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Electroanalytical methods in determination of selected biologically active compounds

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References

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Abstract

The dissertation deals with the development of electroanalytical methods for the determination of selected biologically active compounds in different matrices. The ethylvanillin total amount in foodstuffs, the amount of caffeine, vitamin B6 and disputable β-amino acid taurine were monitored in energy drinks. Finally, voltammetric determination of antiarrhythmic drug, propafenone in its tablets pharmaceutical dosage form. The obtained results can be used for evaluation of the quality and authenticity of the products.

Keywords

Vanillin, energy drinks, caffeine, vitamin B6, taurine, propafenone, voltammetry, pharmaceutical analysis.

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Introduction

Natural "vanilla extract" is a mixture of several hundred different compounds in addition to vanillin. Artificial vanilla flavoring is a solution of pure vanillin, usually of synthetic origin. Because of the scarcity and expense of natural vanilla extract, there has long been interest in the synthetic preparation of its predominant component. The first commercial synthesis of vanillin starts with the more readily available natural compound eugenol, artificial vanillin is made from either guaiacol or from lignin, a constituent of wood which is a byproduct of the pulp industry, Lignin-based artificial vanilla flavoring is alleged to have a richer flavor profile than oil-based flavoring; the difference is due to the presence of acetovanillone in the lignin-derived product (Brenes, et al., 1999).

The molecular structure of vanillin offers some special features, including hydrophobicity, capability to form the hydrogen bonds and reactive carbonyl group that could influence the fate of vanillin during various handling. Biological activity of vanillin can be, in a sum, characterized by antioxidant, antimicrobial, anti-diabetic, anti-inflammatory properties (Kumar, et al., 2012).

The desirable flavour and aroma properties of both methyvanillin (MVA) and ethylvanillin (EVA) have led to their widespread application in food technology, pharmacies, and perfumes. Moreover, it was found that MVA has many beneficial health properties such as inhibition of the oxidation of human low density lipoproteins which causes lower rates of cardiac disease mortality (Teissedre and Waterhouse, 2000) as well as an anti-sickling effect in sickle cell anemia sufferers (Farthing, et al., 1999). It can be assumed that EVA has identical biological activity like MVA due to their very close molecular structures. On the other hand, it should be noted that very high periodical intake of synthetic MVA and EVA can lead to headaches, nausea, and vomiting. A toxic effect on liver and kidney was also described.

Caffeine (CA) is one of the naturally occurring alkaloids that is widely contained in plant products and beverages. CA is a natural stimulant contained in many sources like coffee, tea, chocolate, soft drinks, and tablets for the treatment of many diseases such as asthma, nasal congestion, and headache and even for improving athletic endurance and facilitating weight loss. Many of CA consumers get it from multiple sources, their CA content varies with the type of source and its amount (Barone and Roberts, 1996; Christian and Brent, 2001; Frary, et al., 2005; Knight, et al., 2004).

However, reports on studies with human beings and animals showed that CA produces mental and behavioural effects like those of typical psychomotor stimulant drugs such as amphetamine and cocaine (Garrett and Griffiths, 1997). Stimulation of the central nervous system, diuresis, gastric acid secretion, and increased blood pressure are among the reported physiological effects associated with CA (Heaney, 2002). Among the different possible sources, coffee is known to have the highest CA concentration and yet the most utilized source of it (Knight, et al., 2004). However, the high amounts of CA can cause trembling, nausea, nervousness and seizures (Gaytan and Pasaro, 2012) and mutation effects such as inhibition of DNA (Barrès, et al., 2012).

CA is very attractive compounds for analytical chemists, thus its beverages (various type of coffee, tea, cola, cocoa, energy drinks) and drug formulations belong to the significant economic products in which the highest quality in international business is demanded (Murthy and Naidu, 2012). Owing to its common use, the eventual abuse, the important effects in human system and in respect to the ascending number of samples, the novel and perspective analytical methods providing rapid, sensitive and reliable detection and determination of CA are still necessary.

It is always needful to find such method that is the most appropriate for determination of CA in specific matrix, in the presence of interfering agents, as well as specific concentration range under the minimal elaborateness, the lowest economic and time difficulties (Guardia, et al., 2011). Moreover, with respect to the above-mentioned facts the detection and quantification of CA is important from analytical point of view and does not have the significance only in food and drug chemistry, but it can also give beneficial advice to people's health and life. Numerous studies aimed towards the development of analytical methods for determination of CA in different matrix (beverage, food, environmental, biological etc.) have been published.

Vitamin B6 (VB6) is a water-soluble substance that is converted inside the body into essential coenzymes for more than 100 enzymes in the human body. VB6 has three natural forms: pyridoxine, pyridoxal and pyridoxamine; all of which transform into its active forms in the body, which is the coenzyme pyridoxal 5-phosphate (Snell, 1958; Rabinowitz and Snell, 1947). VB6 deficiency can be observed clinically as seborrheic dermatitis, microcytic anemia, dental decay, glossitis, epileptiform convulsions, peripheral neuropathy, electroencephalographic abnormalities, depression, confusion, and weakened immune function (Vech, et al., 1975; Mueller and Vilter, 1950; Hawkins and Barsky, 1948). There is some proof of VB6 being effective in suppression of lactation as well as in relieving side effects of oral contraceptives such as depression and nausea.

