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Electroanalytical methods in determination of selected biologically active compounds

PhD DISSERTATION

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2021

Author's Declaration

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In Pardubice on ……...

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I would like to thank, of course my parents for everything that I am and other friends who gave me during my studies constantly help and support. Thanks to my wife Azza Abdellatif for her immense patience and emotional support.

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It was an honor to be granted this opportunity to work in UPCE, I am thankful for all that was given and for all that I have taken. I thank you all for the support and generosity that had helped me so much.

SUMMARY

This thesis is concerned with the development of electrochemical methods for determination of different biologically active organic compounds (caffeine, vitamin B6, ethylvanillin, methylvanillin, taurine and propafenone). It is divided into five basic parts. The individual sections contain critical comments to theoretical parts, biological significance of different analytes and their electrochemical activity.

The first part describes voltammetric determination of ethylvanillin total amount as a sum of contribution from ethylvanillin and methylvanillin. The attention was paid to the laboratory preparation of working carbon paste electrode, selection of the best modification of electrode surface, its extraction on electrode surface properly and investigation of the optimal conditions for measurement. The application of the modified electrochemical sensor is demonstrated in various analyses of real samples.

The second part focuses on developing a new electroanalytical method suitable for simultaneous determination of caffeine and vitamin B6 in different commercial energy drinks at glassy carbon electrode modified with a thin layer of Nafion® which resulted in improving the sensitivity and reproducibility of the measurements. It is also necessary to mention that presented method is considered as the first reported electroanalytical method for simultaneous determination of these biologically active compounds.

The third part is mainly dedicated to the determination of food additive taurine after its derivatization into o-phthalaldehyde-ethanthiol. The spot is placed on taurine derivatization reaction using o-phthalaldehyde and ethanthiol to produce highly electroactive isoindole derivative which can be voltammetrically oxidized at bare glassy carbon electrode, finding optimal working parameters and possibility of using developed method for analysis of samples dominantly containing only one amino acid.

The fourth part mentions the electrochemical sensing of antiarrhythmic drug propafenone in aqueous electrolyte using glassy carbon electrode modified with NH_2-functionalized multi-walled carbon nanotubes and successful application of developed method on dosage form without any complicated steps.

The fifth final part summarizes the results and discussion of all presented electrochemical methods mentioned throughout the dissertation.

Table of Contents

Chapter 4. Voltammetric determination of propafenone in pharmaceutical dosage form

Chapter 5. Summary of presented work

List of abbreviations and symbols

AcB	Acetate buffer
AdSV	Adsorptive stripping voltammetry
AgNPs	Silver nanoparticles
AuNPs	Gold nanoparticles
BDDE	Boron-doped diamond electrode
BRB	Britton–Robinson buffer
CA	Caffeine
cAMP	Cyclic adenosine monophosphate
CE	Capillary electrophoresis
CHD	Coronary heart disease
CNTs	Carbon nanotubes
CPC	Cetylpyridinium chloride
CPE	Carbon paste electrode
CV	Cyclic voltammetry
DME	Dropping mercury electrode
DMF	Dimethylformamide
DPV	Differential pulse voltammetry
EtSH	Ethanthiol
EVA	Ethylvanillin
GC	Gas chromatography
GCE	Glassy carbon electrode
GCPE	Glassy carbon paste electrode
HOPG	Highly ordered pyrolytic graphite
HPLC	High performance liquid chromatography
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MCPE	Modified carbon paste electrode
MVA	Methylvanillin
MWCNTs	Multi-walled carbon nanotubes
NH_2*f*MWCNTs	Amine functionalized multiwalled carbon nanotubescarbon nanotubes
OPA	*o*-phthalaldehyde
PB	Phosphate buffer

PC	Paper chromatography
PLP	Pyridoxal 5'- phosphate
PPF	Propafenone
PG	Pyrolytic graphite
QD	Quantum dot
RSD	Relative standard deviation
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SPE	Screen printed electrode
SWCNTs	Single walled carbon nanotubes
SWV	Square wave voltammetry
Tau	Taurine
TLC	Thin layer chromatography
UV	Ultraviolet
VB6	Vitamin B6

Introduction: Targets of dissertation

This thesis is focused on the development of new electroanalytical methods for determination of different biologically active compounds. During its elaborating, it was necessary to accomplish the following tasks to achieve the planned targets:

- Literature review about the analyzed compounds including key information about their biological activity and beneficial influence on human health.
- Description of electrochemical methods based on voltammetric techniques.
- Study of electrochemical behaviour of the compounds studied.
- Laboratory preparation of the working electrodes based on carbon materials and their use in determination of compounds of interest with potential applicability in food and pharmaceutical analysis.
- Optimization of important working parameters to obtain satisfactorily accurate and precise results.
- Comparing the results obtained with the already established standard methods to assure suitability and reliability of methods developed for analysis of real samples.

Chapter 1:

Voltammetric determination of ethylvanillin and methylvanillin

Theoretical considerations

The development of electrochemical cells intended for electroanalytical applications depends mainly on the working electrode. The material selected can enhance or hinder the desirable characteristics sought by researchers since all the electrochemical processes of interest take place at the working electrode surface. The choice of material used for the working electrode depends heavily on the system being electrochemically investigated (Švancara, et al., 2012). A wide variety of noble metal electrodes are available, the most common utilized being gold and platinum as they offer favorable electron rate kinetics and a large potential range. However, elemental metals can be highly reactive, undergoing electrochemical processes or forming oxides, which limits their electrical conductivity and results in the appearance of high background currents; such metal-oxide films strongly affect the rate kinetics of the electrode reaction, resulting in irreproducible data. Additionally, rather expensive noble metal electrodes make them unfavorable for use as disposable electrodes.

Carbon based electrodes are widely utilized due to their availability, a relative low cost (depending on its form), easily modification and high chemical inertness. There is a large range of carbon-based forms available for use as an electrode material, with various allotropic forms offering attractive properties (Švancara, et al., 2001). Of particular note and interest for this thesis, there are a whole host of commercially available working electrodes which utilize a large variety of graphite/carbon products, such as amorphous carbon, glassy carbon (GC), carbon black, carbon fibers, powdered graphite, pyrolytic graphite (PG) and highly ordered pyrolytic graphite (HOPG), each with different chemical and physical properties (Vytřas, et al., 2009)

The low cost and ease of manipulation of graphite has established its use as an electrode material in electrochemistry. The structural conformation and thus the degree of hybridization determines the physical, chemical and electronic characteristics of carbon-based electrodes. The orbital configuration of carbon's six electrons (in its ground-state) is 1s2, 2s2, 2p2. The small energy gap between the 2s and 2p electron orbitals aids in the promotion of one orbital electron to the higher energy p orbital that is unoccupied in this ground state. Conditional to the bonding interactions with adjacent atoms, the promotion credits carbon with the ability to hybridize its electron orbitals into a sp, sp2 or sp3 configuration. Covalent bonding with adjacent atoms tenders increased stability, compensating for the higher energy state of this electronic

configuration. This return is approximately equal for the sp2 and sp3 hybridization states after the out-of-plane π bonding among un-hybridized p orbitals is considered (Razeghi, 2019).

Hybridization of the carbon atom changes the crystal structure of the material. In the case of sp2 hybridization, the carbon atom remains with one free electron in the 2p orbital. Each of the sp2 hybridized orbitals then combines with other hybridized atoms/orbitals to form a series of planar hexagonal structures (Fig. 1). The free delocalized orbital is orientated perpendicular to this plane. Thus, the electron can move easily from one side of the carbon atom layer to the other but cannot easily move from one layer to the other. This phenomenon makes the material anisotropic.

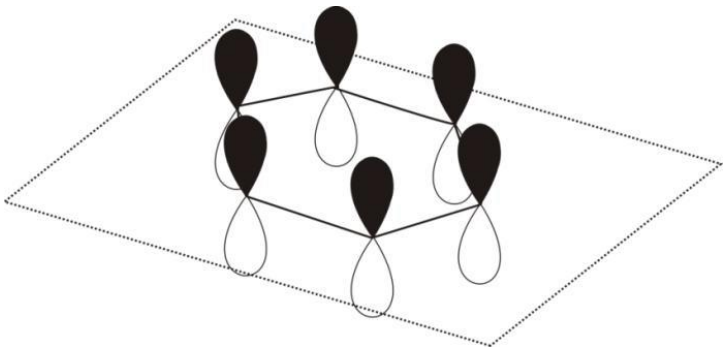

Fig. 1. *Schematic presentation of the sp2 hybridized structure of graphite*.

Graphite is comprised of a series of parallel planar layers, termed basal planes. Graphite has a perfect (defect free) hexagonal, crystallographic structure and should not be confused with other graphitic materials. The stacking of the basal plane occurs in two ordered structures, hexagonal or rhombohedral. The most found stacking order is hexagonal (or alpha) with a –ABABAB– sequence, superimposing the carbon atoms of alternating basal planes as shown in Fig. 2.

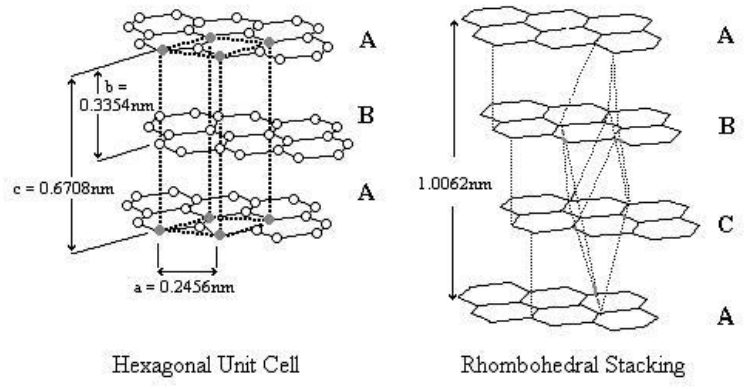

Fig. 2. *Schematic presentation of the sp2 hybridized structure of graphite.*

Carbon paste electrodes (CPEs) in voltammetry compromise the properties of a carbon powder mixed with a pasting liquid. The CPE is characterized by the decreased residual current and a better reproducibility of current response compared to the pure carbon material, thus improving detection limits and reproducibility of measurements (Adams, 1969). The key properties of the CPE depend on the specific components employed, the manner of preparation and maintenance. Different types of carbon powder had been tested when a multi-crystalline graphite was found to be preferable (Olson and Adams, 1960). Two carbon powders, GP-38M and UCP-1-M were compared without a matrix, both carbon powders were found to give high reaction rates for the oxidation of hexacyanoferrate (II) and 3,4-dlhydroxyphenylacetic acid. Further, oxidative pretreatment of carbon particles by chemical oxidants prior to paste preparation increased these rates (Rice, et al., 1983). Other carbon powders have also been used for CPEs (Neeb, et al., 1972), as well as for other carbon composite electrodes (McLean, 1982).

Practical investigations revealed that different pasting liquids are used for an optimal performance depending on the reactions studied (Lindquist, 1974). The influence of the shorter carbon-chain length of aliphatic hydrocarbons as pasting liquids on the rate of the electrode reactions was also studied (Rice, et al., 1983), when shorter chain length diminished the reaction rates. Despite examination of various liquid hydrocarbons, their commercial blend known as Nujol® was found most satisfactory.

Although CPEs have great utility in aqueous solvents, non-aqueous solvents are sometimes encountered in electrochemical methods and a problem arises due to the solubility of the binder in the solvent used. Typical binders such as Nujol or mineral oil have an appreciable solubility in non-aqueous solvents such as acetonitrile (Švancara, et al., 2012). Certain success has been accomplished by incorporating a surfactant, sodium lauryl sulfate, into the carbon paste mixture (Marcoux, et al., 1965), having prevented paste decomposition in acetonitrile, nitromethane, and propylene carbonate but being found less effective in other solvents, such as dimethylformamide, dimethylsulfoxide, benzonitrile, and glacial acetic acid. Ceresin wax can be used in organic solvents that are polar to medium polar. In this case it is beneficial to pre-treat the electrode surface with a surfactant to improve electron transfer rates (Albahadily and Mottola, 1987)

Other binders such as polyethylene (Armentrout, et al., 1979), Kel-F (Anderson and Chesney, 1980; Weisshaar, et al. 1981; Anderson, et al., 1978), teflon (Klatt, et al.,

1975), show good results in non-aqueous solvents like acetonitrile and methanol which are common effluents in liquid chromatography (LC) and high-performance liquid chromatography (HPLC).

