

An Efficient Electrochemical Sensing of Caffeic Acid at Thermolysis Prepared Urea-formaldehyde Resin Modified with Fe(III) and Ti(IV) Oxide Particles

Branka B. Petković^{1,*}, Marija Kostić², Suzana Samaržija-Jovanović¹, Aleksandra Ivanović³,
Bojana Laban¹, Djordje Veljović⁴, Dalibor M. Stanković⁵

¹ Faculty of Sciences and Mathematics, University of Priština-Kosovska Mitrovica, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia,

² Faculty of Technology Novi Sad, University of Novi Sad, cara Lazara 1, 21000 Novi Sad, Serbia,

³ Mining and Metallurgy Institute Bor, Zeleni bulevar 35, 19210 Bor, Serbia,

⁴ University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia,

⁵ University of Belgrade, Faculty of Chemistry, Studentski trg 12-16, 11000 Beograd, Serbia,

*E-mail: branka.petkovic@pr.ac.rs

Received: 8 September 2022 / Accepted: 31 October 2022 / Published: 17 November 2022

Urea-formaldehyde resin (UF) is primarily used as an adhesive applied in the wood composites industry. In this work, we found that this resin modified with metal compounds could be a good starting point to produce high-performance electrode material applied for the construction of electrochemical sensor for important antioxidant, such as caffeic acids (CA). The conductivity of carbon paste electrodes (CPE) modified by materials produced by thermolysis of *in situ* synthesized urea formaldehyde resins with Fe(III) and Ti(IV) compounds (mixed metal compounds and individually) were examined. For comparison, pure CPE and CPE modified with material produced from the physical mixture of UF resins and metal compounds in the same combinations were also electrochemically characterized by impedance spectroscopy and cyclic voltammetry. The most conductive material, which consists of a combination of metal oxide particles *in situ* synthesized with UF (SynFe+Ti/UF-TP@CPE), was used as a CPE modifier and proved itself in sensitive differential pulse voltammetric determination of caffeic acid at a wide working range of 0.5-100 μM and limit of detection of 0.046 μM . The proposed electrode was successfully applied for the determination of the content of CA in spiked urine samples and the estimation of total phenol content, based on CA equivalents, of honey, liqueur and juice samples.

Keywords: voltammetric sensor, caffeic acid, carbon paste electrode, urea-formaldehyde resin, metal oxide particles, antioxidant capacity

1. INTRODUCTION

Until now, urea-formaldehyde resin (UF) has been the best and the most used adhesive applied in the production of wood composites industry due to its good performance and low cost [1, 2]. The main disadvantage of this aminoplast applied on wood panels and boards are formaldehyde (FA) subsequent emission which could be significantly reduced by applying UF resins modification with FA scavengers like different organic compounds, natural and synthetic polymers, clays, metal (nano)particles etc [3]. In that manner, a lot of research was done in our laboratory to design low molar ratio UF resins modified with different fillers and procedures in order to improve the thermal, hydrolytic and radiation stability of produced polymer composites [4-9].

By exploring the other general applications of UF resin, the uses of this aminoplast can find in textile, papers, cotton blends, artificial snow and even coating for electrical devices. As far as we know, there is no literature data about the usage of UF resins in any way in constructing electrochemical sensors before.

The electrochemical sensors are modern, low-cost, replaceable and miniature analytical devices that can compete with expensive apparatus and giant complex analytical systems in many ways [10]. They can recognize chemical species of interest at electrodes made from metal, carbon material or electrode surfaces modified by various and new materials in order to improve sensitivity and selectivity of determination [11]. Thermal degradation of selected polymer resins, such as phenol-formaldehyde resin on special conditions resulted in the production of glassy carbon, one of the best electrode materials [12]. On the other hand, metal micro- and nanoparticles and their oxides were used as efficient electrode modifiers. Iron and iron oxide particles possess specific physical, especially magnetic and chemical properties. Because of that, they are widely applied in many research fields, and one of them is sensors in electroanalysis [13]. Among many reasons for the application of TiO₂ nanoparticles in electrochemical sensors and biosensors, the efficient electronic charge properties and good electronic connectivity can stand out [14].

The goal of this work was to find other possible and effective applications for material produced by thermal degradation of modified UF composite obtained in our laboratory. Our intention was to produce high-conductive and good-performance electrode modifiers by thermolysis of UF composite modified by iron and titan compounds. The composites were produced by: a) physical mixing of synthesized UF resins, first with an iron(III) salt and then with a combination of iron(III) salt and Ti(IV) oxide for unifying their properties; b) by *in situ* synthesis metal compounds and UF resins, using the same combinations as it was done in case of the physical mixing. Then, the materials for modification of the carbon paste electrode were obtained by thermolysis of synthesized modified UF composites. All prepared modified UF composites and materials obtained by calcification were characterized by SEM and electrochemical techniques. The best performance of thermal decomposed material, prepared from UF resin modified by *in situ* synthesis with both metal compounds, was used for electrochemical sensing of caffeic acid, one of the most important polyphenols, which can be used as a standard for determining the total phenol content (TPC) and antioxidative capacity (AC) of various analytes [15-18]. As the spectral methods for estimation of TPC and are interfered by the background color of juices and many actual samples [19], the electrochemical methods should provide

an efficient, simple, fast and sensitive procedure for determinations for this purpose. Caffeic acid could absorb in the human body from food and supplements and excretes by urine [20, 21]. The possibility of urine analysis on CA content by the proposed electrochemical sensor was also the task of this research. With all this in mind, the thermolysis prepared material from modified UF resin is intended to demonstrate its potential in real sample determinations, such as electrochemical CA sensing in human excretions, but also in the study of food and natural product antioxidant properties.

2. EXPERIMENTAL

2.1 Chemical, reagents and standard solutions

In this work, the following chemicals were used: Urea, $(\text{NH}_2)_2\text{CO}$, (Alkaloid- Skopje, North Macedonia); 35% Formaldehyde, CH_2O ; $\text{Fe}(\text{NO}_3)_3 \times 9 \text{H}_2\text{O}$; nano- TiO_2 , $\text{C}_6\text{H}_2(\text{OH})_3\text{CO}_2\text{H}$, caffeic acid (Sigma-Aldrich). Glassy carbon powder for preparation of CPE and paraffin oil were also products of Sigma-Aldrich. All the other materials and solvents used in this study were of analytical grade. For preparing all solutions, ultra-pure water was used ($18 \text{ M}\Omega \text{ cm}$), obtained from Millipore Simplicity 185.

Britton-Robinson buffer, used in all electrochemical experiments, was made from 0.04 M phosphoric, acetic and boric acid adduced to the appropriate pH value by 0.2 M NaOH.

The stock standard solution of caffeic acid (in the concentration of 1 mM) was prepared by dissolving the proper amount of this compound in water and stored in the fridge. The working solutions were made from standard solution by dilution before measurements.