Taurine (Tau) is a conditionally essential amino acid. While it can be synthesized by healthy adults from methionine and cysteine and it can be absorbed from food (generally skeletal muscle) that contain it, infants and children on taurine-free diets have less Tau in their blood plasma because they have not developed the capacity to synthesize it (Gaull, 1986). One effect of Tau deficiency is retinal dysfunction (Geggel, et al., 1985).

Energy drinks are type of non-alcoholic functional beverage that increase alertness and enhance the psycho-physiological responses in human (Rai, et al., 2016). They are relatively new consumable products that are similar to soft drinks, with additional additives and higher doses of caffeine (Howard and Marczinski, 2010).

Most energy drinks are consumed by children, adolescents, and young adults (Malinauskas et al., 2007; Heckman et al., 2010; Wolk et al. 2012). There are health concerns for children and young adults using these products, with a heavy focus on the concerns related to cardiovascular functioning, even though brain development should also be a consideration for these demographic groups.

It is essential to control the maximum limits of Tau in beverage and food. The European Authority for Food Safety reported that, acute health and fatal problems occurred due to the high consumption of energy supplements (Tsvetkova, et al., 2015).

Therefore, development and validation of accurate analytical methods for analysis of Tau in energy drinks is very important issue.

While energy drinks are often consumed alone, energy drinks are also often mixed with alcohol. While this topic has been reviewed elsewhere (Marczinski and Fillmore, 2014; Marczinski, 2015) and is not the central focus of this manuscript, it should be noted that the use of energy drink mixers increases the abuse potential of alcohol. Elevated rates of binge drinking and risk of alcohol dependence have been associated with alcohol mixed with energy drinks versus alcohol alone.

Results from laboratory studies indicate that when an energy drink (or caffeine) is ingested with alcohol, the desire to drink more alcohol is more pronounced in both humans and animals than with the same alcohol dose alone (Marczinski et al., 2013, Marczinski et al., 2016).Warning of a “grave danger” of adolescents drinking more alcohol than intended, and being more likely to drive after drinking alcohol mixed with energy drinks has been revealed. The U.S.

Food and Drug Administration took protective action in November 2010 by sending letters to four manufacturers of caffeinated alcohol beverages. The letters warned that caffeine could not be considered generally regarded as safe when combined with alcohol, and the products were reformulated to remove caffeine, guarana, and Tau.

Propafenone (PPF), chemically named, 1-{2-[2-hydroxy-3-(propylamino) propoxy] phenyl}-3-phenylpropan-1-one.

Because PPF was subjected to genetic polymorphic metabolism, the parent drug and metabolite(s) formation showed greater variability as documented by the data from the cardiac arrhythmic patient population (Siddoway et al., 1987). The extensive metabolizers of propafenone exhibited shorter mean elimination half-life (5.5 h) as compared with poor metabolizers of propafenone (Siddoway et al., 1987).

While the presence of 5-hydroxypropafenone was confirmed in all extensive metabolizers, it was not observed in poor metabolizers (Siddoway et al., 1987). Moreover, since PPF was subjected to a saturable biotransformation during first pass, the pharmacokinetics of PPF was dependent on the dose size and type of formulation used in the extensive metabolizers of the drug (Lee et al., 1990). Therefore, PPF was expected to show a high degree of inter-individual variability owing to the genetic make-up of individuals, especially with the expression levels and activity of CYP2D6 enzyme (Hii, et al., 1991; Lee et al., 1990).

Experimental

Chemicals

Analytical standards of MVA and EVA originating from Sigma-Aldrich (St. Louis, MI, USA.), 50% ethanol, Sodium dodecyl sulfate, cetylpyridinium chloride, and Triton X-100. Other reagents needed for preparation of supporting electrolytes such as 0.1 M Britton–Robinson buffer (boric acid, 98% glacial acetic acid, 85% phosphoric acid, and sodium hydroxide) were purchased from Lach-Ner. Analytical standards of pyridoxine, caffeine, taurine, calcium panthetonic, nicotinic amide, and ascorbic acid, with Nafion® and 99.9% methanol suitable for HPLC were purchased from Sigma-Aldrich (Prague, Czech Republic). Carbon nanomaterials (CNs), namely multiwall carbon nanotubes (MWCNTs). ≥99% o-phthalaldehyde, ≥97% ethanthiol, 99.99% lithium perchlorate, 99% sodium tetraborate and ≥99% boric acid were purchased from Sigma Aldrich (Prague, Czech Republic). NH_2*f*MWCNTswere obtained from DropSens (Llanera, Spain). The standard propafenone purchased from RECORDATI Pharm. Ind., Turkey. Ultrapure water (ρ=18.3 MΩ cm; Milli-Q system, Millipore) was used for preparing all the solutions.