It is noted, however, that these electrodes may require an electrochemical pretreatment for sufficient electron transfer rates. They also show much less paste consistency and more of a solid, hard consistency that resembles that of a glassy carbon or pyrolytic graphite electrode. Although the preparation is still simple and straightforward, it is questionable as to how easily the surface can be renewed.

There are basically two methods for modifying CPEs: (i) the modifier is added into the supporting electrolyte solution and thus chemically attached to functional groups on the graphite or (ii) modifier can be admixed with the carbon paste during its preparation.

The first method to retain the modifier at the electrode surface is to chemically attach it to the graphite particles. This prevents modifiers that are soluble in the solvent from dissolving into solution which would defeat the purpose of modifying the electrode. This also permits more intimate contact between the modifier and the graphite surface, enhancing electron transfer rates. In order to chemically attach the modifier, the graphite surface must contain reactive functional groups available for coupling. Graphite exists as carbon sheets sandwiched in a roughly parallel orientation. Oxygen containing functional groups such as carboxylic, phenolic, quinones, and lactones are believed to exist on the edges of these stacked layers (Coughlin and Ezra, 1968). The type and distribution of these groups depend on the previous history of the material, i.e. manufacturing and pretreatment processes (Mattson and Mark, 1971). In order to obtain a higher loading of modifier onto the graphite particles it is advantageous to further oxidize the graphite surface thus increasing the amount of oxygen functionalities such as carboxylic, phenolic, lactones, and quinones.

The second method of modification is more popular than the previously mentioned one because the amount of modification can be easily controlled as a weight percentage of the paste, a wide range of modifiers can be incorporated provided that they are compatible with the solvent and the binding material and the modified carbon paste electrode (MCPE) is extremely easy to prepare because neither chemical reactions nor special equipment is required.

The modifier should be insoluble or, in the worst case, minimally soluble in the electrolyte solution. This can be accomplished by using the modifier in the form of a

reasonably insoluble salt, by chemically derivatizing to decrease solubility, or by using a binding material such as wax. The effect of modifier solubility on the usefulness of the electrode should be mentioned and studied (Ravichandran and Baldwin, 1983). CPEs modified with phenylenediamine or N,N,N',N'-tetramethyl phenylenediamine showed loss of activity after prolonged standing in the electrolyte solution. This is due to the dissolution of the oxidized form of the modifier.

A simple modification process can result in a very powerful tool. A wide utility of MCPEs, ease of preparation, and enhancement of sensitivity and selectivity make them useful as electrochemical detectors and/or reactors. Their utilization is rapidly expanding because of these favorable characteristics and no doubt their presence will be felt for some time. This chapter focuses on electrode modification using surfactant inside measuring cell and its adsorption into CPE surface prior to measurements.

1.1 Biological activity of vanillin derivatives

Vanillin is the primary chemical component of the extract of vanilla bean. It is a phenolic aldehyde with the molecular formula $C_8H_8O_3$. Its functional groups include aldehyde, ether and phenol. It is the primary component of the extract of the vanilla bean. It is also found in *Leptotes bicolor* (Adahchour, et al., 1999) roasted coffee and the Chinese red pine (Blank, et al., 1992). Synthetic vanillin, instead of natural vanilla extract, is sometimes used as a flavoring agent in foods, beverages, and pharmaceuticals. Closely related is ethyl vanillin (EVA) having a stronger flavor compared to the native derivative, thus it is used widely in the food industry. It differs from vanillin by having an ethoxy group ($–O–CH_2CH_3$) instead of a methoxy group ($–O–CH_3$).

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 shown in Fig. 3 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).

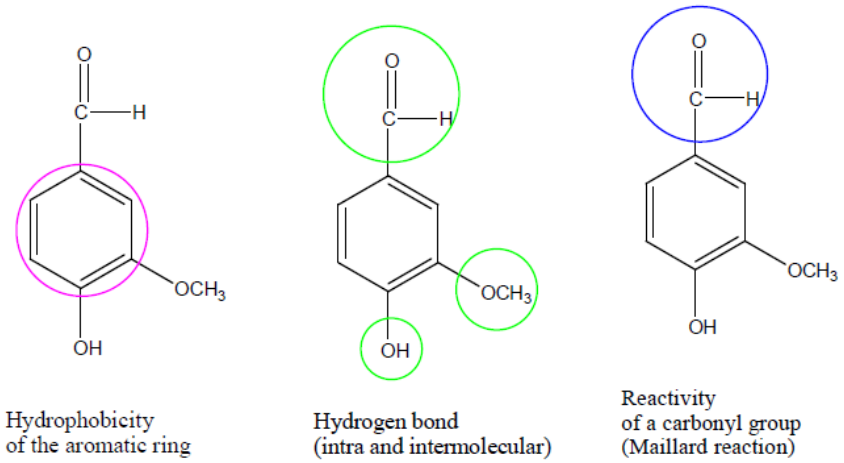

Fig. 3. *Chemical structural activity of vanillin.*

1.2 Modification of the electrodes with surfactants

Surfactant is a substance which tends to accumulate onto a surface or interface. For substances to be surface active, it is necessary to be adsorbed at the interface between bulk phases, such as oil and water or electrode and solution. The distinct structural feature of a surfactant is the hydrophilic region of the molecule or the polar head group and the hydrophobic region or a tail that consists of one or more hydrocarbon chains, usually with 6–22 carbon atoms. Thus, they are also called amphiphiles, i.e., compounds having both polar and nonpolar regions in their molecules. Depending on the chemical structure of the hydrophilic moiety bound to the hydrophobic portion, the surfactant may be classified as anionic, cationic, nonionic, and zwitterionic.

Surfactants can modify and control the properties of electrode surfaces. For decades, surfactants are used to alter or enhance reaction rates (Fendler and Fendler, 1975). More recently, surfactant have been used to control reaction pathways. Research on the influence of active surface substances upon the kinetics of electron transfer reactions at electrodes was rather frequent (Adamson, 1990; Heyrovsky and Kuta

1966; Franklin and Mathew, 1989; Georges and Desmettre, 1986; Franklin et al., 1988; Franklin and Sidraous, 1977; Shinozuka and Hayano, 1979). There was evidence as early as in the 1950s that surfactants could be used to control electrochemical reactions and solubilize organic compounds for electrochemical studies in water. They are found to control the electrochemical catalysis through their microstructures. Research since the late 1970s has demonstrated that coulombic and hydrophobic interactions with surfactants can stabilize various electrochemically produced ion radicals (McIntire, 1990).

More recent work concerning surfactants adsorbed from micellar solutions has focused on elucidating, or utilizing, aggregate structures formed on the electrode. Surfactant molecules generally adsorb at the interface between two bulk phases such as air and water, oil and water or electrode and solution. Adsorption of surfactants on electrodes can have a profound influence on electrochemistry in fluids. (Vittal, et al., 2006).

From a large choice of literature concerning chemically modified electrodes, it is clear that surface modification is an important area of both theoretical and applied electrochemistry and any research carried out in this direction will be of interest; especially, due to a wide applicability of the respective electrodes modified with surfactants. This can be documented on numerous examples, such as reports by Albahadily and Mottola, 1987; Hattori, et al., 1997; Kawakami, et al., 2000; Yuan, et al., 2009; and Mahanthesha, et al., 2009. In this respect, it is pleasant to see that a lot of work of this kind was also made by electroanalysts from the University of Pardubice, see e.g. Vytřas, et al., 1997; Švancara, et al., 1999; Švancara, et al., 2004; Galik, et al., 2006; and Švancara, et al., 2007.

1.2.1 Types of surfactants

Anionic surfactants

These have a negatively charged end of the molecule that gives it the hydrophilic part of the molecule, which is usually the case of sulfonates, sulfates, or carboxylates often neutralized by positively charged metal cations, such as sodium or potassium. Examples include sodium alkylbenzene sulfonates, sodium stearate (a soap), and potassium alcohol sulfates. Anionic surfactants are ionic and are made up of two ions: positively charged, usually metal ion and a negatively charged, organic ion phenolic

compounds such as sulfonate, phosphate, sulfate and carboxylates. Alkyl sulfates include ammonium lauryl sulfate, sodium lauryl and the related alkyl-ether sulfates, sodium laureth sulfate, also known as sodium lauryl ether sulfate, and sodium myreth sulfate. These are the most common surfactants and comprise the alkyl carboxylates (soaps), such as sodium stearate. The stearates comprise >50% of the global usage of surfactants. Many of these find utilization in emulsion polymerization.

Cationic surfactants

These are positively charged molecules usually derived from compounds containing the so-called quaternary nitrogen atom, N^+, bearing either long alkyl chains or being a part of hetero rings. Such compounds are not commonly used as cleaning agents in hard-surface cleaners because of the tendency of the cationic positively charged molecule to be attracted to hard surfaces (that usually have a net negative charge). Many cationic surfactants have bactericidal or other sanitizing properties useful for creating disinfectants that leave a cationic disinfectant film on the surface. Examples are cetylpyridinium chloride, benzalkonium chloride, and benzethonium chloride. The cationic nature of the surfactants is not typically consistent with the non-ionic and anionic charges, and they disrupt cell membranes of bacteria and viruses. Permanently charged quaternary ammonium cations include alkyl trimethylammonium salts: cetyl trimethylammonium bromide and cetyl trimethylammonium chloride.

Non-ionic surfactants

These are surfactants that do not form the ions. They derive their polarity from having an oxygen–rich portion of the molecule at one end and a large organic molecule at the other. The oxygen component is usually derived from short polymers of ethylene oxide or propylene oxide. Here, however, one should be aware of the fact that only in water chemistry, the oxygen is a dense electron-rich atom that gives the entire molecule a partial net-negative charge which makes the whole molecule polar and able to participate in hydrogen bonding with water. Examples of nonionic surfactants are alcohol ethoxylates, polyethylenoxy alcohols, and ethylene oxide/propylene oxide.

Zwitterionic surfactants

Zwitterionic (amphoteric) surfactants possess both cationic and anionic centers attached to the same molecule. The anionic part can be variable and include sulfonates, as in the sultaines CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1-

propanesulfonate). Betaines such as cocamidopropyl betaine have a carboxylate with the ammonium. The cationic part is based on primary, secondary, or tertiary amines or quaternary ammonium cations. Zwitterionic surfactants are often sensitive to pH and will behave as anionic or cationic based on pH.

Different types of surfactants showing major differences in their compositions are illustrated in Fig. 4.

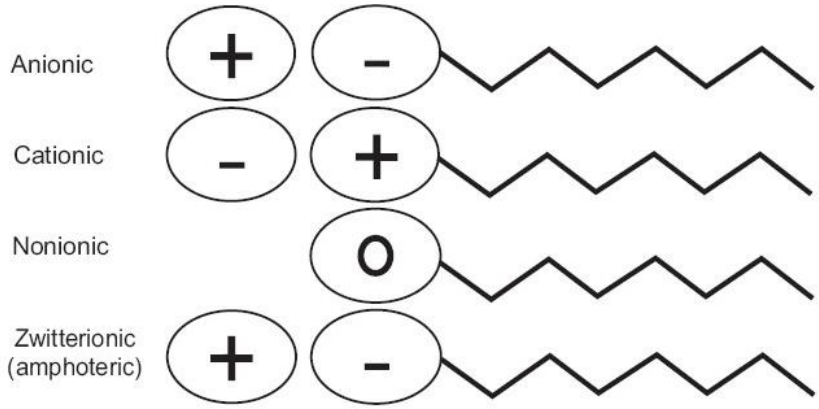

Fig. 4. *Schematic illustration of various types of surfactants.*

1.3 Electrochemistry of vanillin

Vanillin is an electroactive compound and it is possible to quantify its amount in vanilla and in the final products through the study of its oxidation. Procedures based on electroanalytical methods [amperometry, differential pulse voltammetry (DPV) or square-wave voltammetry (SWV)] for the detection of vanillin in food samples have been reported in literature (Luque, et al., 2000; Agüí, et al., 1999; Hardcastle, et al., 2001). It can be assumed that EVA has identical biological activity like MVA due to their very close molecular structures. The respective methods were successfully applied to selected foodtuffs containing vanillin and the results were more than satisfied on the basis of accuracy and reproducibility.

However, the electrochemical analysis of vanillin was limited by the problem of electrode surface fouling, and the need of time and reagents to regenerate the electrode surface. Numerous voltammetric methods which have been developed to determine the methyl vanillin (MVA) only (Li, et al., 2015; Filik, et al., 2017; Wu, et al., 2017; Sýs, et al., 2017) offer some valuable advantages like economically available instrumentation, manipulation with small volumes, high sensitivity, and relatively rapid analysis.

