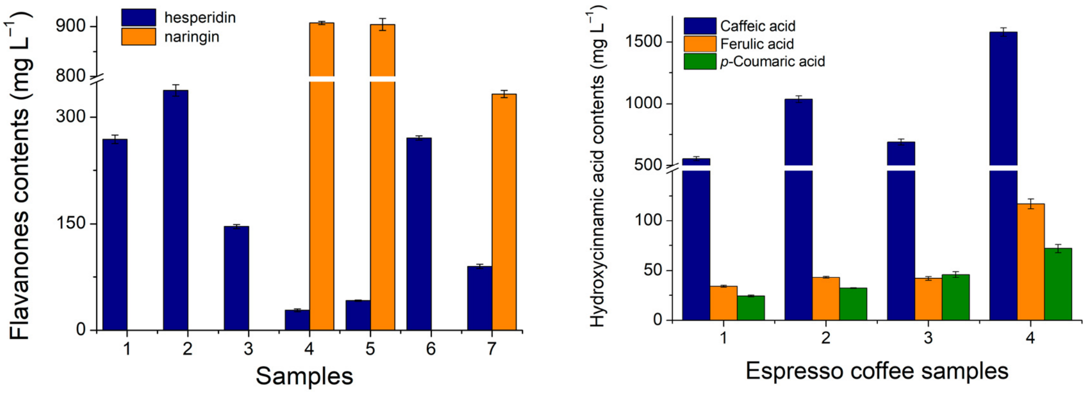


(**a**) (**b**)

**Figure 1.** Baseline-corrected response of polymer-based sensors to the natural phenolics in differential pulse mode: (**a**) flavanons equimolar mixtures at the polyaluminon/f-SWCNT/GCE in PB at pH 5.0. $\Delta E_{pulse}$ = 0.100 V, $t_{pulse}$ =0.025 s, and $\upsilon$ = 0.010 V $s^{-1}$; (**b**) equimolar mixtures of CA, FA, and *p*-CA at the polyPR/f-SWCNT/GCE in BRB at pH 2.0. $\Delta E_{pulse}$ = 0.050 V, $t_{pulse}$ =0.025 s, and $\upsilon$ = 0.010 V $s^{-1}$.

Selectivity in the presence of other natural phenolics has been achieved. A 10-fold excesses of gallic, caffeic, and chlorogenic acids, 5-fold excess of tannic acid, 1.0 μM catechin, quercetin, and rutin, <1.0 μM of FA, sinapic acid and *p*-CA did not affect determination of hesperidin and naringin at polyaluminon-based sensor. PolyPR-based sensor is less selective toward hydroxycinnamic acids in the presence of other phenolics. Chlorogenic acid is oxidized at the same potential as CA, therefore, total contents of these acids are determined in the case of real samples containing both compounds.

Quantification of hydroxycinnamic acids in espresso coffee and flavanones in orange and grapefruit juices has been successfully realized. Recovery values are close to 100% indicating the absence of matrix effects and practical applicability of the sensors developed. The data obtained are presented in Figure 2.



(**a**) (**b**)

**Figure 2.** Determination of natural phenolics in beverages: (**a**) flavanones contents in orange (samples 1–3 (commercial) and 6 (fresh)) and grapefruit juices (samples 4, 5 (commercial)and 7 (fresh)) using polyaluminon/f-SWCNT/GCE; (**b**) hydroxycinnamic acid contents in espresso coffee using polyPR/f-SWCNT/GCE.

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