2.2 Synthesis of modified UF composites and electrode preparation

Four samples of modified urea-formaldehyde (UF) composites with a formaldehyde to urea (F/U) ratio (0.8) were synthesized. Two samples were physical mixtures of pure UF resin with 15% of iron (using Fe(III) nitrate) and a combination of 15% of Fe and 15% of Ti (using Fe(III) nitrate and nano- TiO_2) - hereinafter abbreviated as UF/Fe and UF/Fe+Ti. The other two samples were prepared by adding the same amount of Fe(III) nitrate and nano- TiO_2 (in the same combinations as for physical mixtures) *in situ* during the synthesis (abbreviated as SynUF/Fe and SynUF/Fe+Ti). The synthesis of SynUF/Fe was done with 4.36 g of $\text{Fe}(\text{NO}_3)_3 \times 9 \text{H}_2\text{O}$ by the following procedure [22]. For the synthesis of SynUF/Fe+Ti, in the prepared solution of urea 1.00 g nano- TiO_2 was added along with 4.36 g of $\text{Fe}(\text{NO}_3)_3 \times 9 \text{H}_2\text{O}$, and the proper amount of FA and then prepared by the previously described procedure. For two samples of physical mixtures, UF/Fe and UF/Fe+Ti, the synthesis procedures were the same except the same amount of $\text{Fe}(\text{NO}_3)_3 \times 9 \text{H}_2\text{O}$ (and TiO_2) were mixed and homogenized with pure UF resins after curing.

Electrode material was prepared by thermolysis of all four prepared modified UF composites by procedure and conditions described before [22], and CPEs for electrochemical measurements were modified with 5% of obtained materials.

2.3 Apparatus and measurements

The morphology of UF composites modified with metal compounds, before and after thermolysis, was observed by TESCAN MIRA 3 XMU field emission scanning electron microscope (FE-SEM) at magnifications of 10,000× and 30,000×, operated at 20 keV. Before analysis, materials were prepared by deposition of a thin gold layer. Software ImageJ was used for determining the average particle size.

Attenuated total reflection Fourier transform infrared (ATR-FTIR) measurements were performed on Nicolet IS 50 FTIR spectrometer for infrared measurements. Spectra were collected from a powdered sample at room temperature in the spectral region from 4000 and 400 cm^{-1} and a nominal resolution of 4 cm^{-1} .

Electrochemical measurements were done at CHI 760b (USA) potentiostat/galvanostat. The electrochemical working cell consisted of a three-electrode system: CPE with material obtained by thermolysis of modified UF composites was the working electrode, Ag/AgCl (saturated KCl) was the reference electrode and a Pt-wire was used as a counter electrode. Glassy carbon electrode (GCE), a product of CHInstruments, was used for comparative selectivity study and antioxidative capacity determinations. Electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of a stationary 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ (1:1) mixture as a redox probe in 0.1 M KCl solution with the frequency changed from 1×10^5 Hz to 1×10^2 Hz with a signal amplitude of 5 mV at the potential of 0.05 V. Differential pulse voltammetry (DPV) parameters were varied from 25 to 90 mV for pulse amplitude, 20 to 70 ms for pulse time and 1 to 11 mV/s for step potential.

The pH measurements were performed using a Lab pH Meter inoLab (WTW, Germany) with a combined glass electrode (SenTix 41). All experiments were done at room temperature.

2.4 Sample preparation

The urine sample for analyses was collected from a healthy volunteer, filtered through 0.45 μm PTFE 117 membrane and stored at 4 $^{\circ}\text{C}$ in the fridge until electrochemical measurement. Analysis of the urine sample was done after 1:5 dilution with supporting electrolyte and adding a spiking amount of CA.

For the determination of total phenolic content, different food and beverage samples were electrochemically tested, practically without any sample preparation. Two honey samples were prepared as follows: 0.5 g of each was mixed with 50 mL of BR buffer pH 2, homogenized in an ultrasonic bath for 10 min at room temperature, filtered through 0.45 μm PTFE 117 membrane and analyzed by differential pulse voltammetry at proposed electrodes. The commercial apricot yogurt and apricot juice samples were diluted (1:10) with double-distilled water and homogenized in an ultrasonic bath for 10 min. The test solutions for DPV measurements were prepared by adding 100 μL of those sample solutions into 10.0 mL of BR buffer pH 2 to the electrochemical cell. For the analysis of cherry liquor, the same procedure was used but without previous dilution. As comparing DPV measurements at GCE were expected to be less sensitive [23] than DPV measurements on modified

CPE, they were done by the same following procedure and supporting electrolyte as described before, except that it was added 2.5 g of honey or 1 mL of the other samples.

3. RESULTS AND DISCUSSION

3.1 Electrochemical characterization of modified CPE

High electrical conductivity is one of the main requirements that an electrochemical sensor (and biosensor) should fulfill [24]. In that order, the conductivity study of carbon paste electrodes modified with four materials obtained by calcinations of UF composites modified by iron(III) nitrate and a combination of iron(III) nitrate and titan(IV) oxide, prepared by physical mixing or *in situ* synthesized, was performed. EIS measurements of CPE (pristine and modified with obtained materials), recorded in 0.1 M KCl containing 5 mM of $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$ mixture (1:1), are presented in the Fig. 1a. These measurements shown that all four prepared materials, used as electrode modifiers, significantly promote electron transfer at the CPE. By comparing the conductivity of materials to each other, it can be seen that adding the nano- TiO_2 in a material already decorated with iron oxide improved electron transfer on the surface of the electrode. Bering in mind different procedures of UF resin modification, it is obvious from the same graph that thermolysis prepared *in situ* synthesized UF composite showed much better electrode performance than material obtained by thermolysis of a physical mixture of UF resin and metal compounds. The smallest diameter of the obtained semicircle at the high frequencies corresponds to the largest charge transfer, which is recorded at CPE modified with thermolysis prepared UF composite *in situ* synthesized with both metal compounds (SynFe+Ti/UF-TP@CPE). This confirms that the formation of the Fe/Ti heterojunction helped to synergistically increase the physicochemical properties of the prepared UF resin.

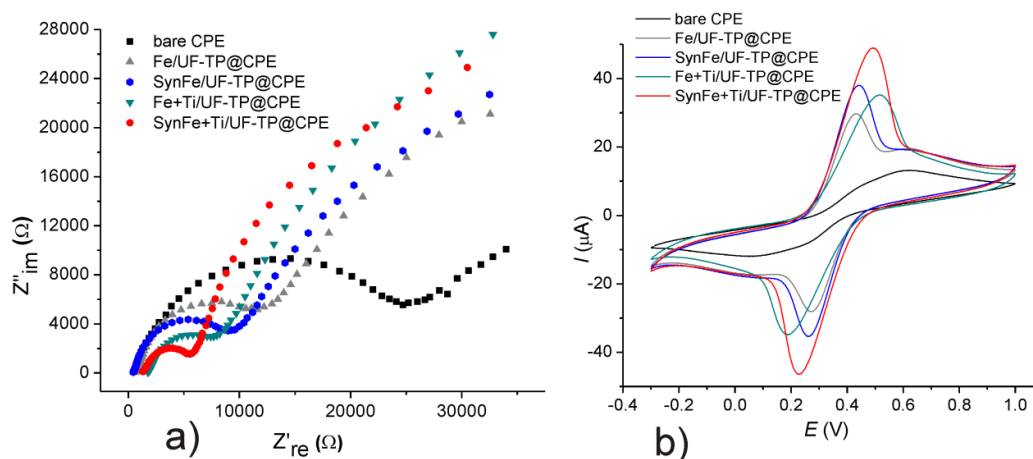


Figure 1. a) EIS measurement in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ test solution at bare CPE and modified CPEs (physical mixture of UF and Fe compound (marked with Fe/UF-TP@CPE), UF mixed with Fe and Ti compounds (Fe+Ti/UF-TP@CPE), *in situ* synthesized UF with Fe compound (SynFe/UF-TP@CPE) and *in situ* synthesized UF with both metal compounds (SynFe+Ti/UF-TP@CPE); b) Cyclic voltammograms at bare CPE and the same modified CPEs at the same test solution (scan rate 50 mV/s)