However, it should be emphasized that both compounds differ structurally only by similar substituents (methyl in MVA, ethyl in EVA), which does not affect their electrochemical behavior; nevertheless, voltammetry is unable to distinguish these compounds and their content has to be expressed as a mass equivalent of EVA. Furthermore, it is important to note that their direct electrochemical oxidation at the bare working electrodes lacks the required reproducibility and this drawback is probably due to non-specific adsorption of oxidation products. This is the reason why electrode surfaces are often modified, which seems to the most rational way to overcome this obstacle (Bettazzi, et al., 2006; Kong, et al., 2010; Peng, et al., 2012; Shang, et al., 2014; Zheng, et al., 2010; Deng, et al., 2015; Crevillen, et al., 2007). Such sophisticated electrochemical sensors are usually based on composite electrode materials (Sýs, et al., 2017) or on a glassy carbon electrode modified with various kinds of nanomaterials, such as nanotubes, metal nanoparticles and their combinations or, newly, with graphene.

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Voltammetric determination of caffeine and vitamin B6

Chapter 2.

Theoretical considerations (Chapter 2)

Alkaloids are broad category of nitrogen-containing organic metabolites produced by plants; the plants that produce these alkaloids make their leaves unattractive to eating by insects and higher animals (Cauli and Morelli, 2005). The well-known toxic compounds morphine, quinine, cocaine, and codeine belong to this group of compounds. Caffeine (CA), chemically known as 3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione (Fig. 5a) 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 behavioral effects similar to 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). A fatal dose of CA has been evaluated to be more than 10 g (about 170 mg kg^{-1} of body weight). It is also considered to be a risk species for cardiovascular diseases, kidney malfunction and may also cause hyperactivity (Wardle, et al., 2012). CA is usually prescribed as analgesic adjuvant in drug formulations for the treatment of headache and pain related to postpartum, postoperative, and dental surgery and therapeutically used for the treatment of migraine in combination with other drugs such as aspirin, paracetamol and ascorbic acid (Fernández-Dueñas, et al., 2008). Many contributions dealing with impact studies of CA on human health have been reported (Peck, et al., 2010; Glade, 2010; Taylor, et al., 2011, Jura, et al., 2011; Moy and McNay, 2013).

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) chemically named, 4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol (Fig. 5b) 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.

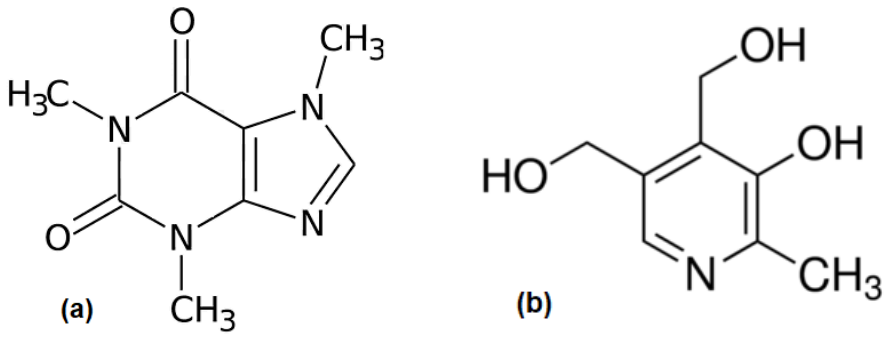

Fig. 5. *Chemical structure of (a) caffeine and (b) vitamin B6.*

2.1 Biological activity of caffeine and vitamin B6

Natural products are ancient promising sources used in traditional medicines. Plants have tremendous capacity to synthesize extraordinary secondary metabolites which possess different biological activities. Use of plant metabolites with antimicrobial activity can be of great importance as far as therapeutic treatments are concerned. Herbs of tea and coffee are the ones that have been studied earlier for the same (Stefanović and Comic, 2012). Tea and coffee are the most popular beverages all over the world and are being consumed since ancient times.

The biological properties of tea and coffee attributed to the presence of methylxanthins such as caffeine and phenolic compounds like catechins. Caffeine (1,3,7-trimethylxanthine) is one of such compounds present in coffee as well as tea. Amongst the biological activities of caffeine include immunomodulatory activity, central nervous system stimulatory activity, antimicrobial, anticancer and antioxidant activity. (Adwan and Mhanna, 2008). CA is known to exhibits various biological functions. Some of its reported actions include inhibition of phosphodiesters, which increases the level of intracellular Cyclic adenosine monophosphate (cAMP), CA has direct effect on intracellular concentration of calcium and indirect effect with response to membrane hyperpolarisation. CA also blocks enzymes such as 5’ nucleotidase and alkaline phosphatase. It has been reported that CA changes the inhibitory effect of antimicrobial agents. In recent times there has been lot of interest increasing about anti-infective activity of such bioactive compounds (Archana and Abraham, 2011). This can be an alternative approach to combat bacterial pathogens which selectively disrupt pathways that mediate virulence in them. The ability of such compounds to act synergistically with antibiotics could be a new approach to solve the problem of antimicrobial resistance.

To explain the mode of action of CA against microorganisms, it has been found that caffeine can pass easily to the cell wall of the bacteria. Then, CA can begin to inhibit DNA synthesis. Lower DNA causes to lower activity in all bacteria cells. Therefore, enzyme synthesis and protein synthesis does not happen (Sundarraj and Dhala, 1965). However, this inhibition process of CA in bacteria can be stopped when it is converted into theobromine and para-xanthine by bacteria enzymes (demethylases) before going to oxidation process (Blecher, et al., 1977). In mold, CA can inhibit its spore germination (Kumar et al., 1995). Therefore, the lag phase of mold spore contamination is extended

and is observed. CA degradation of molds (*Aspergillus tamari, A. niger, A. fumigatus and P. commune*) is started when the nitrogen source is insufficient. Aspergillus sp. showed the most efficient caffeine degradation (92%) (Hakil et al., 1998). Furthermore, CA can inhibit aflatoxins from molds by stopping the synthesis of glucose, fructose, and maltose. These sugars are substrate for aflatoxin produce of mold (Buchanan et al., 1983; Hasan, 1999; Aneja and Gianfagna, 2001; Holmes et al., 2008).

On the other hand, the B vitamins are a group of water soluble, chemically quite distinct compounds to which other than vitamin B6, vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin or niacin amide), B5 (pantothenic acid), B7 (biotin), B9 (folic acid), and B12 (various cobalamins) also belong (Roje, 2007). Historically, it was believed that only one vitamin B existed with a critical function for maintenance of growth and health and prevention of characteristic skin lesions in animals and human (Birch, et al., 1935). However, with ongoing research it became obvious that vitamin B comprised a group of compounds that was collectively called the 'vitamin B complex'.

Vitamin B6 (VB6) itself is an enzymatic co-factor required for more than 140 biochemical reactions including transaminations, aldol cleavages, α-decarboxylations, racemizations, β- and γ- eliminations, and replacement reactions. Most of these reactions are related to amino acid biosynthesis and degradation, but VB6 is also involved in other processes including sugar and fatty acid metabolism (Percudani and Peracchi, 2009). It comprises a set of three different pyridine derivatives called pyridoxine, pyridoxal and pyridoxamine. Furthermore, all three B6 family are phosphorylated by a kinase, which is a requirement for their role as cofactors in enzymatic reactions. While pyridoxamine-5'-phosphate has been reported to function as a co-factor, it is pyridoxal 5'-phosphate that is the biologically most active form (Brouwer, et al.,1998; Amadasi, et al., 2007). Since its discovery in 1932, VB6 has been discussed in relationship to health issues (Ohdake, 1932). VB6 was associated with pellagra, a skin disease that is based on multi-vitamin deficiencies that mostly occurs in context with niacin undersupply (Oka, 1999; Nogueira, et al., 2009; Hendricks, 1991).

Furthermore, a recent U.S. study, which tested the blood pyridoxal 5'-phosphate (PLP) levels in around 8,000 patients, demonstrated a widespread deficiency of the vitamin among all tested subgroups, and the authors suggested an increase of the daily allowance from around 2 mg to 3 to 4.9 mg per day (Morris, et al., 2008). It has been

reported for animal models, that continuous uptake of very high doses (e.g. 400 mg/kg) can lead to peripheral sensory neuropathy and nerve degeneration (Perry, et al., 2007; Albin, et al., 1987). These problems are generally reversible when supplementation is stopped. Additionally, some studies have suggested that increased levels of VB6 and some derivatives can generate toxic photoproducts as a result of UV irradiation (Lu, et al., 2008; Wondrak, et al., 2004; Maeda, et al., 2000). However, the applied daily dosages were far beyond any physiological concentrations an organism is normally exposed to, making it unlikely that such VB6 induced impacts will be observed. Because of the great interest in VB6 as a therapeutic and pharmaceutical compound, its reactive capability, and its potent antioxidative characteristics.

Other aspects in which VB6 is directly discussed to play an important role are cardiovascular disease and high blood pressure. Coronary heart disease (CHD) is one the major reasons for death worldwide. It is caused by atheroma, which are swollen artery walls due to the accumulation of cell debris containing e.g., fatty acids and cholesterols that negatively affect blood flow. Though the impact of VB6 is controversially discussed a variety of works indicate positive effects of VB6 on CHD (Pires, et al., 2008; Ishihara, et al., 2008). For instance, a large study in Japan, comprising 40,803 subjects, recently showed that VB6 has the potential to reduce the risk of CHD, and especially nonfatal myocardial infarction, among middle-aged (40–59 years) non-multivitamin supplement users (Ishihara, et al., 2008). Here, an increase of daily supplementary VB6 intake from 1.3 to 1.6 mg already significantly reduced the number of affected patients with reported CHDs and MIs (Ishihara, et al., 2008). Similarly, the Coronary Health Project and other studies indicate a correlation between increased vitB6 intake and reduced risk of CHD [Gonzalez, et al., 2007; Czeizel, et al., 2004; Merrill, et al., 2008; Booth and Wang, 2000). It is noteworthy that often other vitamins like folates or cobalamins are tested in these studies as well with similar positive effects in reducing the risk of CHD.

The precise reasons for the beneficial impact of VB6 are unclear. One suggested reason is that VB6, like folates and cobalamins, can lower homocysteine levels in the blood by converting the amino acid to cysteine or methionine, respectively. VB6 is required as a cofactor for cystathionine-β-synthase, a PLP-dependent enzyme that converts homocysteine to cysteine via a cystathionine intermediate (Nozaki, et al., 2001). Because high levels of homocysteine are often associated with an increased chance for

atherosclerotic diseases, it is considered a risk factor like high blood pressure, active smoking, or adverse blood lipid profiles (Graham, et al., 1997). But it is not generally accepted whether VB6, folates, or cobalamins do indeed reduce the blood homocysteine levels, as one review indicates, thus still awaiting additional proof (Marti-Carvajal, et al., 2009).

2.2 Nafion® as a modifier in electroanalysis

Glassy carbon (GC) has been extensively studied since it had been first produced in the mid 1960's originally as the so-called or reticulated or vitreous carbon (Noked, et al., 2011) , and is the most extensively employed carbon material for the electrodes due to its low density, high conductivity, chemical inertness, and impermeable to gas, as well as wide availability and inexpensiveness (Harris, 2005; Zoski, 2007; Zittel and Miller, 1965; Shigemitsu, et al., 1979; Walker, 1972; Yamada and Sato, 1962). GC exhibits relatively low background currents and wide potential range (a.k.a. window) compared to conventional metal electrodes (Zoski, 2007). A major disadvantage of GC electrodes is its high sensitivity or even vulnerability towards surface mechanical damage, surface pre-treatment as well as the electrode history; this leads to a variation in the observed electrochemistry (Van der Linden, et al., 1980; McCreery, 2008). The exposed graphitic edges and oxygen containing functional groups have been proposed to be the active sites for certain reactions (Chen and McCreery, 1996; Zoski, 2007).

GC electrode material has been extensively employed to study the electron transfer kinetics of a wide range of mediators; where the effect of surface termination as well as surface pre-treatment have been comprehensively studied (Kneten and McCreery, 1992; Rice, et al., 1990; Chen and Swain, 1998). The resistance of GC to corrosion and chemical inertness under a wide variety of conditions, has led to its use in extremely corrosive environments over a wide range of electrical potentials (Van der Linden, et al., 1980). On the other hand, although GC is chemically inert, its sp2 content and high density of oxygen containing functional groups leaves it eager to the adsorption of molecules, especially biomolecules, via intermolecular forces (McCreery, 2008). This means the GC surface is highly susceptible to surface deactivation.