Working electrodes preparation

CPE/SDS; Unmodified carbon paste electrode (CPE) was prepared by mixing 0.4 g graphite powder type CR-5 with 0.1 g silicon oil (SO) type MV 8000 in a ceramic mortar for 10 min. Chemically, SO is polydimethylsiloxane of low viscosity and is used commonly as pasting liquid. Resulting homogeneous carbon paste was packed into the cavity of Teflon® piston holder. Freshly prepared CPE should not be employed generally in any experiments due to their rather unstable electrochemical behaviour caused by an incomplete homogenization, especially when SO was used. Fresh CPE has therefore been ripened in laboratory conditions for 1 day.

GCE/Nafion®; Surface of solid GCE with diameter 3 mm from Metrohm (Prague, Czech Republic) was renovated on a polishing pad with water suspension of Al2O3 powder (particle size 1.0 μm) for 30 s. Dispersions (2 mg/mL) of each carbon nanomaterial in 1% Nafion® neutralized by 8% ammonia solution were prepared using ultrasonic bath for 30 min. The modification of GCE surface was made by dropping of corresponding 20 μL CNs dispersion and left to dry at laboratory conditions at least one hour.

GCE; Surface of the GCE (type 6.1204.600) with a diameter of 3 ± 0.1mm (Metrohm Prague, Czech Republic) was renewed on polishing pad in the presence of water suspension of Al2O3 powder of particle size 1.0 μm for 30 s. After the subsequent rinsing of the surface with demineralized water, the GCE was ready for next electrochemical experiment.

GCE/NH_2*f*MWCNTs; GCE surface of the GCE was prepared and renewed by polishing with alumina powder (0.05 μm) on a polishing pad and rinsed with distilled water. Then, 10 μL of NH_2*f*MWCNT suspension (containing1 mg/mL in DMF) was

added onto the GCE. Surface characterizationstudies were done by scanning electron microscopy (SEM) coupledwith energy dispersive X-ray (EDX) probe using a ZEISS EVO 40 apparatus (Merlin, Carl Zeiss).

Electrochemical setup

All electrochemical measurements were carried out in a 50 cm^3 glass cell at laboratory conditions. Typical three electrode system consisting the working electrode, Ag/AgCl/3.0 M KCl (reference electrode), and platinum sheet (auxiliary) and connected to potentiostat Autolab PGSTAT101 (Metrohm) compatible with software Nova version 1.11 was used.

Other apparatus

scanning electron microscopy (SEM) coupledwith energy dispersive X-ray (EDX) probe using a ZEISS EVO 40apparatus (Merlin, Carl Zeiss). Reciprocating shaker from Heidolph Instruments, Germany. Measurements in ultraviolet region was carried out with UV–Vis spectrophotometer UV2450 from Shimadzu (Kyoto, Japan) using 1 cm quartz cuvette from Fisher Scientific (Pardubice, Czech Republic) in the range of wavelengths from 400 to 200 nm at scanning speed of 0.5 nm s^{-1}. In the prepared solutions, values of pH were determined at pre-calibrated pH meter from Metrohm (Prague, Czech Republic).

Selected samples for analysis

Commercially available foodstuffs in Czech stores: vanilla sugar, vanilla biscuits and absolute vanilla vodka. Randomly selected energy drinks available in Czech markets: Red Bull, Semtex, Kong Strong and Crazy Wolf. Marketed samples, Rytmonorm®tablets containing 150 mg PPF per tablet, were manufactured byAbbott, Turkey.

Results and discussion

Voltammetric determination of ethylvanillin and methylvanillin

MVA and EVA are considered p hydroxybenzaldehyde differing by methoxy groups in ortho position. Using CV at SDS/CPE it was found that this difference does not have any great impact on their electrochemical behaviour (Fig. 1). In the first scan, both compounds provide only one sensitive oxidation signal at +0.66 V in PBS of pH 6.0 which corresponds to $2e^-$ and $2H^+$ transfer with nucleophilic addition of water and sequent release of appropriate alcohol, namely methanol (MVA) and ethanol (EVA). In subsequent scans, less sensitive redox couple (o-quinone/ catechol) is produced. According to this finding, it is necessary to state that it is impossible to recognize the MVA from EVA by voltammetric techniques. Therefore, only sum of these compounds can be determined in foodstuffs.