The next electrochemical study of the bare CPE and modified CPEs in the same $[\text{Fe}(\text{CN})_6]^{3-/4-}$ test solution was performed by cyclic voltammetry technique ranging from -0.3 to +1.0 V within the scan rate of 50 mV/s (Fig 1b), with the aim to investigate and confirm nature of the interfacial charge transport at the interface electrodes/electrolyte. The analysis of obtained voltammograms corresponds to previously recorded EIS measurements. Compared to the recording of the same test solution at bare CPE (Fig. 1b), it can be seen that the redox peak is shifted by 0.1 V to more positive potentials due to promoted catalytic activity as a result of modification of CPE with thermolysis prepared UF composites. The best electrode performance was also recorded at SynFe+Ti/UF-TP@CPE due to the highest anodic and cathodic peak current ($I_a = 49\mu\text{A}$; $I_c = -46.6\mu\text{A}$) and the largest active electrode surface area of 0.42 cm^2 determined by using Randles-Sevcik equation:

$$I_p = 2.687 \times 10^5 n^{3/2} A D^{1/2} \nu^{1/2} C_0$$

where I_p is the peak current, n is the number of electrons involved ($n = 1$ in the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox system), A is the surface area of the electrode, C_0 is the concentration of the reactant, D_0 is the diffusion coefficient of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ($6.7 \times 10^{-6}\text{ cm}^2 \cdot \text{s}^{-1}$) and ν is the scan rate.

Hence, SynFe+Ti/UF-TP@CPE was selected for further electrochemical examinations and analysis.

3.2 ATR-IR and SEM characterization of modified UF composite and prepared electrode modifiers

Due to better electrical conductance of thermolysis prepared modified composites material with combined metal compounds, composites SynUF/Fe+Ti and UF/Fe+Ti before calcinations were studied by ATR-IR spectroscopy. This method should offer certain evidence that adding the metal compounds during the synthesis (*in situ*) produces certain differences in the structure of obtained composite.

The absorbance ATR-IR spectra of SynUF/Fe+Ti, UF/Fe+Ti and unmodified UF (for comparison) are presented in Figure 2. ATR-IR spectra of UF possess a strong band at 3324 cm^{-1} assigned to the hydrogen bond between N-H and -OH. The methylenization reaction leads to a more cross-linked structure of UF, which is created by the reduction in the extent of hydrogen-bonded interactions. This could be indicated by the sharpness of the bonds which were previously discussed. [25]. A broadening of the bands around 3320 cm^{-1} and 1630 cm^{-1} is the consequence of chemisorbed water on the surface of the TiO_2 and bonding vibration of -O-H bond [26, 27]. The stretching of the carbonyl group ($>\text{C}=\text{O}$) and -CN stretching of secondary amines were recorded around 1630 cm^{-1} and around 1560 cm^{-1} respectively, for all UF composites [28]. The intensity of the band at around 1384 cm^{-1} which is attributed to the vibrations of the hydroxymethyl ($\text{HOCH}_2\text{NH}-$) decreases depending on which synthesis was applied. In the case of *in situ* synthesis, the band shifted to a value of 1374 cm^{-1} . The decrease in the intensity of that band is related to the reduced content of hydroxymethyl. Two small bands at 3031 cm^{-1} and 2960 cm^{-1} and multiple bands between $1600 - 500\text{ cm}^{-1}$ are assigned to symmetrical -C-H stretching vibrations from -CH₂- group of ether, -CH₂OH and N-CH₂- groups. The band at 1381 cm^{-1} in pure UF originates from stretching valence vibrations of the -CH₂OH group as well as from bending vibrations of $\delta(\text{NH})$ and NH-CO and 2°-amine (amide II). ATR-IR spectra of SynUF/Fe+Ti and UF/Fe+Ti composites show that this band shifts to 1371 cm^{-1} , the intensity of the

bands increases and overlaps with the band originating from iron(III) nitrate nonahydrate, which is defined with a peak at 1317 cm^{-1} for the UF/Fe+Ti composite. In addition to this new band in the modified UF composites, there are two more bands at 1180 cm^{-1} and 753 cm^{-1} that can be attributed to -OH deformation and bending in $\gamma(\text{Fe-O})$ -group [29].

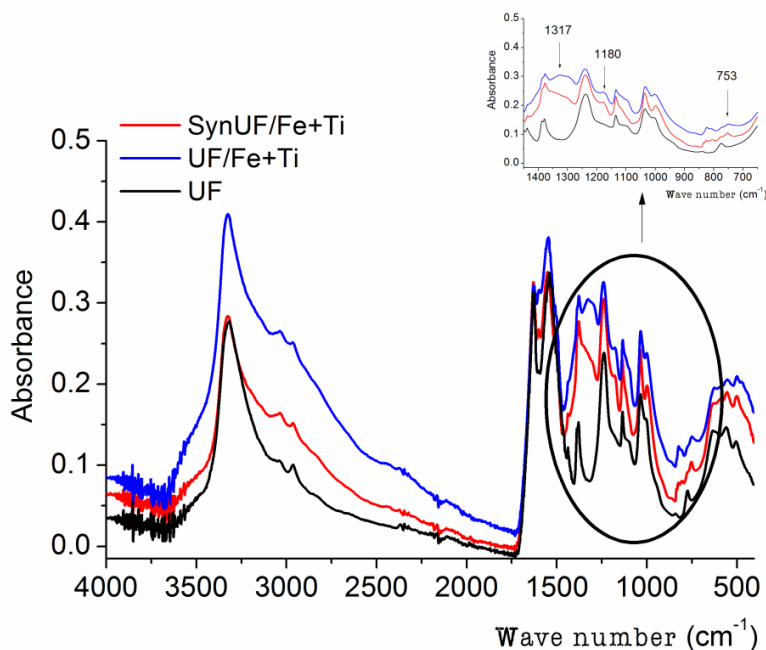


Figure 2. ATR-IR spectra of unmodified UF resin and modified SynUF/Fe+Ti and UF/Fe+Ti composites.

Surface morphological characterization of prepared UF composites with iron(III) nitrate and nano- TiO_2 (*in situ* synthesized and physical mixture) and electrode material prepared by thermolysis of those UF composites are shown in Fig. 3.

Observing the SEM micrographs of modified UF resins obtained by different methods of preparation (*in situ* synthesis and physical mixing of UF resins and metal compounds) (Fig. 3a,b) certain characteristics of the surface morphology could be noticed. The granule of UF/Fe+Ti was $5 - 10\ \mu\text{m}$ with visible particles on the surface of the composite. Those particles at the surface were most likely metal compound particles (or agglomerates) with an average size of approximately 780 nm . They were probably immersed through the organic polymer but also visible at the surface of the modified UF/Fe+Ti composite. Contrary to modified UF resin obtained by physical mixing, *in situ* synthesized modified composite, SynUF/Fe+Ti, possessed a more granular structure with a smaller size of about $1.3\ \mu\text{m}$. In the modified composite SynUF/Fe+Ti metal compound particles interacted in some way with the polymer matrix (as an interpretation of ATR-IR spectra suggested before), or they were encapsulated in overlapping UF resin. At a magnification of $10,000\times$, they are practically imperceptible on the recorded smooth surface texture. This output of *in situ* synthesis was also noticed for UF composites only modified with iron compounds [22]. After thermolysis of both composites, metal oxide particles and carbon were probably obtained. Contrary to plate-like nuggets of Fe+Ti/UF-

TP at the same SEM magnification, a more divorced and layered structure was seen by observing SynFe+Ti/UF-TP. The largest number of studied Fe+Ti/UF-TP particles was about the size of 580 nm (from the histogram, not presented), while a significant amount of much smaller particles was observed for SynFe+Ti/UF-TP, which were close to nano-size of 100-200 nm, providing a high specific surface area of the obtained material. Those morphological characteristics of SynFe+Ti/UF-TP were probably crucial for better conductivity and other performances of this potential electrode material intended for a successful electrochemical sensor.