The rate of electron transfer at the GC surface is, in general, satisfactorily rapid, but it can be yet improved via various activation techniques: electrochemical pre-treatment (Engstrom, 1982; Alsmeyer and McCreery, 1991), mechanical polishing (Hu, et al.,

1985; Kamau, et al., 1985), laser treatment (Rice, et al., 1990, Pontikos and McCreery, 1992), plasma treatment (Kusano, et al., 2007; Miller, et al., 1981), and vacuum heat treatment (Fagan, et al., 1985; Stutts, et al., 1983) which lead to an adventitious change in either the surface termination or/and microstructure (McCreery, 2008; Van der Linden, et al., 1980; Kamanu, 1988). Yet another way is mechanical polishing with alumina is the easiest method for renewing a clean and activated electrode surface, where oxygen containing functional groups are incorporated physically (Sato, 2003; McCreery, 2008; Zoski, 2007). However, this means the surface characteristics and/or area of the electrode may change over time, with each polishing of the electrode surface revealing a new layer of material, with potentially different properties (Engstrom, 1982).

Nafion®, a copolymer of tetrafluoroethylene and sulfonyl fluoride vinyl ether, is one of the most interesting solid polymer electrolytes for technological and scientific purposes. It is used as ionomeric permselective membrane for a variety of electrochemical applications including fuel cells, water electrolyzers and chlor-alkali cells,(Xing and Savadogo, 2000; Pillai, et al., 2000) chemically modified electrodes (White, et al., 1982; Martin and Dollard, 1983), ion-selective and potentiometric sensors (Martin and Freiser, 1981; Ugo, et al., 2002), voltammetric sensors (Ugo and Moretto, 1995), and electrochromic devices(Sabatani, et al., 1996; Bertoncello, et al., 2002).

For electroanalytical purposes, Nafion® coated electrodes has been prepared mainly by depositing a film of the polymer prepared from water-alcohol solutions (Ugo and Moretto, 1995). Such modified electrodes were widely used for preconcentrating and determining trace concentrations of electroactive cations (Ugo and Moretto, 1995; Ugo, et al., 2002). Voltammetric signals relevant to the preconcentrated analytes are, in fact, increased by a factor dependent on the partitioning within the film (Ugo and Moretto, 1995). However, the much lower values for the apparent diffusion coefficients that rule mass and charge transport phenomena within the coating (Buttry and Anson, 1981), can sometimes be in contrast with the increase in sensitivity expected only on the basis of pre-concentration effects.

In general, fluorocarbon polymers are hydrophobic in nature, but Nafion® is an exception due to its partial solubility in water determined by the presence of sulphonic groups, which exhibit a very strong acidic character. The water content of the membrane

affects greatly its properties, since it determines the type of counter ions incorporated within the polymer matrix (Gebel and Lombard, 1997, Mauritz, 1998) as well as the dynamics of diffusive processes within the coating layer (Martin and Dollard, 1983). Also, the ionic conductivity in Nafion® is understood to be strongly dependent on the morphology and local structural order of the film (Okada, et al., 1998). All these evidences support the relevancy of developing deposition methods able to control the film architecture down to the molecular level.

2.3 Electrochemistry of caffeine and vitamin B6

Generally, the electrochemical methods have rarely been used for the determination of CA. The major drawback in the use of conventional bare electrode materials (e.g. GC, graphite, CP) at which the oxidation of CA occurs at a very positive potential; typically, at more than +1.3 V vs. Ag/AgCl electrode). Thus, the determination may be limited by usable potential range of electrode material or by overlaps with the potential of the electrolytic solution in anodic side often leading to less reproducible results (Brunetti, et al, 2007; Spătaru, et al., 2002; Martínez-Huitle, et al., 2010). This fact must be considered when one optimizes experimental conditions (selection of appropriate working electrode material and supporting electrolyte). In order to avoid the overlapping of the oxidation peak of CA with the electrolyte, several types of carbon-based electrodes have been examined using electrochemical techniques. Nevertheless, rapid growth of reports dealing with electrochemical determination of CA can be observed for the last years. The main reason is the use of miscellaneous modifiers and applicability of boron-doped diamond (Švorc, et al., 2012; Da Silva, et al., 2017; Yiğit et al., 2016) as the versatile electrochemical tools largely improving the sensitivity and selectivity. Recently, the carbon-based electrode materials are frequently used in the electrochemical laboratory because of their availability in various forms and shapes, and usefulness over the relatively wide potential range in cathodic and anodic area (Centi and Perathoner, 2010). The most commonly used carbon-based material in the analytical laboratory known for its chemical stability and relatively large over-potential of oxygen and hydrogen evolutions is glassy carbon electrode (GCE) (Geremedhin, et al., 2013). The first report dealing with the determination of CA at the GCE without the sample pretreatment was presented (Sontag and Kral, 1979). However, in this case, the strong adsorption of CA had required the mechanical and electrochemical pretreatment of GCE surface. Consequently simple, rapid and accurate differential pulse voltammetric (DPV)

method for the determination of CAF simultaneously with PAR and AA in drug formulations on GCE was reported. (Lau, et al., 1989). Unmodified pseudo carbon paste microelectrode (CPME) by mixing graphite powder with paraffin wax was prepared and studied the electrochemical behavior of CA (Mersal, 2012). This electrode showed the good sensitivity and high selectivity for the direct determination of CA using square wave voltammetry (SWV). The oxidation of CA and its irreversible electrochemical behavior on bare BDDE using cyclic voltammetry and linear sweep voltammetry (LSV) were studied (Spătaru, et al., 2002). They proposed the oxidation scheme of CA which is similar to mechanism supposed by the first authors investigated the oxidation of CA (Hansen and Dryhurst, 1971). It can be concluded that overall process involves four electrons ($4e^-$) and four protons ($4H^+$) as depicted in Fig. 6.

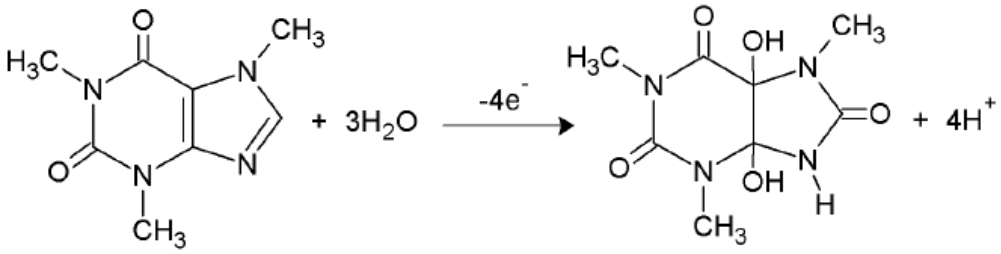

Fig. 6. *Mechanism of overall oxidation of caffeine*

As it is mentioned previously, the oxidation of CA occurs at the very positive potential overlapping with the oxidation of the background medium, which gives the analysis the low reproducibility. Several solutions including the use of bare electrodes with wide range of polarization or modified electrodes were proposed. Over the years, many types of modified working electrodes have been developed and used in various ways for voltammetric measurements. In order to enhance the sensitivity and stability of the measurements, the electrodes modified with designable molecules have been used in electrochemical determination of CA. Although the majority of modified electrodes successfully quantified the concentrations of CA, the fabrication of the electrochemical sensor is still one of the challenging tasks for the researchers interested in this research work. On the other hand, cyclic voltammetric experiments were made to study the redox behavior of VB6 at a conventional GCE and the observed voltammetric pattern was in agreement with previous literature findings (Hernandez et al., 2003; Qu et al., 2004; Desai et al., 2008; Wu et al., 2005): the oxidation system is characterized by an anodic peak in the forward step and by the absence of any peak on the reverse scan, indicating

that the reaction is irreversible. The detailed electro-oxidation mechanism of pyridoxine may involve complex multi-step/multi-electron reactions (Crevillen et al., 2008; Hernandez et al., 2003; Wang et al., 2005). It is also known (Hernandez et al., 2003; Wang et al., 2005; Gonzalez Rodriguez et al., 2011; Pineda et al., 2000) that the process is pH-dependent due to the complex distribution of species resulting from acid-base and hydration equilibria. It has been reported (Hernandez et al., 2003; Tan et al., 2004; Pineda et al., 2000; Cao et al., 2004; Pournaghi-Azar et al., 2007) that the oxidation proceeds with the formation of the aldehyde (pyridoxal) and the subsequent oxidation to pyridoxic acid, according to the scheme reported in Fig. 7.

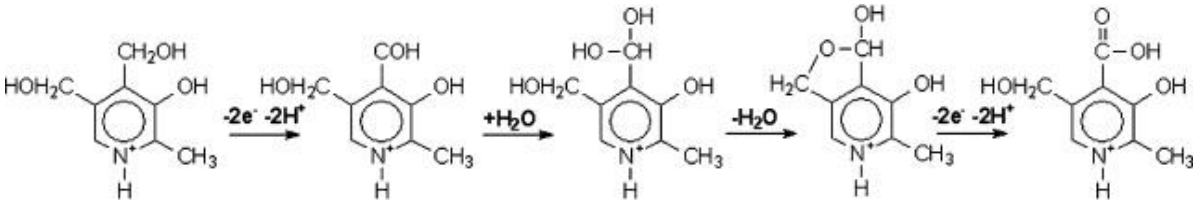

Fig. 7. *Mechanism of overall oxidation of vitamin B6*

In some cases (Hernandez et al., 2003; Cao et al., 2004) only an overall process was observed. This feature was explained (Hernandez et al., 2003) by the fact that, in the reported experimental conditions, pyridoxal is present as hemiacetal form but the occurrence of the free aldehyde (the electroactive one) is negligible, then a previous chemical transformation at the electrode is needed during the voltammetric experiment.

A simple DPV method based on Nafion-modified GCE for the quantitative determination of CA in cola beverages was developed (Brunetti, et al.,2007). The modified electrode exhibited a distinct enhancement of the current response in comparison to bare GCE due to incorporation of CA into the polymer layer, until the equilibrium conditions were achieved (after 10 min). This effect is similar to that employed in stripping voltammetry (Economou, 2010) as for this case it is based on chemical pre-concentration step instead of electrochemical one.

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Voltammetric determination of taurine after derivatization reaction

Chapter 3.

Theoretical considerations (Chapter 3)

Taurine (Tau), known as 2-aminoethanesulfonic acid (Fig. 8) 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).

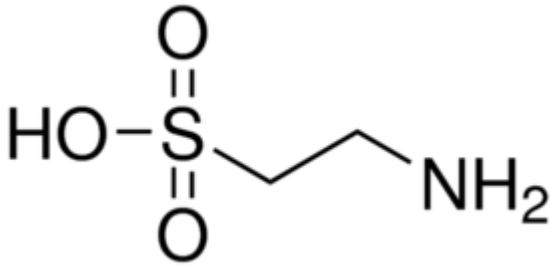

Fig. 8. *Chemical structure of taurine.*

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). While typical ingredients found in energy drinks and shots can vary by brand. Most energy drinks are sweetened carbonated beverages containing 80–320 mg of caffeine per serving, similar to a range seen in a prototypical cup of coffee. In addition to the primary psychoactive ingredient of caffeine that is included in energy drinks to enhance consumer alertness and energy, there are a variety of other compounds in energy drinks, including Tau, ginseng, glucuroncolactone, guarana, and vitamins (Marczinski, 2015).

These compounds stimulate brain activity, memory and attention (Tsvetkova, et al., 2015). Sales of energy drinks continue to climb worldwide, with sales reportedly nearing $50 billion. They represent 63% of the functional beverage category, with traditional sports drinks comprising only 27% of sales. As a category with exponential sales growth, the use of energy drinks has been extremely controversial. The majority of these products 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. For cardiovascular health, it is known that the acute effects of caffeine and consumption of energy products can moderately increase blood pressure and heart rate (Mesas et al., 2011; Seifert et al., 2011; Higgins and Babu, 2013; Marczinski et al., 2014). While moderate elevations in blood pressure after use of energy products may be relatively innocuous in healthy adults, they can be concerning in developing children, who have smaller body sizes and no developed tolerance to stimulant drugs. For this reason, numerous countries have banned the sale of energy drinks to minors, and others are seriously considering doing the same.

Hence, 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.