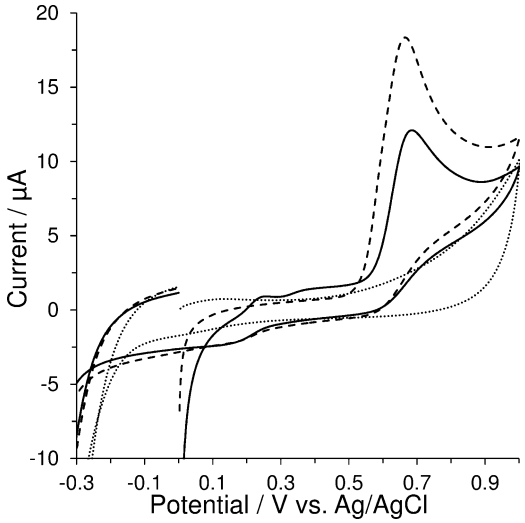

Fig. 1 *Cyclic voltammetry of blank (dotted line), 5.0 × 10 [4] M MVA (dashed line), and 0.5 mM EVA (solid line) at SDS/CPE performed in 0.1 M PBS pH 6 at 50 mV·s [1]*

In our approach, utilizing modification of the electrode surface by functional group of surfactants with the subsequent extraction of an adduct with the surfactant nonpolar alkyl chain into the lipophilic binder of CPE. All that is schematized in Fig. 2. Three different types of surfactants such as anionic sodium dodecyl sulfate (SDS), cationic cetylpyridinium chloride (CPC), and nonionic Triton X-100 were tested within optimization which resulted in immersing CPE into 3.0 × 10-4 M SDS as optimum condition for all measurements because of the maximum peak current response obtained.

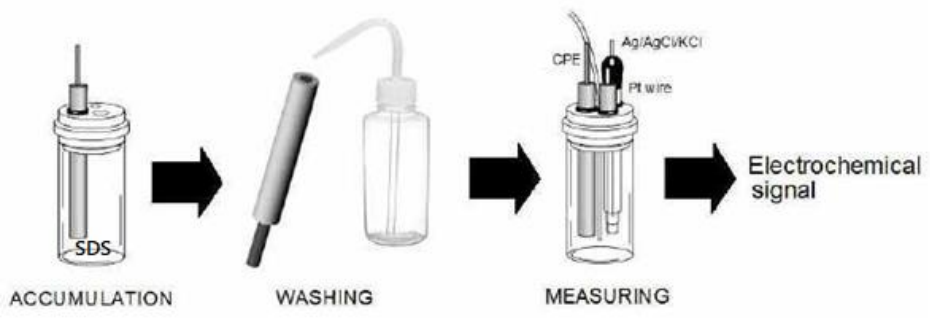

Fig. 2. *Proposed procedure for EVA and MVA electrochemical determination after extraction of SDS into CPE.*

Figure 3 shows typical voltammograms with corresponding calibration curve. Limits of detection (LOD) and quantification (LOQ) were calculated according to the already known equations LOD=3σ/k and LOQ=10σ/k, respectively, where σ is the standard deviation of measurement of 1.0×10^{-6} M EVA (N=10) and k is the slope of corresponding calibration curve Ip=0.435c–0.095 with coefficient of determination R^2=0.9965. Values 3.0×10^{-8} M and 1.0×10^{-7} M EVA for LOD and LOQ were calculated, respectively. For MVA, a calibration curve described by the equation Ip=2.52c–0.432 with coefficient of determination R2=0.9958 for linear range from 7.0×10^{-8} to 2.0×10^{-5} M and value of LOD 2.0×10^{-8} M were also determined.

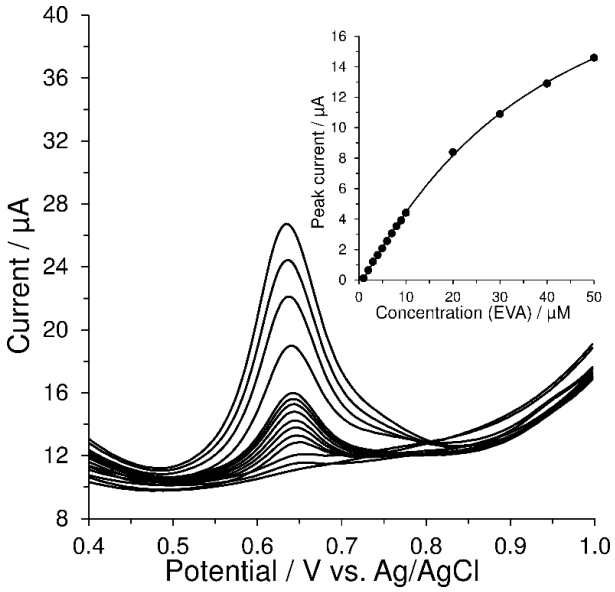

Fig. 3. *Voltammograms for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40 and 50 × 10^{-6} M EVA with corresponding calibration curve obtained at SDS/CPE measured in 0.1 M PBS pH 6 using SWV at potential step 5 mV, potential amplitude 70 mV, and frequency 50 Hz.*

In comparison with the standard HPLC method, the electrochemical approach offers some significant benefits. For example, one can appreciate a lower consumption of organic solvents, easier sampling preparation, shorter time of analysis, as well as simpler instrumentation.

Voltammetric determination of caffeine and vitamin B6

Experiments based on GCE/Nafion® modification for simultaneous determination of CA and VB6 have provided only one oxidation peak in 0.1 M BRB of pH 4.5 at peak potential values +0.882 and +1.349 V vs. ref., respectively (Fig. 4).