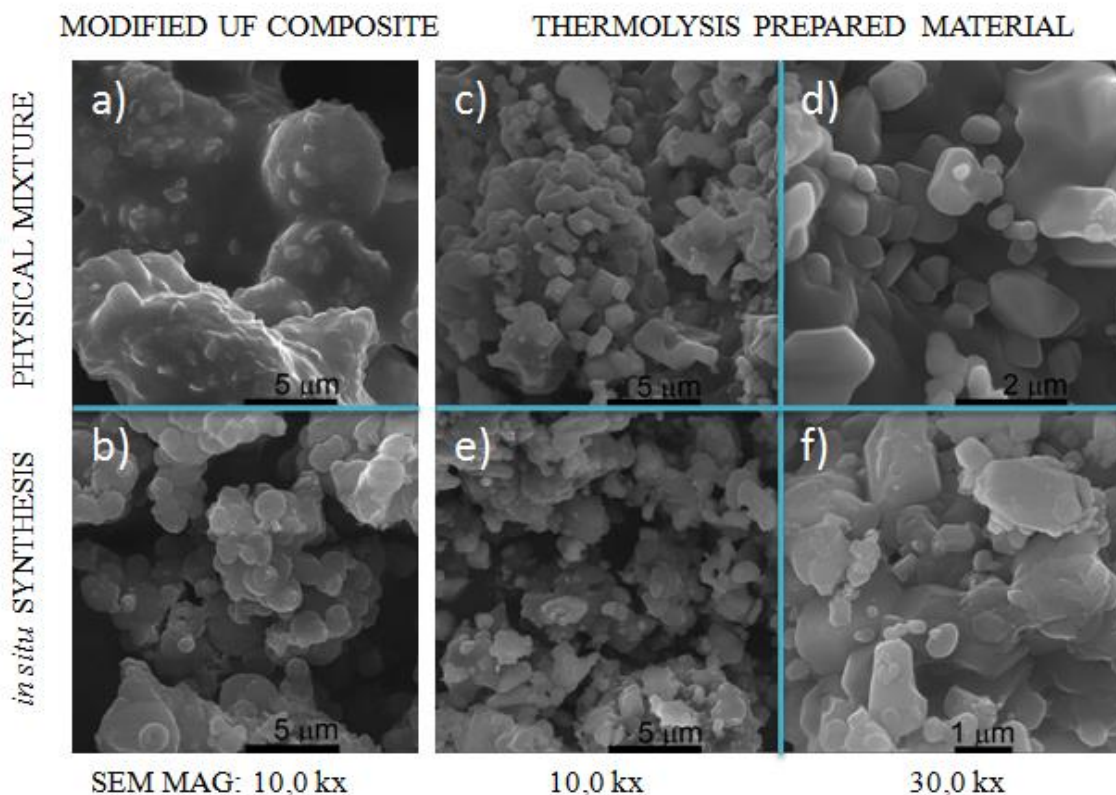


Figure 3. SEM micrograph of: a) modified UF/Fe+Ti at magnifications of 10,000 \times ; b) modified SynUF/Fe+Ti at magnifications of 10,000 \times ; c) and d) Fe+Ti/UF-TP prepared by thermolysis from UF/Fe+Ti at magnifications of 10,000 \times and 30,000 \times , respectively; e) and f) SynFe+Ti/UF-TP prepared by thermolysis from SynUF/Fe+Ti at magnifications of 10,000 \times and 30,000 \times , respectively.

3.3 Electrooxidation of caffeic acid at proposed modified CPE

Thermolysis prepared the most conductive material with combined metal oxide particles was applied as an analytical platform to investigate the electrochemical behavior of the prominent polyphenol, caffeic acid. Caffeic acid, besides gallic acid, is one of the most potent polyphenols and antioxidants. It is synthesized by all plant species and it is present in food and food products [30]. Like gallic acid, it is usually used in electrochemical determinations as a standard (or model compound) for evaluating the antioxidant properties of different samples, based on CA equivalents [31]. CA has the capacity to produce the highest oxidation current among the other polyphenols recorded as one sharp

reversible redox peak [31]. Hence, in this work, this polyphenol compound was chosen for analyte and standard for studying antioxidant properties of honey and selected beverage samples at obtained electrode high-performance material.

The cyclic voltammetry investigation was scrutinized to determine the electrocatalytic possibilities of the selected electrode toward electrochemical sensing of caffeic acid. By studying the electrochemical behavior of 100 μM of caffeic acid in BR buffer pH 2 at SynFe+Ti/UF-TP@CPE characteristic, well-defined, sharp anodic peak at 0.52 V and reverse cathodic peak at 0.43 V were recorded (Fig. 4). The ratio $I_c / I_a = 0.956$ was very close to 1, confirming that the system is chemically reversible, while the peak potential difference, ΔE_p , between peaks I_c and I_a is about 90 mV indicating a quasi-reversible reaction in BR buffer pH 2 at SynFe+Ti/UF-TP@CPE. The formal potential ($E^{0'}$) of CA at SynFe+Ti/UF-TP@CPE calculated as $(E_{pa} + E_{pc})/2$ was 0.475 V. The same electrochemical behavior was noticed for CA at bare GCE before [32].

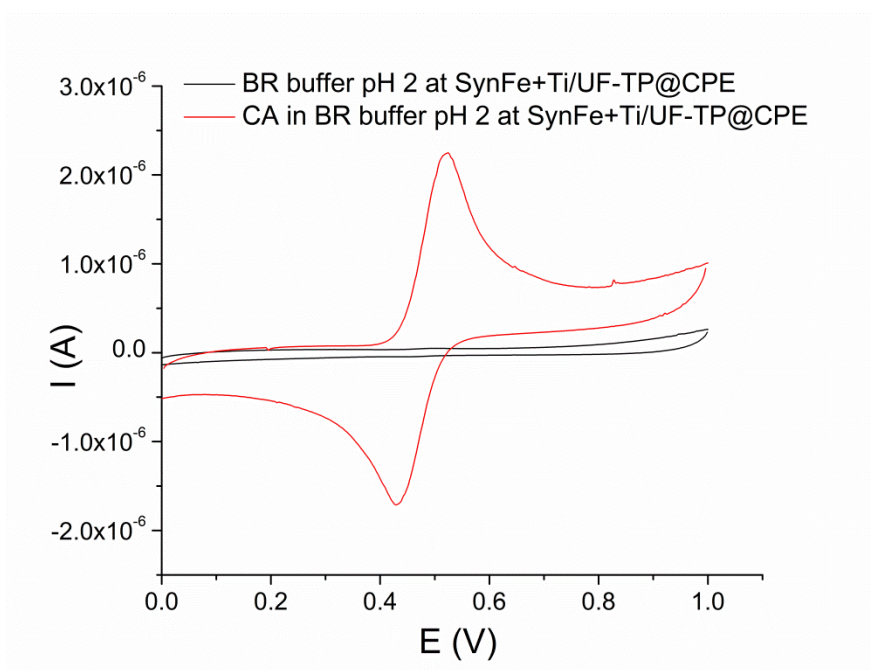


Figure 4. Cyclic voltammograms recorded with and without caffeic acid in Britton-Robinson buffer pH 2 at SynFe+Ti/UF-TP@CPE. Scan rate of 20 mV/s.