The U.S. Food and Drug Administration limits the caffeine content of carbonated beverages to 71 mg/serving and regulates caffeine in over-the-counter products. However, energy drinks are sold as food supplements, and do not undergo the same regulatory process in the United States in part because caffeine is considered GRAS (generally recognized as safe) and does not require testing before placement in new products (FDA). Therefore, the caffeine content of many energy drinks is often unknown or difficult to determine, especially for newer products. A single energy drink could push 70% of children and 40% of teenagers past the adverse effect level of 3 mg/kg/day when combined with other dietary sources. Accidental consumption is also an issue. Roughly half of all energy drink-related calls to the United States National Poison Data System (NPDS) between October 1, 2010 and September 30, 2011 involved children under the age of 6 (Seifert et al., 2013). Similarly, more than 45% of caffeine intoxication reports involve children or teenagers (Seifert et al., 2011). However, it remains unclear if caffeine toxicity is the primary reason that energy drinks can be risky products for children and adolescents.

(McClellan and Lieberman 2012) reviewed the most common ingredients, other than caffeine, which are found in the most popular energy drinks. They reported levels of the amino acid Tau between 750 and 1,000 mg per serving. Tau is a non-essential amino acid, and the normal diet typically contains 40 to 400 mg per day (Shao and Hathcock, 2008). The Mayo Clinic recommends no more than 3,000 mg per day. Tau can modulate calcium release, so there are potential impacts on the brain, heart, and skeletal muscle

(Seifert et al., 2013). Cardiac effects are exacerbated when Tau and caffeine are ingested together which can be a concern, given that caffeine alone can increase blood pressure and heart rate.

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.

3.1 Biological significance of taurine

Because Tau is not incorporated into proteins, it is less familiar to many scientists. A brief survey of general biology textbooks does not find mention of it, and a brief survey of biochemistry textbooks highlights only taurine's ability to conjugate cholic acid to form the bile salt, as glycine does. Still, it is one of the most prevalent free amino acids in the body, concentrated in the heart, central nervous system, retina, skeletal muscle, and liver (where it conjugates bile acids to form bile salts).

Concentrations in blood plasma average around 60 μM; the concentration in enhanced areas is in the 2–40-mM range (Geggel, et al., 1985; Oja, et al., 1983). While its role in the body is not fully understood, it has been studied extensively and a group of international researchers has held a Tau symposium approximately every two years since 1975. An excellent review of the subject is available (Huxtable, 1992).

Tau plays an important role in brain development and the development of eyesight (Geggel, et al., 1985); Tau is also used in the treatment of congestive heart failure and

epilepsy (Oja, et al., 1983). It is a suspected neurotransmitter (Hayes, et al., 1975). It is present in human milk, but not cow milk (except early in lactation), and has been added to infant formula since the mid-1980s, partly as a result of work by Hayes in which kittens fed a taurine-free diet did not develop sight (Hayes, et al., 1975).

Disruptions in Tau homeostasis have been reported in numerous studies of neurological disorders, including epilepsy and autism (Fukuyama and Ochiai, 1982; Junyent et al., 2009; Kuwabara et al., 2013). Tau levels increase in the brain after stress in an apparent compensatory mechanism (Huxtable, 1992). It has been observed that Tau is released from swollen cells as normal volume gets re-established. This change has been observed in patients with a traumatic brain injury, whereby Tau levels in the cerebral spinal fluid of patients increased to almost double from controls with Tau levels, returning back to control values within 3 days (Seki et al., 2005).

Interestingly, the levels of Tau in the brain decreased significantly with age, which led to numerous studies investigating the potential neuroprotective effects of supplemental Tau in several different experimental models (Taranukhin et al., 2012; Ananchaipatana-Auitragoon et al., 2015; Zhang et al., 2017).

3.2 Derivatization in electrochemistry

Analytical chemists have borrowed well-known reaction techniques from organic and organometallic fields to perform derivatization reactions to enhance detection of the analytes of interest. The derivatization technique should be simple to perform, fast, selective, and that the reagent of choice should react quantitatively with the analyte and only give the only final product. Some derivatization reactions are indeed fast and some are not; some being very selective and some more universal. However, with a certain care and careful control of optimized conditions, quantitative enhancement outcomes and increased separative power and detection can be very worthwhile. The number of derivatization reagents commercially available itself suggests one that there must be some merit to derivatization, that overcomes the associated extra costs: in time (to incorporate the extra reaction step), and in the expense of acquiring, handling, and storing derivatizing reagents.

The fundamental idea of derivatization is that the basic chemical or physical structure of the analytical moiety is changed through a suitable reaction. For example, by labeling compounds for a specific detection purpose, or by changing a functional group to

enhance certain characteristics, better detection and separation can be both achieved via the most suitable instrumental techniques. The type of derivatization is highly dependent upon the analytical method and upon the nature of the compound analyzed.

The derivatization step can be carried out before, in combination with, or after sample pretreatment in a sampling scheme and can be used for metals, nonmetals, and organic analytes. Derivatization in electrochemistry can be accomplished by addition of electrochemical tag to the target analyte improving the delectability of the analyte.

The quantitation of amines in various samples is a problem that has prompted the development of numerous analytical schemes. The most successful approaches have been those which couple derivatization of amines with a separation based on liquid chromatography, as illustrated by the amino acid method (Spackman, et al., 1958). Their amino acid analyzer was basically a special liquid chromatograph, utilizing ion-exchange separation and derivatization. With the advent of high-performance liquid chromatography, newer systems have emerged capitalizing on the higher level of chromatographic capability. Generally, detection of chromophoric derivatives combined with continuous two-component gradients on hydrophobic octadecylsilyl or hydrophilic cation exchange stationary phases have been employed. For amino acids, derivatization schemes include those based on ninhydrin (Rubenstein, et al., 1979), o-phthalaldehyde (Hill, et al., 1979; Davis, et al., 1979; Hogan, et al., 1982; Lindroth and Mopper, 1979), fluorescamine (Rubenstein, et al., 1979), dansyl chloride (Dejong, et al., 1982), dabsyl chloride (Chang, et al., 1982), and 7-fluoro-4-nitro-2,l,3-benzoxadiazole (Watanabe and Imai,1981).

In particular, fluorescence methods utilizing o-phthalaldehyde as a derivatizing reagent in both the pre- and postcolumn modes have become popular, although they continue to face certain limitations. The reaction of o-phthalaldehyde (OPA) with amino acids in the presence of a thiol reducing agent to produce fluorescent products (Roth, 1971); the fluorescent products were subsequently determined (Simons and Johnson, 1976) to be l-(alky!thio)-2-alkylisoindoles (I). The S-substituted isoindoles formed by the reaction of amines with OPA and either ,3-mercaptoethanol or ethanethiol are amenable to reversed-phase liquid chromatography in conjunction with precolumn derivatization. However, a significant problem with precolumn OPA approaches has been the instability of the derivatives (Hogan, et al., 1982; Lindroth and Mopper, 1979; Turnell and Cooper, 1982), causing careful timing or even instrumental automation to ensure reproducibility. Postcolumn derivatization schemes utilizing OPA must scrupulously

avoid impurities in the reagents and mobile phase buffers which can contribute to high background fluorescence (Böhlen and Schroeder, 1982). Postcolumn schemes also result in some loss of both resolution and sensitivity due to mixing the mobile phase with diluent (Böhlen and Mellet, 1979; Kucera and Umagat, 1983). (Joseph and Davies, 1983) reported that OPA//3-mercaptoethanol derivatives of amino acids undergo anodic oxidation at moderate potential, permitting the use of liquid chromatography /electrochemistry (LCEC) for their determination.

3.3 Electrochemistry of taurine derivatives

From electrochemical point of view, Tau is an aliphatic amine and so, it does not exhibit a significant electroactivity which explains poor literature about electrochemical method used for determination of Tau. At the bare electrode substrates such gold (Hasanzadeh, et al., 2014) or glassy carbon (Wang and Chen, 2009), Tau undergoes the polymerization reaction to form polytaurine film which is utilized for construction of highly selective sensors. However, it has been shown that this polymerization reaction cannot be used in Tau determination. In 2018, a group of Iranian scientists developed a α- cyclodextrin with silver nanoparticles composite voltammetric sensor (α-CD/AgNPs/GCE) for direct determination of Tau in the human plasma (Hasanzadeh, et al., 2018).

Unlike this, another indirect possibility for Tau voltammetric determination could exist. These methods are to be based on Tau derivatization to produce an electrochemically active derivative. A few publications focused on HPLC with electrochemical detection suggest this assumption (Joseph and Davies, 1983; Tcherkas, et al., 2001).

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Voltammetric method for determination of Propafenone in pharmaceutical dosage form

Chapter 4.

Propafenone (PPF), chemically named, 1-{2-[2-hydroxy-3-(propylamino) propoxy] phenyl}-3-phenylpropan-1-one (Fig. 9)

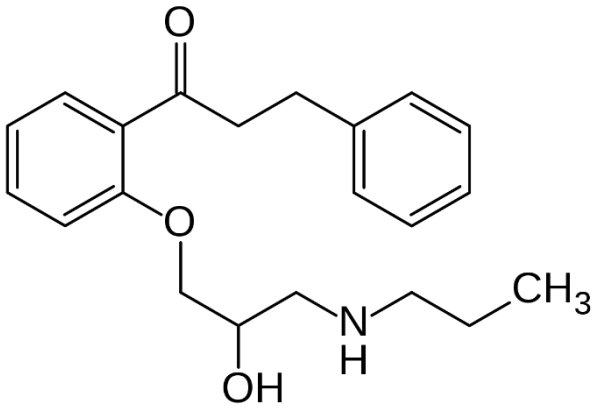

Fig. 9. *Chemical structure of Propafenone*

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).

4.1 Biological significance of propafenone

PPF is a potent and well-known antiarrhythmic agent used in clinical therapy for a number of decades now (Connolly, et al., 1983; Prystowsky et al., 1984). One of the earlier reports showed the effectiveness of propafenone in the management of patients with recurrent paroxysmal supraventricular tachycardia (Waleffe et al., 1981).

Clinically, PPF has been used in the management of a number of cardiac arrhythmias because it possesses multiple modes of action, via ß-adrenergic receptor blockade and calcium antagonistic activity (Dukes and Williams, 1984). Additionally, 5-hydroxypropafenone, an active metabolite, was shown to have a higher inotropic effect, an enhanced calcium influx antagonism and lower ß-adrenergic blocking activity relative to propafenone (McLeod, et al., 1984; Von Philipsborn et al., 1984).

4.2 Electroanalytical methods in pharmaceutical analysis

Analytical measurement techniques play an indispensable role in area of medicinal, clinical as well as pharmaceutical research. Modern analytical chemistry must satisfy the diversified demands of regular monitoring of pharmaceutical substances and analyses of complex biological systems, organic and inorganic compounds often at trace levels mainly in the area of drug analysis as well as in biological samples.

The application of electroanalytical techniques to pharmaceutical and biomedical analysis has grown vastly in recent decade as biological reactions in human beings and electrochemical reactions at solution-electrode interface follow same set of electron transfer pathways. Many important physiological, enzymatic and other biochemical processes work on principle of electrochemical oxidation/reduction mechanism (Ozkan, et al., 2015; Kim, et al., 2014). Electroanalytical techniques gain supreme importance over other techniques such as titrimetry, spectrophotometry, chromatography and capillary electrophoresis in terms of elucidation of the mechanism for new drug substances, wide spectrum of the analyte (drug compounds) and high enantioselectivity for the enantiomers in case of chiral drugs. Though the above-mentioned techniques are effective, the quantization of trace and ultra-trace components in complex analyte suffers from disadvantages in terms of extraction and separation, significant effect of the matrix from endogenous substances in biological fluids and pharmaceutical dosages. Therefore, electrochemical methods present a great alternative in detection and analysis of biological fluids, pharmaceutical substances as well as in discrimination of enantiomeric drugs due to their high sensitivity, selectivity, reproducibility, portability and low cost of the instrumentation.