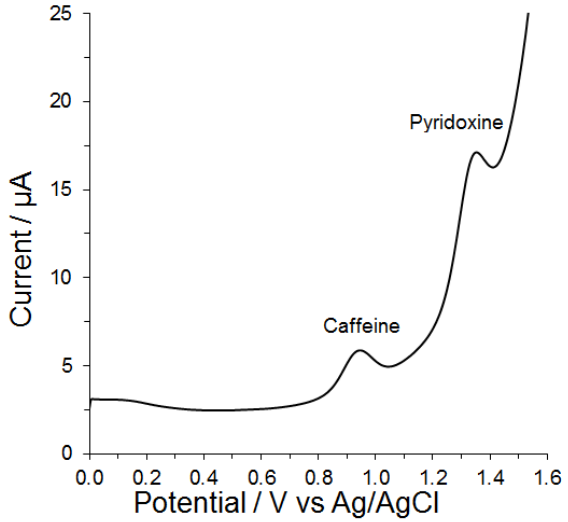

Fig. 4. *Differential pulse voltammograms of 200 µM caffeine and 100 µM pyridoxine 0.1 M BRB of pH 4.5.*

The optimization of electrochemical detection by DPV was focused on finding the proper pulse amplitude and scan rate, which both had highly affected the peak current. the effect of potential amplitude on the height of their anodic peaks is studied where no evident increase of peak current was obtained at amplitudes higher than 70 mV. The similar behaviour was observed under scan rate study. Setting values higher than 50 mV/s did not cause any further increase.

Typical oxidation responses to various concentrations of CA and VB6 recorded under optimized experimental parameters are shown in Fig. 5. Limits of quantification (LOQ) and detection (LOD) were calculated according to the equations LOQ=10σ/k and LOD=3σ/k, respectively, where σ is the standard deviation of minimally five repetitions (N=5) of chosen concentrations 0.3 mM CA and 20 µM VB6 in the linear ranges and k is the slope of corresponding calibration curves. Obtained linear ranges can be described by following equations, namely Ip(µA)=0.024c(µM)-0.181 with coefficient of determination (R^2) 0.9964 for CA and Ip(µA)=0.017c(µM)-0.250 with R^2=0.9976 for VB6. Relatively low and negative values of intercepts (q) allow the use

of the standard addition method for determination of CA and VB6 in selected energy drinks.

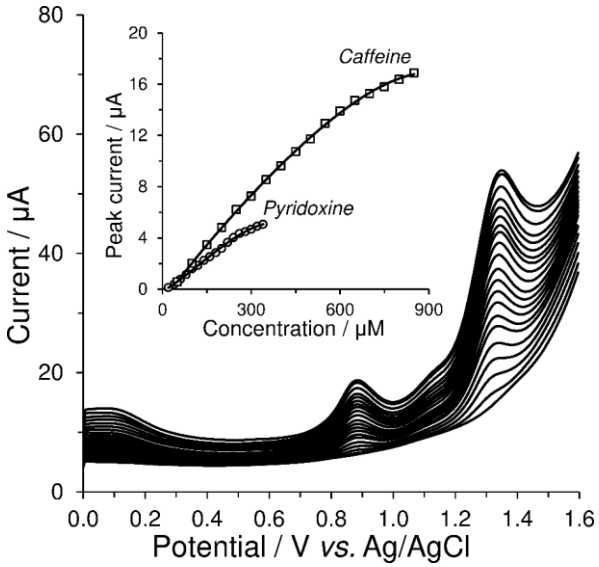

Fig. 5. *Voltammograms for 0 to 400 µM pyridoxine and 0 to 850 µM caffeine with corresponding calibration curves obtained at Nafion®/GCE. Measured using DPV at E_{step}=5 mV, E_{ampl}=70 mV, and v=50 mV/s*

Declared contents of CA and VB6 in selected energy drinks were compared with the results obtained using DPV and HPLC and good agreement between both methods was found. Moreover, it was observed that values of CA and VB6 corresponded well to the declared amounts listed by producers. Several samples of energy drinks purchased from Czech stores were analyzed by developed electrochemical method at optimum working conditions.

Electrochemical methods offer the practical advantages including operation simplicity, satisfactory sensitivity, wide linear concentration range, low expense of instrument, possibility of miniaturization, suitability for real-time detection and less sensitivity to matrix effects in comparison with separation and spectral methods.

Voltammetric determination of taurine after derivatization

A completely new voltammetric method has been developed for quantitative determination of food additive Tau in energy drinks. This electroanalytical method is based on voltammetric oxidation of o phthalaldehyde-ethanthiol derivative of Tau at glassy carbon electrode in 95% methanol containing 0.1 mol L^{-1} lithium perchlorate. Working conditions necessary for quantitative Tau derivatization reaction and electrochemical detection using square wave voltammetry were optimized. Linear range from 1.0×10^{-5} to 1.0×10-4 mol L^{-1} characterized by coefficient of determination 0.9998, limits of quantification 6.81×10^{-6} mol L^{-1} and detection 2.07×10^{-6} mol L^{-1} were obtained at pulse amplitude 50 mV and frequency 80 Hz. Analytical method of

calibration curve was used for monitoring of Tau in several commercially available energy drinks. This method was also compared with the reference RP HPLC method utilizing Tau pre-column derivatization with PITC with spectrophotometric detection. As well, it could find its application in the routine food analysis for small laboratories which cannot afford to acquire chromatographic instrumentation.