3.4 Effect of pH of the supporting electrolyte and sweep rate

The optimal pH value of BR buffer, as a supporting electrolyte, was found by recording cyclic voltammograms of 100 μM of CA in different pH values from 2 to 7 (Fig 5a). The increase in the pH value in this range resulted in a substantial decrease in the peak currents for both electrode reactions – anodic and cathodic. Based on this, BR buffer pH 2 was used in further experiments. A negative shift of anodic peak has occurred by increasing pH, indicating an increase in the nucleophilicity of the organic compound [33]. Additionally, pH-dependent variation in the current response reveals that the analyte's proton affinity is vital in activating charge transport for effective detection.

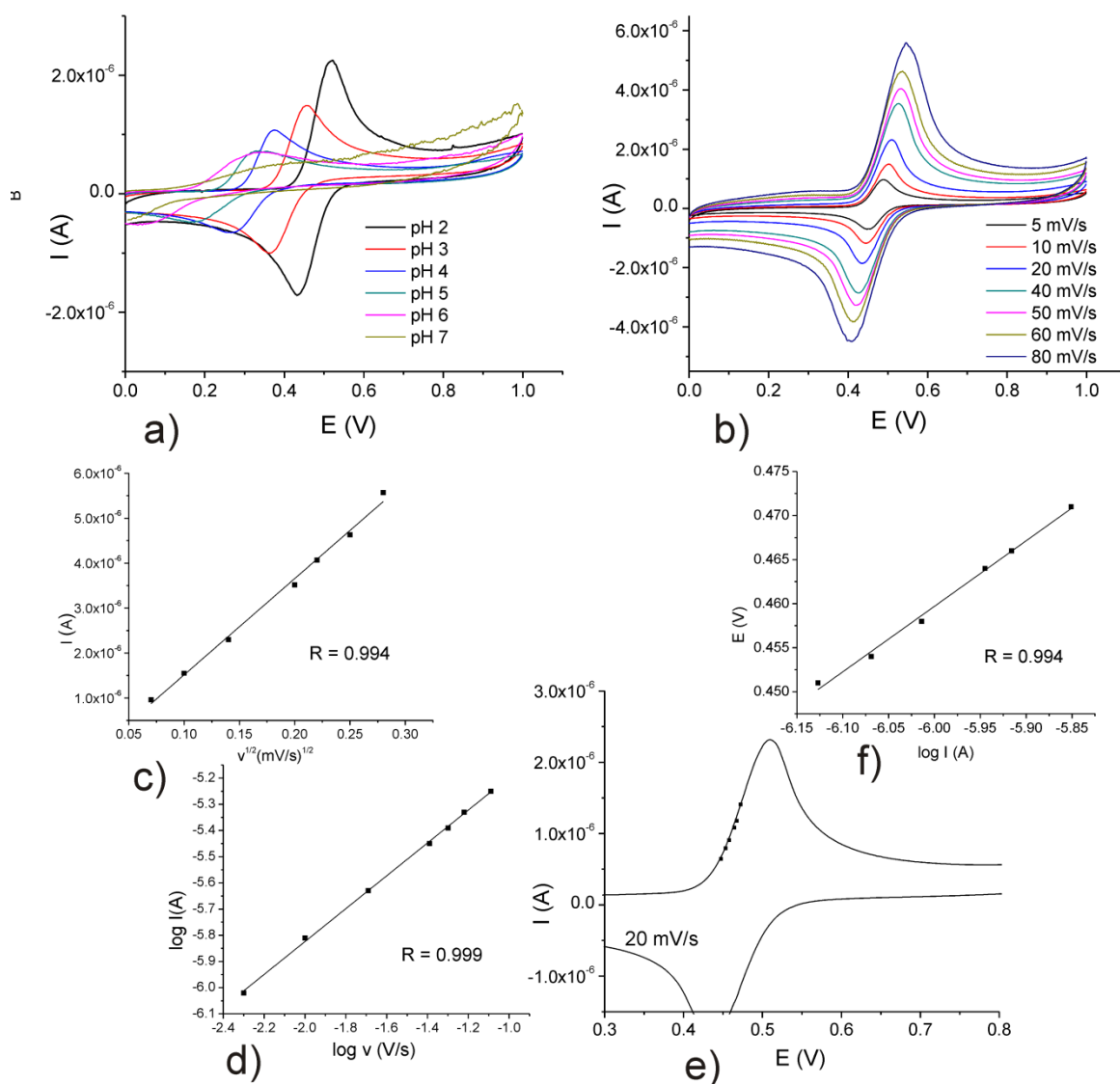


Figure 5. a) Cyclic voltammograms of 100 μM of CA in different pH values of Britton-Robinson buffer at SynFe+Ti/UF-TP@CPE. b) Effect of scan rate (in range of 5 to 80 mV/s); c) The peak current vs scan rate derived from graph b; d) The logarithm of peak current in the function of the logarithm of scan rate derived from b; e) Tafel region at cyclic voltammogram of 100 μM of CA at a scan rate of 20 mV/s; f) corresponding Tafel plot.

In order to investigate the reaction mechanism, cyclic voltammetry with repetitive cycles at different scan rate from 5 to 80 mV/s were recorded (Fig. 5b). By analyzing the effect of scan rate on cyclic voltammograms of CA, a linear relationship between peak current, I , and the square root of scan rate, v , was found (Fig. 5c). This upsurge is noticeable for both processes, cathodic and anodic. However, slight shifts in the peak potentials toward less negative/more positive values for the bot redox process indicate negligible support of the adsorption of the analyte at the electrode interface. The linear relationship between $\log I$ and $\log v$, expressed by regression equation $\log I \text{ (A)} = -4.56 + 0.58 \times \log v \text{ (V/s)}$, ($R = 0.999$) can be seen from Fig. 5d. It was observed that $\log I$ vs $\log v$ resulted in

regression line with slope 0.58, and this value is very close to the theoretical value of 0.5 for the diffusion-controlled process [34]. Tafel region (the linear raising part of the voltammetric profile of CA recorded at a scan rate of 20 mV/s), which is affected by electron transfer kinetics between the electrode surface and caffeic acid (Fig. 5e,f), has shown the regression relationship E (V) = 0.072 log I (A) + 0.986. The slope of the regression line was found to be 72 mV indicating that two electrons were involved in charge transfer in the rate-determining step at SynFe+Ti/UF-TP@CPE, bearing in mind that the theoretical value for Tafel slop should be close to 120 mV for one electron and about 60 mV for two electrons participating in the electrochemical reaction [35]. According to Giacomelli et al [32], at pH 2.0, CA exists 99% in solution in the form of H₃CA and only 1% in a f H₂CA, and we suggested that the origin of CA redox peak involves a reversible transfer of two electrons and two protons (as it was found before [32] for solutions of pH up to 5.5), in the one step-two electron mechanism depicted scheme presented in Fig. 6:

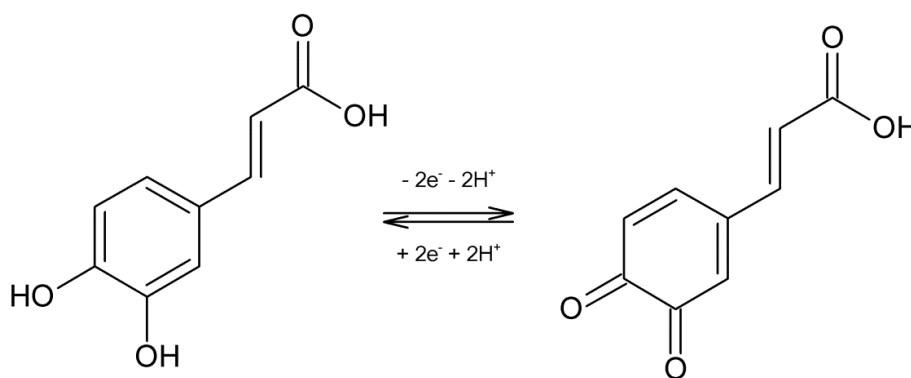


Figure 6. The suggested reaction mechanism of electrooxidation of CA

3.5 Analytical performances and selectivity

Optimal experimental conditions and characteristics of the electrochemical process were chosen and studied by cyclic voltammetry technique in previous experiments. For analytical application, the other more suitable and sensitive electrochemical technique was selected. Pulse voltammetric techniques are frequently used for this purpose due to good discrimination against background current and low detection limits of voltammetric measurements [36]. In this work, preference was given to the differential pulse voltammetry because the significantly distinct oxidation peak was observed by recording with differential pulse voltammetric technique (DPV) than with square wave voltammetry (SWV). The optimization of DPV parameters was done by changing one parameter in the selected range, while others were kept constant (see section 2.3). The optimal values applied for the construction of the calibration curve, selectivity experiments and sample analysis were: a pulse amplitude of 80 mV, a pulse time of 50 ms and a potential step of 7 mV/s.

In order to investigate range of analytical applicability and statistical parameters of CA determination, DPV profiles of different concentrations of caffeic acid (0, 0.5, 0.7, 1, 3, 5, 7, 10, 15, 30, 40, 50, 70, 90, 100 μ M) were recorded in BR buffer pH 2 at SynFe+Ti/UF-TP@CPE (Fig. 7 a,b). The analytical signal was linear in a whole wide concentration range, as it can be seen from the

calibration graph (Fig. 7 c). The linear relationship was expressed by regression equation $I(A) = 1.84 \times 10^{-7} + 5.72 \times 10^{-8} C_{CA} (\mu M)$ with Pearson correlation coefficient $R = 0.996$. The detection limit of $0.046 \mu M$ was calculated from the equation $LOD = 3s/m$, where s was the standard deviation for the supporting electrolyte and m was a slope used from the regression equation of the calibration curve. The repeatability of the method, expressed by the relative standard deviation (RSD) of five times repeated measurements of 1 and $15 \mu M$ of LA at the same working and experimental conditions were 3.4 and 2.8 %, respectively. A long-term stability test showed a 95.5% of initial oxidation peak for CA after 30 days. According to the obtained data, the developed method meets the criteria of stability for electrochemical sensors (RSD does not exceed 5%) and the selected material with a large surface area and synergetic effect of the Ti and Fe-based nanomaterial with highly ordered architectures ascertains the enhancement in electrochemical performance of the CPE.

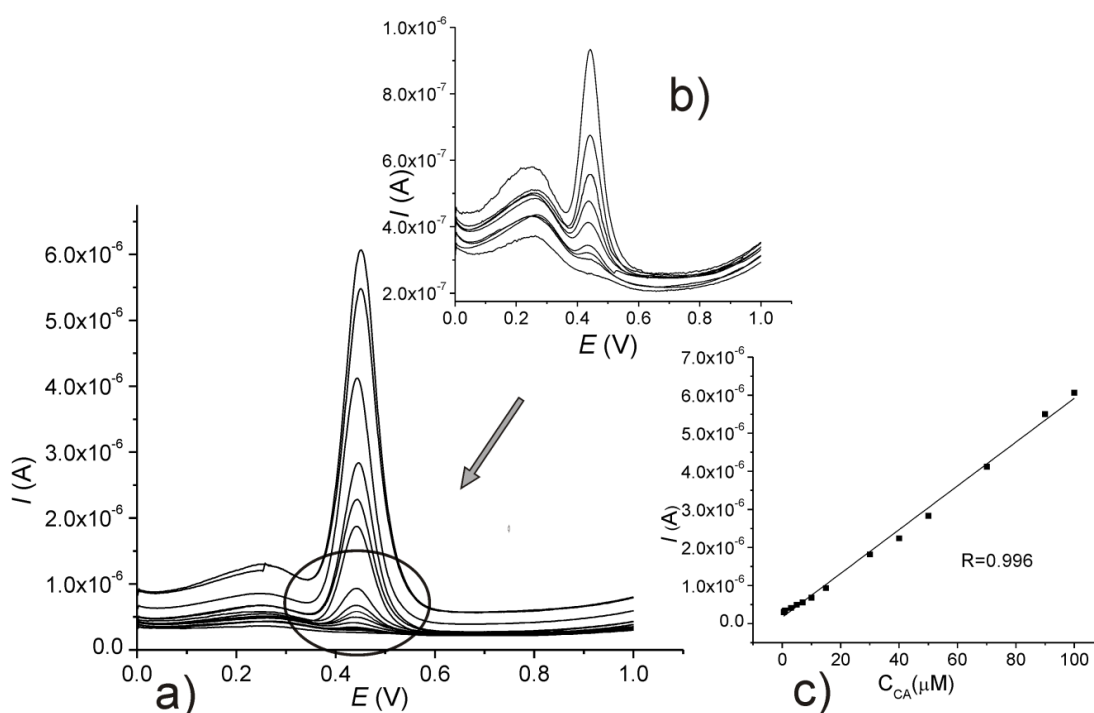


Figure 7. a) DPV profiles of different concentrations of CA ($0.5-100 \mu M$) in BR buffer pH 2 at SynFe+Ti/UF-TP@CPE; b) DPV profiles of low studied concentrations of CA (0, 0.5, 0.7, 1, 3, 5, 7, 10, 15 μM); c) corresponding calibration graph for all range of studies concentrations.

Comparing some important characteristics of this developed method with previously reported electroanalytical methods for determining CA, the proposed modified CPE presented a longer linear range and lower LOD than unmodified GCE [22] and modified GC electrodes (with molecularly imprinted siloxanes [37], electrochemically reduced graphene oxide-nafion composite [38], laccase-MWCNT-chitosan/Au [39], nafion/tyre/sonogel-carbon [40], poly(glutamic acid) [41], fluorine-doped graphene oxide [42] and the smaller linear range and comparable LOD than Co_3O_4 microballs modified screen printed electrodes [43]. For the carbon nanofiber-based screen-printed sensor developed for simultaneous determination of caffeic acid and ferulic acid, the higher LOD and smaller

working range were reported compared to the established method at SynFe+Ti/UF-TP@CPE [18]. Further, as is the case of this sensor, it can be noticed that the employment of iron compounds in the modification of CPE led to the development of sensitive electrochemical sensors for CA. CPE modified with Fe₃O₄ nanoparticles/1-methyl-3-butylimidazolium [44] resulted in a sensor with a wide working range (0.03-410 μ M) and lower LOD (0.008 μ M) than this studied modified CPE platform, while for graphene nanoribbons/CoFe₂O₄ and NiO nanoparticles with 1-ethyl-3-methylimidazolium acetate modified CPE huge linear range was reported (0.1-1000 μ M), with two linear relationships, and no evidence for which concentration ranges they apply [45].

Selectivity of the developed analytical method was studied in order to predict possible analytical application. Uric acid and especially ascorbic acid were noticeable at bare GCE compared to barely a visible change of differential pulse voltammograms at SynFe+Ti/UF-TP@CPE in the presence of those two compounds (Fig. 8a). As can be seen, by adding 12 μ M of caffeic acid, change of analytical signal was practically negligible (much less than 5%) in case of adding of 100 μ M of ascorbic acid (AA), glucose (GLU) and uric acid (UA) (Fig. 8b), while adding of gallic acid (GA) to CA in ratio 1:1 has doubled analytical signal (Fig. 8c).