Voltammetry which is an extremely sensitive electrochemical technique developed by the great Czechoslovakian chemist Jaroslav Heyrovsky in the year the 1920s has found its way for measuring trace amount of pharmaceutical active compound either in

dosage forms or in biological samples. A lot of scientific papers have thoroughly reviewed the application of electroanalytical techniques for analysis of drugs in detail (Ozkan, et al., 2003, Uslu and Ozkan, 2011; Gupta, et al., 2011). Others reviewed some chiral drugs in the form of electrochemical enantioselective sensors (Trojanowicz, 2014; Li, et al., 2016). The commonly used electrodes for voltammetric methods are that of gold, platinum, carbon paste electrode (CPE), glassy carbon paste electrode (GCPE), glassy carbon electrode (GCE), pencil graphite electrode (PGE) and screen-printed electrodes (SPE). The surface of electrode is a very powerful tool in electroanalysis which may suffer from various disadvantages such as larger background current, high detection limits, electrode surface passivation and/or deactivation due to adsorption of macromolecules which greatly affect the performance of electrode response. Many pharmaceutical compounds show no response within a potential window at solid electrodes. The drawbacks mentioned above often can be controlled by modifying the electrode surface by changing the chemical nature due to incorporation of chemical reagents or immobilized functional groups which may improve the stability and performance of the electrode. Examples of some electroanalytical methods using different working electrodes are summarized in table 1.

Table 1. Selected examples of different electroanalytical methods for determination of some pharmaceutical compounds in their dosage forms and biological fluids

Compounds	Electrodes	Technique	Application media	References
Nifedipine	GCE	CV and LSV	Tablets and capsules	Şentürk, et al., 1998
Buprenorphine	CPE	CV	Vials and tablets	Garcia-Fernandez, et al., 1999
Ascorbic acid	SPE	CV	Voltammetric behaviour	Florou, et al., 2000
Albendazole	GCE	LSV	Tablets	De Oliveira and Stradiotto 2001
Colchicine	HMDE	CV	Dosage forms and biological media	Kasim 2002
Alfuzosine	GCE	CV and LSV	Voltammetric behaviour	Uslu 2002
Melatonine	CPE	CV	Dosage forms	Corujo-Antuna, et al., 2003
Naproxen	Pt-Electrode	CV and LSV	Voltammetric	Adhoum, et

			behaviour	al., 2003
Triprolidine HCl	HMDE	LSV	Dosage forms	Zayed and Habib, 2005
Bisoprolol fumarate	GCE/SWCNTs	DPV	Pharmaceuticals and human urine	Goyal, et al., 2008
Abacavir	HMDE	DPV	Pharmaceuticals	Dogan, et al., 2008
Sparfloxacin	GCE	DPV	Pharmaceuticals	Kumar, et al., 2006
Cefixime	GCE	DPV	Pharmaceuticals, urine and breast milk	Golcu, et al., 2005
Resveratol	CPE	SWV	Pharmaceuticals and urine	Zhang, et al., 2007
Ticlopidine	HMDE	SWV	Pharmaceuticals	Turkoz and Onar, 2007
Fluoxetine	GCE	SWV	Pharmaceuticals	Lencastre, et al., 2006
Dopamine	Hg-Electrode	SWV	Pharmaceuticals	Winter, et al., 2007
Metoclopramide	CPE	SWV	Pharmaceuticals and human urine	Farghaly, et al., 2005
Rutin	HMDE	SWV	Pharmaceuticals	Ensafi and Hajian, 2006
Hydroxyzine	GCE	SWV	Pharmaceuticals and human serum	Beltagi, et al., 2008
Amlodipine	CPE	SWV	Pharmaceuticals	Kazemipour et al., 2009

4.3 Carbon nanotubes as electrode modifier

The field of chemically modified electrodes (CMEs) has been initiated in the mid of 1970s (Murray, 1980; Elliott and Murray, 1976; Murray, et al., 1987). Chemical modification of electrode surface can be achieved using different approaches; a) Adsorption and chemisorption: the modifier gets irreversibly adsorbed on the surface of electrode forming self-assembled monolayer, b) Covalent bonding: in this case, the chemical modifiers are attached in such a way that they form multilayer, c) Polymer film coating: electro-conductive as well as non-conductive, organometallic, organic or inorganic polymer films can be attached to electrode surface and d) Composites: in this process, electrode matrix is mixed with modifier of interest which results in the enhancement of the electrochemical properties (Labuda, et al., 2000; Guadalupe and Abrun, 1985; Snell and Keenan, 1979; Durst, et al., 1979; Zen, et al., 2003).

Functionalized nanomaterials on the electrode surface has given nano scale dimension

to the electrochemical methods for the analysis of pharmaceutical and biomedical compounds, which has grown in recent years. Exploitation of nanomaterials immensely contributed to the development of CMEs for electroanalysis of drugs as reviewed by several researchers (Sanghavi, et al., 2015; Shao, et al., 2010; Sharp and Burkitt, 2015; Yu, et al., 2015; Kochmann, et al., 2012; Li and Hu, 2012). Nanomaterials in comparison to other electrode materials have received enormous attention in the recent years in the field of CMEs for analysis of drugs because of their interesting electrical, magnetic, electronic, optical, chemical, and electrochemical properties. The large surface area to volume ratio, high conductivity, electrocatalytic activity and biocompatibility make them almost ideal modifier where they produce a synergistic effect helping in trace level determination of pharmaceutical, biomedical, and medicinal compounds.

Since the discovery of carbon nanotubes (CNTs) in 1991 (Iijima, 1991), there has been growing interest in using CNTs in chemical and biochemical sensing (Gong, et al., 2005; Merkoci, 2006; Wang, 2005; Zhao, et al., 2002) and nanoscale electronic devices due to their remarkable electronic and mechanical properties. CNTs behave as a metal or semiconductor depending on their structures. CNT-modified electrodes have better conductivity than graphite (Li, et al., 2002; Valentini, et al., 2003) and show a superior performance compared with such electrodes as Au, Pt, and other carbon electrodes. CNTs have a hollow core, which is suitable for storing guest molecules. Proteins and enzymes can be immobilized in the hollow core as well as to the surface of CNT without losing biological activity. The electrical conductivity of nanotubes can be improved by modifying the original CNT structure. CNTs possess fascinating mechanical strength and are the strongest and stiffest material known (Ajayan, 1999). CNTs, especially the side walls, are relatively inert. However, the ends of nanotubes are more reactive than the cylindrical parts (Ajayan, 1999; Tsang, et al., 1993). For the application of CNTs to electrochemical sensing, CNTs exhibit the enhanced electrochemical response to some important biomolecules (Zhao, et al., 2002; Musameh, et al., 2002) and promote the electron transfer reactions of proteins (Wang, 2005). These characteristics demonstrate clearly that nanotubes have significant potential for the design of electrochemical sensors. Indeed, in the last decades, there have been extensive studies on the applications of CNTs in electrochemical sensors (Sotiropoulou and Chaniotakis, 2003).

4.3.1 The structure of carbon nanotubes

CNTs are unique tubular structures of nanometer diameter and have large length/ diameter ratio. CNTs are divided into two main groups: single-walled carbon nanotube (SWNT) and multi-walled carbon nanotube (MWNT) as shown in Fig. 10. SWNT can be considered as a long-wrapped graphene sheet by rolling it in certain directions. The properties of the nanotubes are mainly dictated by the rolling direction as well as the diameter. SWNT consists of two separate regions (the side wall and the end cap) with very different physical and chemical properties. The end-cap structure is similar or derived from a small fullerene in which the carbon atoms are in both pentagon and hexagon rings. The side wall only consists of hexagon rings. MWNT can be considered as a collection of concentric SWNTs with different diameters. MWNTs may consist of one up to tens and hundreds of concentric shells of carbons with adjacent shell separations of ~0.34 nm. The carbon network of shells is closely related to the honeycomb arrangement of the carbon atoms in the graphite sheets.

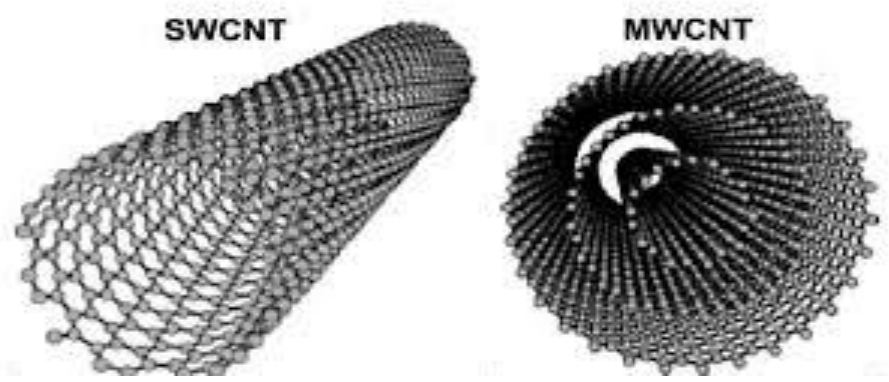

Fig. 10. *Schematic representation of Single Walled Carbon Nanotube (SWCNT) and Multi Walled Carbon Nanotube (MWCNT).*

4.3.2 Properties of carbon nanotubes

The carbon nanotubes possess such properties as high conductivity, excellent strength and stiffness, and chemical inertness. CNTs also show unusual electronic characteristics that are dependent on lattice helicity and elasticity. The density of SWNTs is estimated to be smaller (0.6 g/cm3) than graphite due to the presence of hollow channels in the center of CNTs. As expected for nano-sized materials, the surface area of CNTs is very large, e.g. ~10–20 m^2/g for MWNTs and the value of SWNTs is expected to be an order of magnitude higher.

Mechanical properties

The strength of the CC covalent bond, which is one of the strongest in nature, makes CNTs one of the strongest and stiffest materials. Treacy et al. estimated the elastic modulus of CNTs to be in the terapascal range by measuring their thermal vibrational amplitudes using TEM (Rinzler, et al., 1995). Because of the hollow structure and closed topology, CNTs can sustain extreme strains (40%) in tension without showing plastic deformation of bond rupture (Yakobson, et al., 1996). Under strain, some local bonds are broken, but this local defect is re-distributed over the entire surface due to the mobility of these defects. This process changes the helicity of CNTs and eventually affects its electronic property.

Electronice properties

The relationship between the structure and electronic conductivity of SWNTs using STM/STS images and current voltage curves obtained by tunneling spectroscopy on individual CNTs was explained (Wilder, et al., 1998; Odom, 2001). Their studies indicate that so-called armchair tubes are metallic, and the zigzag tubes and chiral tubes are either metallic or semiconducting depending on the wrapping angle and the length of CNTs. Nevertheless, the SWNT samples exhibit many different structures with no one species dominating. Four-probe measurements of MWNTs reveal that the electrical conductivity of individual MWNTs is metallic, semiconducting or semimetallic (Ajayan, 1999). The electrical conductivity of MWNTs becomes metallic by doping with boron and nitrogen (Carroll, 1998) and the conductivity of SWNTs becomes an order of magnitude higher by intercalating the CNT tubes with alkali and halogen dopants.

Physicochemical properties

It has been known that the basal graphite plane (graphene hexagon) is chemically inert. However, CNTs are susceptive to some chemical reactions due to the π-orbital mismatch in the curvature structures. Oxidation studies have revealed that the tips (caps) of CNTs are more reactive than the cylindrical parts (Ajayan, 1999; Treacy, et al., 1996). *Ab initio* calculations indicate that the average charge density of a pentagon (at the tips) is significantly larger than that at the graphene hexagon in the basal graphite plane and would act as an electrophilic reaction site. There have been numerous reports on the enhanced electron transfer of analytes when CNTs are used as

electrode materials. In carbon nanotubes, it has been suggested that the presence of defects creating overall topological change may inherently increase reactivity compared with their graphite counterparts (Ajayan, 1999). The moderate reactivity made it easy to introduce functional groups to the CNTs (both side wall and end cap) which is essential for sensor design in many cases. For example, during the purification of CNTs with a strong acid, the carboxyl functional groups are introduced to the surface of CNTs, especially at the tips. The carboxy functional groups are involved in nanotube chemical modifi cation with amide-linked groups at the tip ends of the CNTs (Wong, et al., 1997; Wong, et al., 1998).

4.3.3 Advantages of electrochemical sensors based on carbon nanotubes

CNTs have been one of the most actively studied electrode materials in the past few years due to their unique electronic and mechanical properties. From a chemistry point of view, CNTs are expected to exhibit inherent electrochemical properties similar to other carbon electrodes widely used in various electrochemical applications. Unlike other carbon-based nanomaterials such as C60 and C70 (Xie, et al., 1993), CNTs show very different electrochemical properties. The subtle electronic properties suggest that carbon nanotubes will have the ability to mediate electron transfer reactions with electroactive species in solution when used as the electrode material. Up to now, carbon nanotube-based electrodes have been widely used in electrochemical sensing (Britto, et al., 1999; Campbell, et al., 1999; Che, et al., 1998; Luo, et al., 2001). CNT-modified electrodes show many advantages which are described in the following paragraphs.