Tau represents an electrochemically low active amino acid. For potential range from -0.8 to +1.6 V, no voltammetric peak was obtained at GCE in the first cycle. As shown in Fig. 6, it was confirmed that Tau provides two broad oxidation peaks at +1.083 and +1.314 V within repetitive cyclic voltammetry. Their peak current responses increase with number of cycles, indicating an electrochemically controlled polymerization reaction.

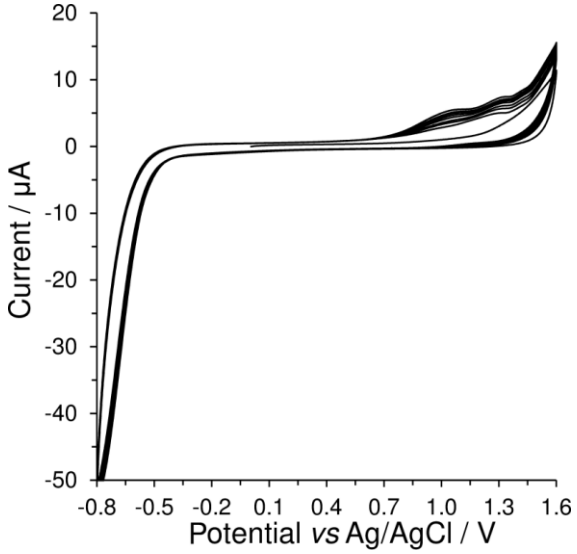

Fig. 6. *Repetitive (10 cycles) cyclic voltammetry of 0.5 mmol L^{-1} Tau performed in 95% methanol containing 0.1 mol L^{-1} $LiClO_4$ at potential step 5 mV and scan rate 50 mV s^{-1}.*

On the other hand, OPA-EtSH-Tau derivative provides one irreversible oxidation peak at +0.554 V. Moreover, it was found that derivatizing agent (OPA with EtSH) also provides very sensitive oxidation peak at +1.41 V. After some addition of Tau, an evident decreasing of its peak current response was observed (Fig. 7). It seems that both oxidation peaks could be used for analytical purpose. However, the firstly mentioned oxidation peak was chosen for determination of Tau in selected energy drinks because an interference of electroactive accompanying substances (pyridoxine and caffeine) is excluded.

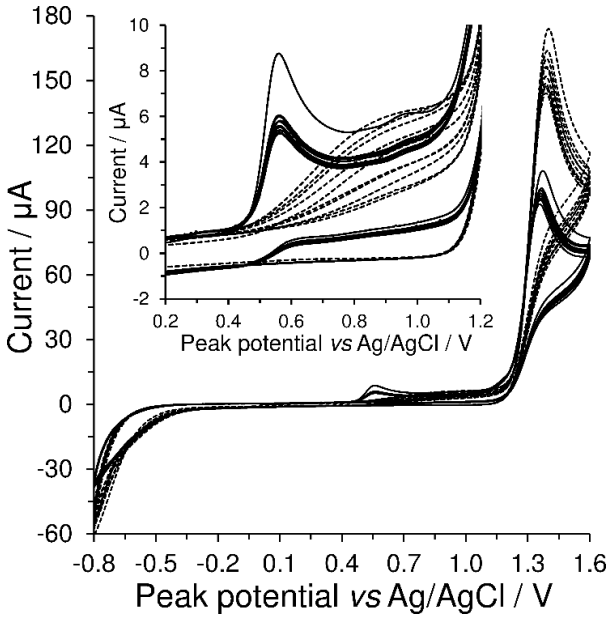

Fig. 7. *Repetitive (10 cycles) cyclic voltammetry of 3.7 mmol L^{-1} OPA with 7.1 mmol L^{-1} EtSH mixture (dashed) and their 0.5 mmol L^{-1} Tau derivate (solid line) performed in 95% methanol containing 0.1 mol L^{-1} $LiClO_4$ at potential step 5 mV and scan rate 50 mV s^{-1}.*

The performance of the proposed voltammetric method was studied at optimal conditions for derivatization reaction and proper SWV parameters. LOQ and LOD were calculated according to the equations LOQ=10s/k and LOD=3s/k, respectively, where s is the standard deviation of minimally ten repetitions (n=10) of chosen concentrations 20 µmol L^{-1} Tau (Ip=1.464±0.056 µA; presented as arithmetic mean and corresponding standard deviation) and k represents the slope of calibration curve from 10 to 100 µmol L^{-1} Tau. Precision of developed voltammetric method was evaluated using above-mentioned repetitive measurement. Value 3.8% of relative standard deviation (RSD) was determined. Linear dependence was characterized with equation Ip(µA)=0.0822c(µmol L^{-1})-0.2159 and coefficient of determination (R^2) 0.9998. Values 6.81 µmol L^{-1} of LOQ and 2.07 µmol L^{-1} of LOD were calculated. Moreover, if calibration curve is prolonged up to 200 µmol L^{-1} Tau, equation Ip(µA)=0.0745c(µmol L^{-1})+0.1293 with R2=0.9965 will be achieved. The calibration curve and corresponding voltammograms representing the linear range obtained are shown in Fig. 8.