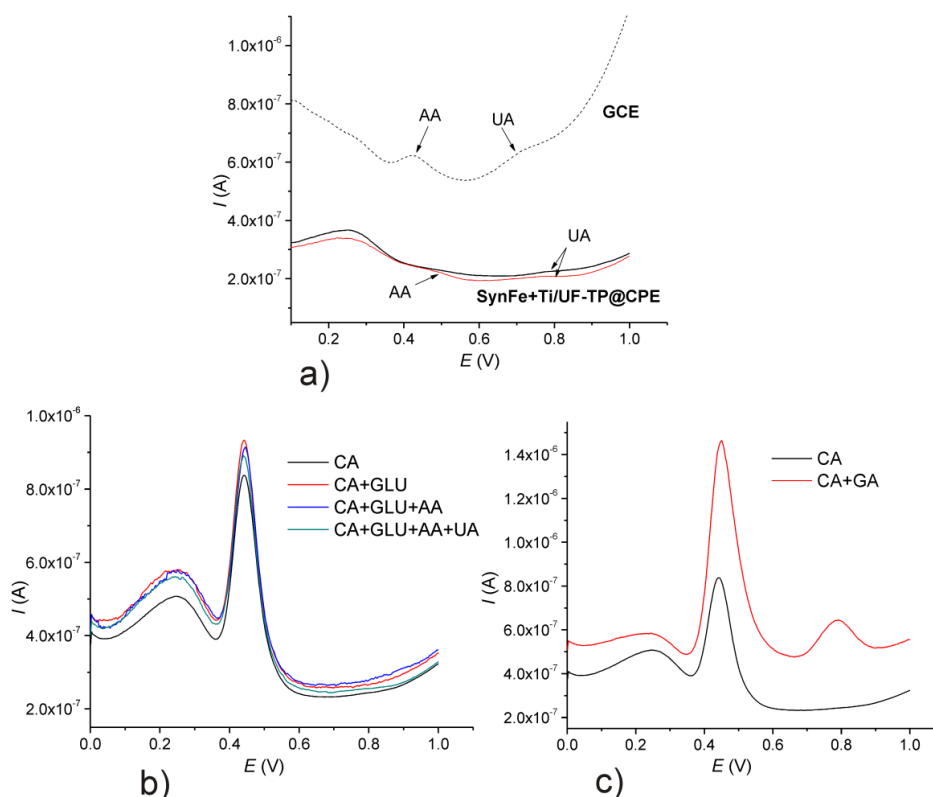


Figure 8. Differential pulse voltammograms at SynFe+Ti/UF-TP@CPE in the presence of possible interfering compounds: a) comparing DPV profiles of AA and UA without the presence of CA at GCE; b) CA with added GLU, AA and UA; c) CA in the presence of GA.

Based on selectivity study results, for analytical testing of the applicability and accuracy of proposed CA determination at SynFe+Ti/UF-TP@CPE, a human urine matrix was selected. Bearing in

mind that quantification of CA and some other polyphenols in human urine was usually done by expensive advanced hybrid techniques, like Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry [46, 47], the proposed electrochemical method at SynFe+Ti/UF-TP@CPE could present a direct, simple, fast and low-cost analytical approach for determination of caffeic acid in human urine samples, almost without previous sample preparation.

Electrochemical measurements of amounts of caffeic acid in a urine sample at the proposed electrode were done at optimized DPV parameters and established analytical procedure and results were presented in Table 1. Spiked recoveries of the determination in the range of 101.7 to 104 % and RSD of measurements from 2.8 to 3.6 % indicated the possibility of an accurate analysis of caffeic acid in urine by this method, considering reported median CA concentration of 3.27 μM obtained from 475 examined human urine samples from the medical study [46].

Table 1. Determination of CA in spiked urine samples using proposed SynFe+Ti/UF-TP@CPE

Sample	Added (μM)	Found (μM)*	Recovery (%)	RSD (%)
Urine	0.50	0.52	104.0	3.6
	1.50	2.08	104.0	3.3
	2.00	4.12	103.0	2.8
	5.00	9.16	101.7	3.0

* Average of 3 measurements

3.7 The total phenolic content in selected samples

In order to examine other possibilities of application, the proposed modified CPE and developed DPV method were used to estimate the antioxidant properties of selected samples on the basis of the caffeic acid oxidation current peak. The origin of the anodic peak could be ascribed to the oxidation of different polyphenols present in samples having the structure with ortho-diphenol groups at B-ring, which are (on this pH) the major contributors to the antioxidant capacity of determined samples [18]. Five different samples were analyzed: commercial apricot juice and apricot yogurt, cherry liquor and two honey samples. The results (mean values of five electrochemical measurements) represent the total phenolic content expressed in mg/L (for juice and liquor) and mg/100mg (for honey and yogurt) of CA estimated from the calibration curve (Table 2). As DPV measurements of caffeic acid were successfully used before in the possibility of determining some polyphenols (caffeic acid was one of them) alone and in fruit juices at glassy carbon electrode [18], this electrode was used for comparison. Taking into account recovery values (in the range of 94.6 to 105.5%) in Table 2, it can be concluded that the values of obtained CA equivalents estimated by the proposed modified CPE were comparable to results obtained at bare GCE. Also, statistical F test-two sample variances was not exceeded the critical value confirming that the developed electrochemical method at SynFe+Ti/UF-TP@CPE can also be used successfully in electrochemical determining of TPC of various food and beverage samples.

Table 2. Determination of the total phenolic content of selected samples by electrochemical methods

Sample	DPV on the proposed modified CPE	DPV on GCE	Recovery (%)	F ($F_{\text{crit at } Q 95\%} = 6.39$)
Honey 1	19.9 ^a	21.0 ^a	105.5	5.23
Honey 2	21.9 ^a	22.8 ^a	104.1	5.44
Cherry liquor	11.7 ^b	11.1 ^b	94.9	6.02
Apricot juice	257.6 ^b	249.6 ^b	96.9	4.98
Apricot yogurt	24.4 ^a	23.1 ^a	94.6	5.19

^a mg of CA in 100g of sample

^b mg of CA in L of sample

4. CONCLUSION

Urea formaldehyde resin modified with Fe(III) nitrate and Ti(IV) oxide was exposed to thermal degradation in order to obtain high conductive performance material used as a carbon paste modifier applied for sensitive determining of prominent antioxidant caffeic acid. The optimal thermolysis prepared material was selected and morphologically and electrochemically characterized. Due to optimizing experimental conditions and parameters of used differential pulse technique, analytical method and procedure for real sample determination were established. Successful quantification of caffeic acid in spiked urine samples by the proposed method at this modified CPE demonstrated the possibility of fast and accurate determination of caffeic acid, which can compete with determinations of this compound on much more complicated and expensive analytical instrumentation. The established electroanalytical method at the proposed modified CPE also proved itself in a simple and fast estimation of the total phenolic content in selected honey and beverage samples. In addition, this study aims to identify the effect of the morphological superiority of hierarchical architectures towards the electrochemical detection of important biological molecules essential to human health and well-being. Therefore, monitoring the concentration of biologically relevant molecules in human fluid samples with selective and stable recognition elements can have a great impact on the control of the quality of human life with the interaction between surface architecture and functionalization.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Agreement Numbers 451-03-68/2022-14/200123 and 451-03-68/2022-14/200052.