First, the CNT-modified electrodes catalyze the redox reactions of analytes. It has been reported that the oxidation of such analytes as dopamine, H2O2, and NADH are catalyzed at the various types of CNT-modified electrodes. Second, the biomacromolecules such as enzymes and DNA can be immobilized on the CNT-modified electrodes and maintain their biological activities. Third, the CNTs are a good material for constructing the electrodes. CNTs are small, straight, and strong, and they also have chemical stability. These characteristics of CNTs are advantageous for constructing CNT-modified electrodes. A couple of examples are vertically aligned CNT modified electrodes and the electrodes based on an individual nanotube. Fourth, CNTs can be functionalized mostly through the carboxyl group on the tips which helps to immobilize the enzymes, etc. for the development of various types of sensors. The

fifth advantage of CNTs is their good electronic conductivity.

The conductivity of CNTs is also affected by the structural changes such as twisting and bending of CNTs which may be applied for the sensing purpose.

All these advantages combined with others such as the porous structure may contribute to having good wetting property for the solvents, a better electrode–electrolyte interface, and a large surface area. The central hollow cores and outside walls are a superior material to adsorb and store gases such as oxygen, hydrogen, and nitrogen oxide (Wang, 2005; Davis, et al., 2003; Valcarcel, et al., 2005). The investigation of gas sensors using the adsorptive properties of carbon nanotubes to detect oxygen and carbon oxide has been reported (Wang, 2005; Adu, et al., 2001). The CNTs in many cases can serve as molecular wires that connect the electrode surface to the active site of enzymes. The direct or enhanced electrochemistry of several proteins and enzymes has been observed without the needs of mediators.

4.4 Electrochemistry of propafenone

Several analytical approaches dealing with the determination of PPF in biological fluids were described. Despite the availability of many applicable methods, there is no previous report on the direct electroanalytical determination of PPF that explains its electrochemical behaviour.

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Summary of presented work

Chapter 5.

5.1 Electroanalysis of vanillin

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. 11). 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.

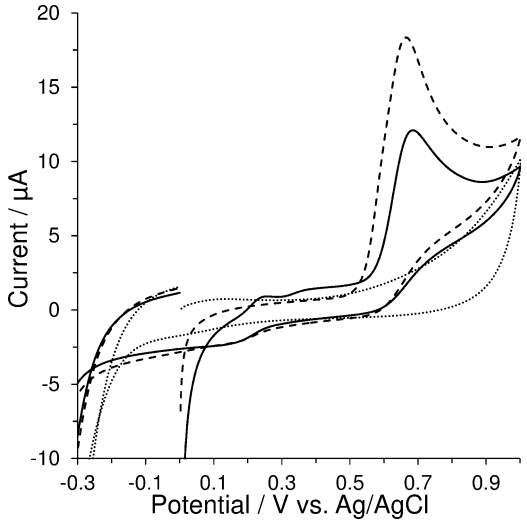

Fig. 11. *Cyclic voltammetry of blank (dotted line), 5.0 × 10 [4] M MVA (dashed line), and 0.5 mM EVA (solid line) at SDS/CPE performed in 0.1 M PBS pH 6 at 50 mV·s [1]*

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. 12. 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.

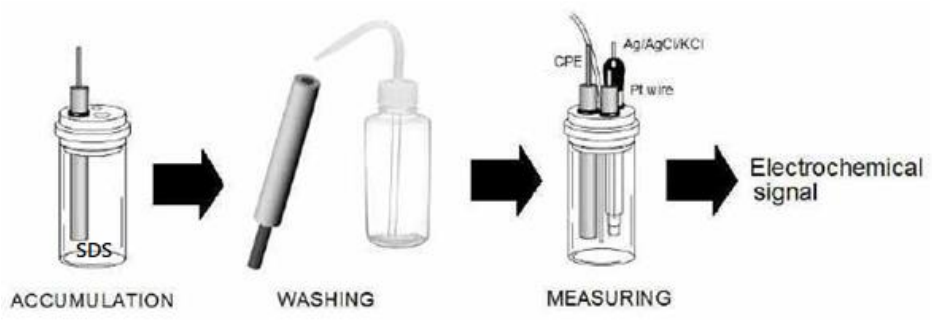

Fig. 12. *Proposed procedure for EVA and MVA electrochemical determination after extraction of SDS into CPE.*

Figure 13 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 I_p=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 I_p=2.52c–0.432 with coefficient of determination R^2=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.

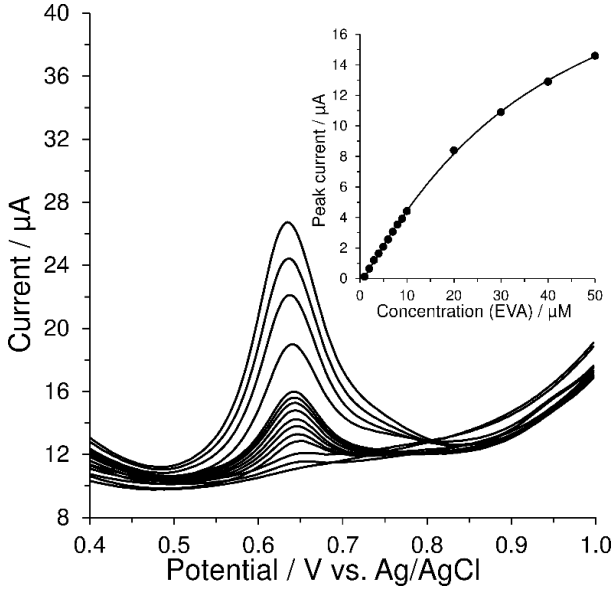

Fig. 13. *Voltammograms for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40 and 50 × 10^{-6} M EVA with corresponding calibration curve obtained at SDS/CPE measured in 0.1 M PBS pH 6 using SWV at potential step 5 mV, potential amplitude 70 mV, and frequency 50 Hz.*

Quantification of EVA and MVA sum in the foodstuffs samples was carried out by standard addition method due to low and negative intercept value (*q*) of calibration curve -0.095 µA. In comparison with a method of calibration curve, it should be generally known that results obtained from an analysis with the participation of sample matrix are often more accurate. Unfortunately, declared amounts were not listed because they are used as food ingredients. For that reason, the results obtained from direct SWV measurements of foodstuffs on the content of EVA were compared with those obtained by the reference standard HPLC analyses. Satisfactory obtained results among these fundamentally different analytical protocols confirm the fact that developed method can be also used in the routine food analysis.

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.

5.2 Electroanalysis of caffeine and vitamin B6

In presented work, 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. 14).

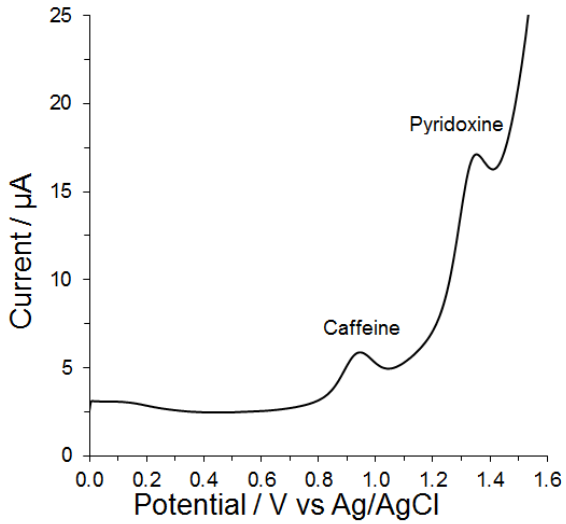

Fig. 14. *Differential pulse voltammograms of 200 µM caffeine and 100 µM pyridoxine 0.1 M BRB of pH 4.5.*

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. 15. 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 I_p(µA)=0.024c(µM) - 0.181 with coefficient of determination (R^2) 0.9964 for CA and I_p(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.

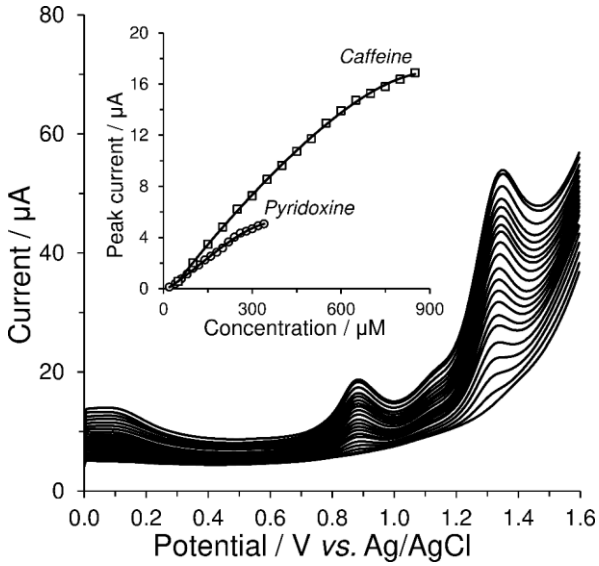

Fig. 15. *Voltammograms for 0 to 400 µM pyridoxine and 0 to 850 µM caffeine with corresponding calibration curves obtained at Nafion®/GCE. Measured using DPV at E_{step}=5 mV, E_{ampl}=70 mV, and v=50 mV/s*

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. For demonstration, relevant voltammograms recorded from analysis of energy drink with trademark ‘‘Semtex” are shown in Fig. 16.

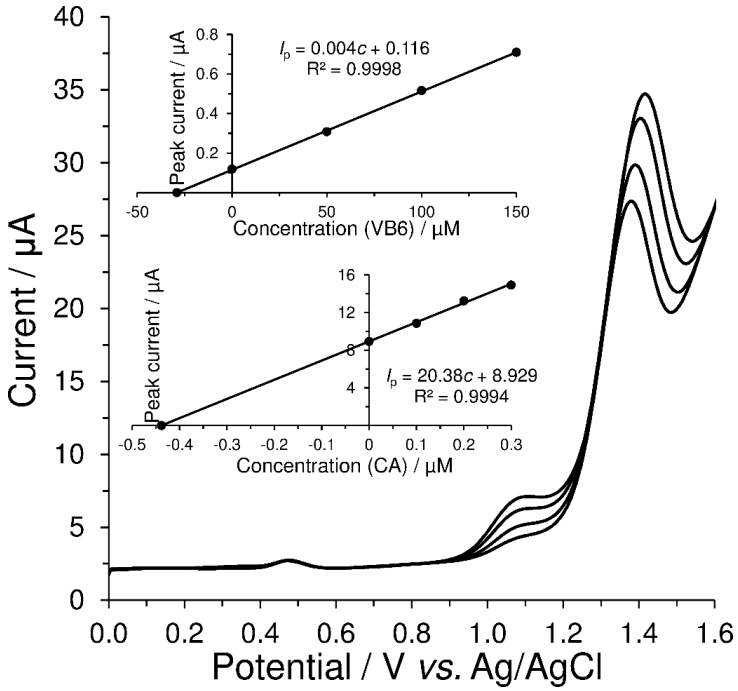

Fig. 16. *Typical anodic voltammograms obtained during electrochemical analysis of energy drink (Semtex) by standard addition method.*

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 (Švorc, et al., 2012; Švorc, et al., 2013). Due to their higher sensitivity and low detection limits, the electroanalytical methods based on stripping processes seems to be promising for trace analysis (Desimoni, et al., 2002).

5.3 Electroanalysis of Taurine

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. 17, 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. In the past, resulting polymer has been used for development of poly(taurine) film based voltammetric sensors (Hasanzadeh, et al., 2014; Wang and Chen, 2009). Initially, there was idea to utilize this polymeric reaction to develop adsorptive stripping voltammetric method, but it was unsuccessful.

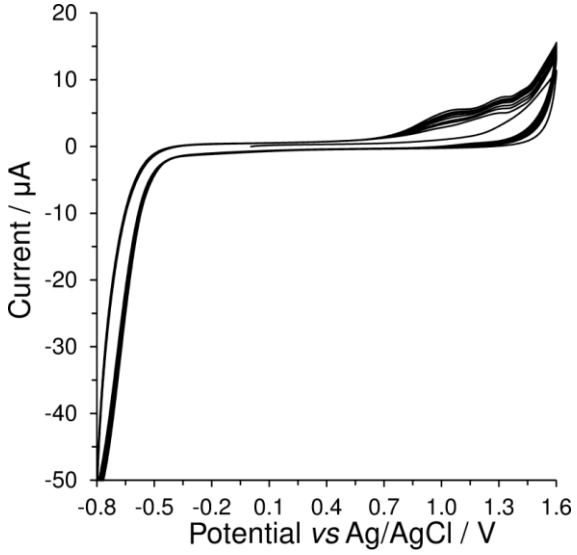

Fig. 17. *Repetitive (10 cycles) cyclic voltammetry of 0.5 mmol L^{-1} Tau performed in 95% methanol containing 0.1 mol L^{-1} $LiClO_4$ at potential step 5 mV and scan rate 50 mV s^{-1}.*

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. 18). 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.