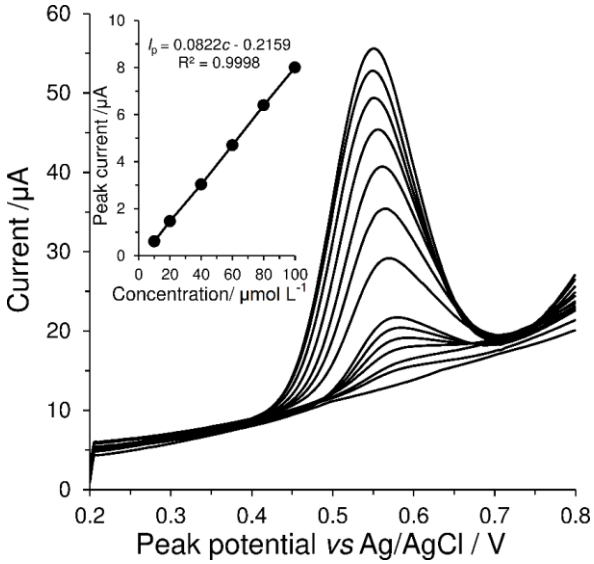

Fig. 8. *Voltammograms for 0 (blank), 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700 and 800 µmol L^{-1} Tau with corresponding calibration curve to 100 µmol L^{-1} Tau obtained at GCE. Measured in 95% methanol containing 0.1 mol L^{-1} LiClO4, 3.7 mmol L^{-1} OPA and 7.1 mmol L^{-1} EtSH using SWV at potential step 5 mV, potential amplitude 50 mV and frequency 80 Hz*

It can be concluded that developed voltammetric method provides comparable analytical results and, therefore can be use in practical laboratories. However, more precision results were obtained at reference RP-HPLC method (lower values of confidence intervals). Nevertheless, a lower consumption of organic solvents, lower initial cost of instrumentation and statistically comparable analytical parameters make the developed voltammetric method attractive. Moreover, it can be assumed that analogical procedures could be developed for analysis of foodstuffs containing dominantly only one amino acid.

Voltammetric determination of propafenone

A novel electroanalytical method for the determination of PPF in pharmaceutical dosage form and biological fluids using GCE/NH2fMWCNTs as a sensitive sensor.

The electrochemical response of PPF showed one well-defined oxidation peak around 0.9 V for NH2fMWCNTs /GCE, bare GCE and GCE /NH2fMWCNTs /AgNPs with scan rate of 100 mV/s. However, the best and sensitive peak was obtained in the case of using of NH2fMWCNTs /GCE; therefore, it was used as the most effective electrode modification for as the most effective electrode modification for building up a novel voltammetric method for its determination in pharmaceutical dosage form without any separation, evaporation, or otherwise difficult sample handling.further investigations (Fig. 9).

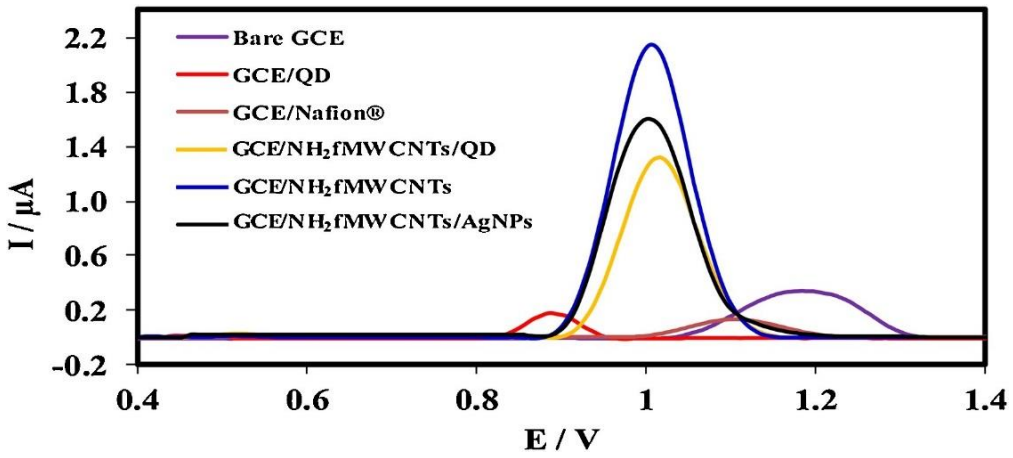

Fig. 9. *DPVs of 10µM PPF at bare GCE and its different modifications in BRB solution pH*

DPAdV of PPF at NH2fMWCNTs/GCE in BR buffer (pH 7.0) was used and the respective voltammograms recorded under optimal conditions. Thus, a linear relationship between concentrations and the corresponding peak currents was obtained in the ranges from 0.1 to 10 µM and from 10 to 100 µM with RSD ±3.9% for five measurements within the lowest concentration range has indicated a reasonable repeatability (Fig. 10). The limit of detection (LOD) and the limit of quantification (LOQ) were estimated to be 0.01 µM and 0.03 µM, respectively, showing rather high sensitivity of the developed method.