References

1. A.H. Conner, Urea–formaldehyde adhesive resins, *Polymeric materials encyclopedia*, 11. CRC. (1996) USA, p. 8497.
2. M.P. Ansell, *Wood Composites*, Woodhead Publishing, Elsevier (2015).
3. N. Ayrlmis, *International Scientific Journal "Innovations"*, 4 (2021) 151.
4. V. Jovanović, S. Samaržija-Jovanović, B.B. Petković, V.S. Dekić, G.S. Marković, M.T.

- Marinović-Cincović, *RSC Advances*, 5 (2015) 59715.
5. B. Petković, S. Samaržija-Jovanović, V. Jovanović, B. Dekić, G. Marković, M. Marinović-Cincović, *Composites Part B: Engineering*, 69 (2015) 397.
 6. S. Samaržija-Jovanović, V. Jovanović, G. Marković, S. Konstantinović, M. Marinović-Cincović, *J. Thermoplast. Compos. Mater.*, 26 (2013) 747.
 7. S. Samaržija-Jovanović, V. Jovanović, B. Petković, T. Jovanović, G. Marković, S. Porobić, J. Papan, M. Marinović-Cincović, *Polym. Compos.* 41 (2020) 3575.
 8. V. Jovanović, S. Samaržija-Jovanović, B. Petković, Z. Milićević, G. Marković, M. Marinović-Cincović, *Polym. Compos.*, 40 (2019) 1287.
 9. V. Jovanović, S. Samaržija-Jovanović, B. Petković, S. Jovanović, G. Marković, S. Porobić, M. Marinović-Cincović, *Polym. Bull.*, 78 (2021) 399.
 10. H. Karimi-Maleh, F. Karimi, M. Alizadeh, A.L. Sanati, *The Chemical Record*, 20 (2020) 682.
 11. C.G. Zoski. Handbook of Electrochemistry, Chapter: Chapter 8: G. A. Edwards, A.J. Bergren, and M.D. Porter, Chemically Modified Electrodes, Elsevier (2007).
 12. S. Sharma, *Materials (Basel)*. 11 (2018) 1857.
 13. B. Šljukić, C.E. Banks, R.G. Compton, *Nano Lett.*, 6 (2006) 1556.
 14. L. Bertel, D.A. Miranda, J.M. García-Martín, *Sensors*, 21 (2021) 6167.
 15. S.S. Al Harthi, B. Pharm, A. Mavazhe, B. Pharm, H. Al Mahroqi, S.A. Khan, *Journal of Taibah University Medical Sciences*, 10 (2015) 346.
 16. A. Sałata, R. Gruszecki, *Acta Sci. Pol., Hortorum Cultus*, 9 (2010) 175.
 17. A.V. Bounegru, C. Apetrei, *Sensors*, 22 (2022) 4689.
 18. C.-V. Popa, L. Lungu, M. Savoiu, C. Bradu, V. Dinoiu, A. F. Danet, *Int. J. Food Prop.*, 15 (2012) 691.
 19. Y. Zheng, H. Karimi-Maleh, L. Fu, *Sensors* 22 (2022) 3238.
 20. F. Guneral, C. Bachmann, *Clin Chem.* 40 (1994) 862.
 21. L.I. Mennen, D. Sapinho, H. Ito, P. Galan, S. Hercberg, A. Scalbert, *Eur. J. Clin. Nutr.*, 62 (2008) 519.
 22. B. Petković, M. Kostić, S. Samaržija-Jovanović, B. Laban, Dj. Veljović, D. Stanković, The First International Conference on Sustainable Environment and Technologies, "Creating sustainable commUNiTy", Belgrade, Serbia, 2021, 147.
 23. A.J. Blasco, M.C. González, A. Escarpa, *Anal. Chim. Acta*, 511 (2004) 71.
 24. D. Grieshaber, R. MacKenzie, J. Vörös, E. Reimhult, *Sensors*, 8 (2008) 1400.
 25. M. J. Safi, I. M. Mishra, B. Prasad, *Thermochim. Acta*, 412 (2004) 155.
 26. Y.L. Zhang, D.J. Wang, G.K. Zhang, *Chem. Eng. J.*, 173 (2011) 1.
 27. G. Zhang, Q. Xiong, W. Xu, S. Guo, *Appl. Clay Sci.*, 102 (2014) 231.
 28. S. Samaržija-Jovanović, V. Jovanović, T. Jovanović, B. Petković, G. Marković, S. Porobić, M. Marinović-Cincović, *J. Therm. Anal. Calorim.*, In press, (2022).
 29. J. Weissenriederz, C. Leygraf, *J. Electrochem. Soc. B*, 151 (2004) 165.
 30. A. Rahman, *Studies in Natural Products Chemistry*, Elsevier (2022).
 31. I.G. Munteanu, C. Apetrei, *Antioxidants*, 11 (2022) 584.
 32. C. Giacomelli, K. Ckless, D. Galato, F.S. Miranda, A. Spinelli, *J. Braz. Chem. Soc.*, 13 (2002) 332.
 33. J. Wang, *Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine*, Wiley-VCH (1988) New York.
 34. D.T. Sawyer, A. Sobkowiak, Jr. J. L. Roberts, *Electrochemistry for Chemists*, 2nd ed., John Wiley & Sons Inc. (1995) New York.
 35. G. Kear, F.C. Walsh, *Corros. Mater.*, 30 (2005) 51.
 36. A. Brett, C. Brett, *Electrochemistry Principles, Methods, and Applications*, (first ed.), Oxford Science Publications, Oxford (1993) New York.
 37. F.R.F. Leite, W.J.R. Santos, L.T. Kubota, *Sens. Actuators B Chem.*, 193 (2014) 238.
 38. H. Filik, G. Çetintaş, A.A. Avan, S. Aydar, S.N. Koç, I. Boz, *Talanta*, 116 (2013) 245.

39. M. Diaconu, S.C. Litescu, G.L. Radu. *Sens. Actuators B Chem.*, 145 (2010) 800.
40. M. El Kaoutit, I. Naranjo-Rodriguez, K.R. Temsamani, M.P. Hernández-Artiga, D. Bellido-Milla, J.L.D. Cisneros, *Food Chem.*, 110 (2008) 1019.
41. D.P. Santos, M.F. Bergamini, A.G. Fogg, M.V.B. Zanoni, *Microchim. Acta*, 151 (2005) 127.
42. V.S. Manikandan, B. Sidhureddy, A.R. Thirupathi, A. Chen, *Sensors*, 19 (2019) 1604.
43. S. Ramki, P. Balasubramanian, S.M. Chen, T.W. Chen, T.W. Tseng, B.S. Lou, *Int. J. Electrochem. Sci.*, 13 (2018) 1241.
44. R. Abdi, A. Ghorbani-HasanSaraei, H. Karimi-Maleh, S. Naghizadeh Raeisi F. Karimi, *Int. J. Electrochem. Sci.*, 15 (2020) 2539.
45. R. Mostafazadeh, A. Ghaffarinejad, F. Tajabadi, *Food Chem. Toxicol.*, 167 (2022) 113312.
46. D. Achaintre, A. Bulete, C. Cren-Olive, L. Li, S. Rinaldi, A. Scalbert, *Anal. Chem.*, 88 (2016) 2637.
47. H. Ito, M.P. Gonthier, C. Manach, C. Morand, L. Mennen, C. Remesy, A Scalbert, *Br. J. Nutr.*, 94 (2005) 500.

© 2022 The Authors. Published by ESG (www.electrochemsci.org). This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).