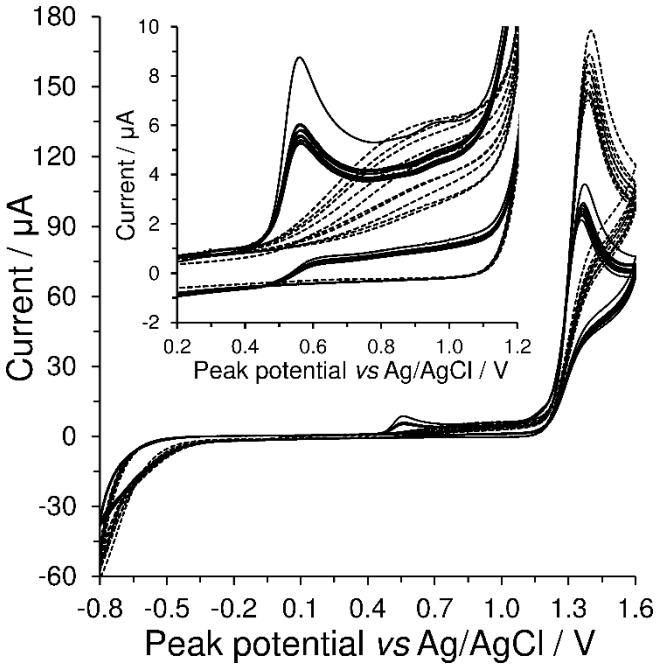

Fig. 18. *Repetitive (10 cycles) cyclic voltammetry of 3.7 mmol L^{-1} OPA with 7.1 mmol L^{-1} EtSH mixture (dashed) and their 0.5 mmol L^{-1} Tau derivate (solid line) performed in 95% methanol containing 0.1 mol L^{-1} $LiClO_4$ at potential step 5 mV and scan rate 50 mV s^{-1}.*

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=10*s*/*k* and LOD=3*s*/*k*, respectively, where *s* is the standard deviation of minimally ten repetitions (n=10) of chosen concentrations 20 μmol L^{-1} Tau (I_p=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

I_p(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 I_p(μA)=0.0745c(μmol L^{-1})+0.1293 with R^2=0.9965 will be achieved. The calibration curve and corresponding voltammograms representing the linear range obtained are shown in Fig. 19.

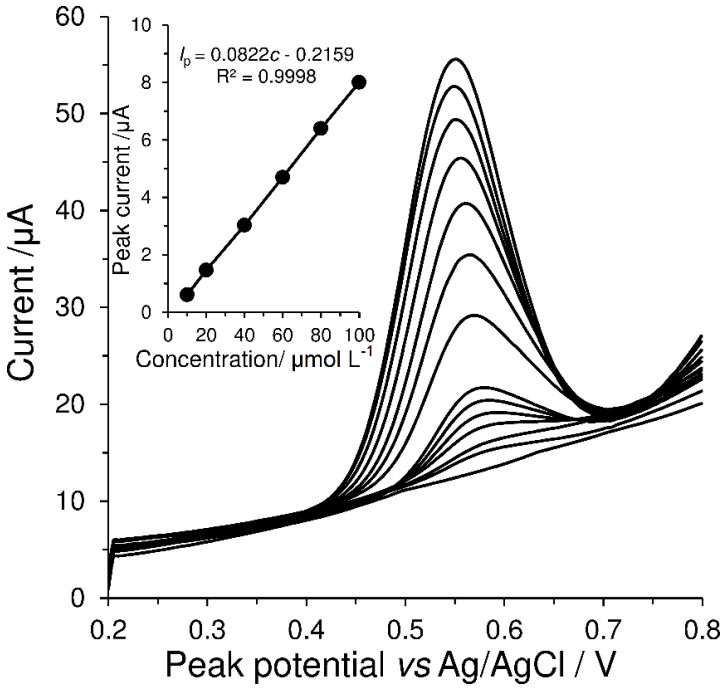

Fig. 19. *Voltammograms for 0 (blank), 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700 and 800 μmol L^{-1} Tau with corresponding calibration curve to 100 μmol L^{-1} Tau obtained at GCE. Measured in 95% methanol containing 0.1 mol L^{-1} LiClO4, 3.7 mmol L^{-1} OPA and 7.1 mmol L^{-1} EtSH using 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.

5.4 Electroanalysis of Propafenone

A novel electroanalytical method for the determination of PPF in pharmaceutical dosage form and biological fluids using GCE/NH_2*f*MWCNTs as a sensitive sensor.

The electrochemical response of PPF showed one well-defined oxidation peak around 0.9 V for NH_2*f*MWCNTs /GCE, bare GCE and GCE /NH_2*f*MWCNTs /AgNPs with scan rate of 100 mV/s. However, the best and sensitive peak was obtained in the case of using of NH_2*f*MWCNTs /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. 20).

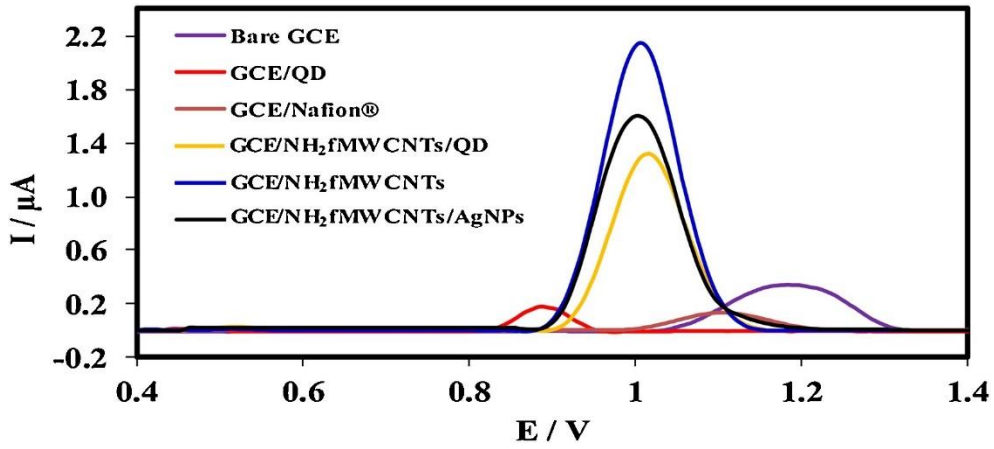

Fig. 20. *DPVs of 10µM PPF at bare GCE and its different modifications in BRB solution pH 7.0*

DPAdV of PPF at NH_2*f*MWCNTs/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. 21). 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.

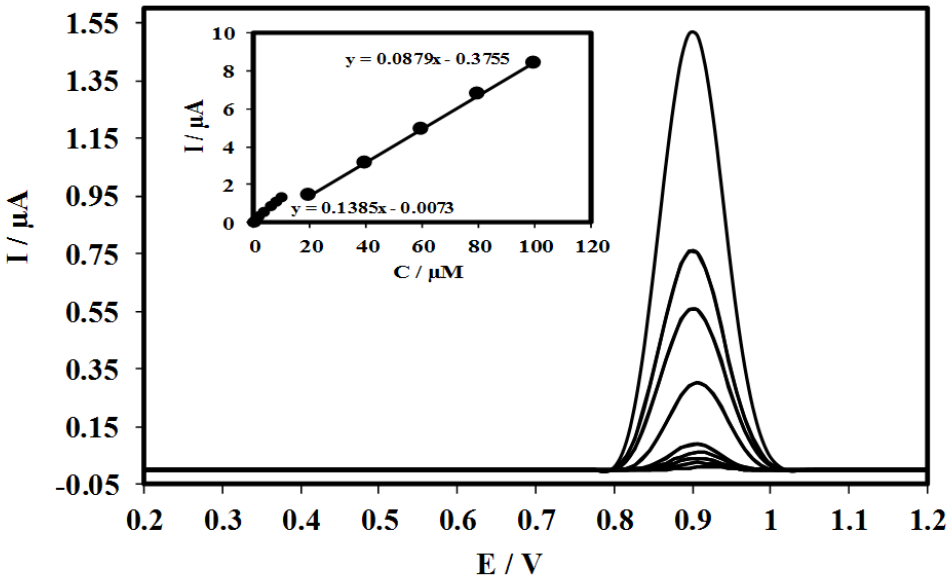

Fig. 21. *DPAdVs of PPF (0.1µM - 10µM), the inset shows the corresponding calibration curve.*

The possible oxidation mechanism of PPF can be explained on the basis of hydroxyl group oxidation and its transformation into the carbonyl group as shown in Fig. 22.

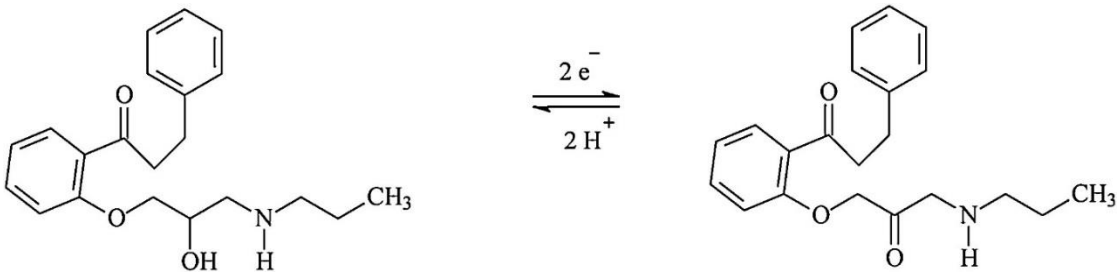

Fig. 22. *Possible oxidation mechanism of PPF at the NH_2fMWCNT/GCE.*

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 theNH_2fMWCNTs/GCE in voltammetric measurements of PPF and for routine pharmaceutical dosage form analysis.

5.5 References (Chapter 5)

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Conclusions

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} $LiClO_4$ 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 NH_2*f*MWCNTs 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.

List of publications

Farag A. S., Sýs M., Hájek T., Vytřas K. **(2018)**. Voltammetric determination of ethylvanillin and methylvanillin sum at carbon paste electrode modified by sodium dodecyl sulfate in selected foodstuffs. *Monatshefte für Chemie, 149,* 1945–1953. **[I_F: 1.349].**

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Farag A. S., Klikarová J., Česlová L., Vytřas K., Sýs M. **(2019)**. Voltammetric determination of taurine in energy drinks after o-phthalaldehyde-ethanethiol derivatization. *Talanta, 202,* 486–493. **[I_F: 5.339].**

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Farag A. S., Bakirhan N. K., Švancara I., Ozkan S. A. **(2019)**. A new sensing platform based on NH_2fMWCNTs for the determination of antiarrhythmic drug propafenone in pharmaceutical dosage forms. *Journal of Pharmaceutical and Biomedical Analysis, 174,* 534–540. **[I_F: 3.209].**

Data for the library database

Title	Electroanalytical methods in determination of selected biologically active compounds
Author	MSc. Amir Shaaban Farag Mohamed NAWAR
Specialization	Analytical Chemistry
Year of Defense	2021
Supervisor	prof. Ing. Ivan ŠVANCARA, Dr.
Annotation	This dissertation thesis describes possibilities and limitations for determination of biologically active substances in foodstuff and pharmaceutical dosage forms at various modified carbon-paste and glassy carbon-paste electrodes using electrochemical techniques. It deals with voltammetric determination of ethylvanillin, methylvanillin as the total content, further with simultaneous determination of caffeine and vitamin B6 in commercially available energy drinks by differential pulse voltammetry. On the other hand, voltammetric determination of taurine was achieved after its derivatization to an electroactive product quantifiable in energy drinks. Finally, the antiarrhythmic drug Propafenone could be successfully determined in its tablet dosage form using adsorptive differential pulse voltammetry. Each analytical procedure proposed was optimized and obtained results found to be in good agreement with reference standard methods.
Key words	Voltammetry Vanillin caffeine Vitamin B6 Taurine Propafenone Energy drinks Pharmaceutical dosage forms