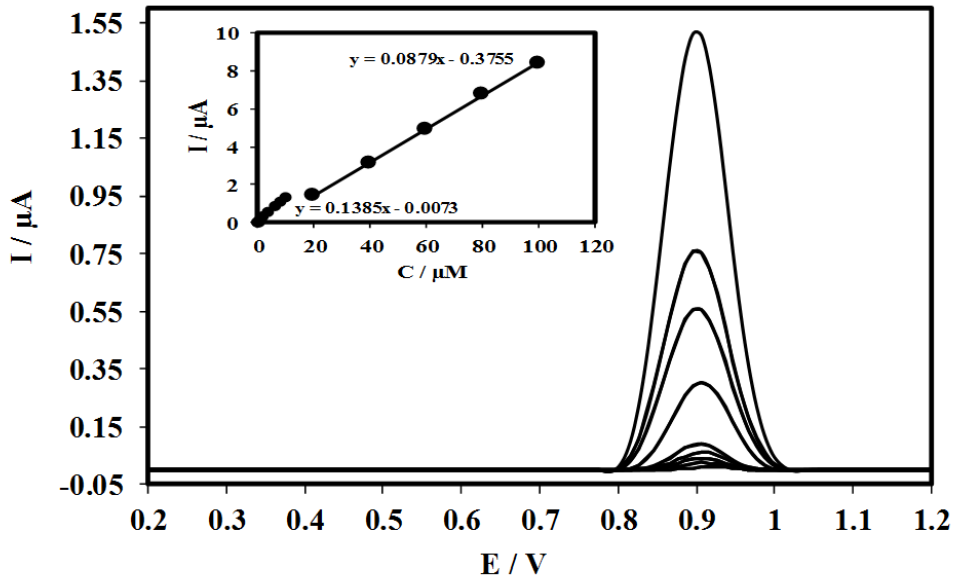

Fig. 10. *DPAdVs of PPF (0.1µM - 10µM), the inset shows the corresponding calibration curve.*

Satisfying results obtained by comparing the tablet-content values with the declared amount using the experimentally obtained calibration plot. A really excellent recovery (100.7%) confirms the reliability and accuracy of the proposed method for tablet dosage form and for the analysis of real samples. Moreover, the obtained results have shown that the developed method is not affected by the presence of different tablet excepients that may occur in the dosage form. Mostly, however, these excipients are not electrochemically active.

The obtained results and their statistical evaluation illustrate the suitability of the method with theNH2fMWCNTs/GCE in voltammetric measurements of PPF and for routine pharmaceutical dosage form analysis.

Coclusion

Different electroanalytical methods, how selected biologically active compounds in various kinds of foodstuffs and pharmaceutical dosage forms could be determined, are presented in this dissertation work. Working electrodes based on carbon materials, such as solid glassy carbon, various kinds of carbon pastes and carbon nanotubes, found a wide application in development of described analytical procedures. The CPE/SDS electrochemical sensor provides a sensitive tool for voltammetric determination of the sum of MVA and EVA in selected food samples. A simple modification was applied for this purpose, based on the immersion of unmodified CPE into an aqueous solution of this surfactant. Compared to the previously described sensors, the SDS/CPE provided significantly better analytical parameters.

An improvement in simultaneous determination of CA and VB6 was proposed at Nafion®/GCE performed using differential pulse voltammetry as electrochemical technique. Surface modification of GCE with thin layer of Nafion® results in high reproducibility and sensitivity of the measurement. The presented method can be considered as the first reported electroanalytical method for simultaneous determination of these biologically active compounds. In comparison with the standard HPLC method, the electrochemical approach offers a lot of advantages such as working without using organic solvents, cost-effective instrumentation, simple sampling preparation, and shorter analysis time. It can be assumed that developed electroanalytical method could be used in routine food quality control laboratories.

A simple and rapid electroanalytical method for Tau determination in commercial energy drinks has been developed. This method is based on direct voltammetric oxidation of OPA-EtSH-Tau derivative at GCE in 95% methanol containing 0.1 mol L−1 LiClO4 as supporting electrolyte. This method was also compared with the standard reference RP-HPLC method and obtained results show that presented voltammetric method provides statistically identical values, and therefore it could completely replace chromatographic method used.

A new sensing platform based on NH2fMWCNTs for the determination of antiarrhythmic drug PPF in pharmaceutical dosage forms representing the first electroanalytical method developed for this purpose with satisfactory analytical performance and short analysis time can be a useful alternative to commonly used separation and spectral techniques and also applying as a suitable reference method.

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Annexes

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