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THE USE OF CARBON-BASED ELECTRODES FOR THE DEVELOPMENT OF NEW ELECTROCHEMICAL METHODS FOR THE DETERMINATION OF AMINONITROPHENOLS

POUŽITÍ ELEKTROD NA BÁZI UHLÍKU K VÝVOJI NOVÝCH ELEKTROCHEMICKÝCH METOD STANOVENÍ AMINONITROFENOLŮ

Ph.D. Thesis

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I declare that all the results which are used and published in this Thesis have been obtained by my own experimental work and that all the ideas taken from work of others are properly referred to in the text and the literature survey. I am conscious that the prospective use of the results, published in this Thesis, outside the Charles University in Prague is possible only with a written agreement of this university.

I also declare that neither this Thesis, nor its significant part, has been submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Prague, 18. 5. 2013 Mgr. Hana Dejmková

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ABSTRAKT

Tato práce byla věnována vývoji metod pro stanovení pěti derivátů aminonitrofenolu technikami diferenční pulsní voltametrie (DPV) a HPLC s amperometrickou detekcí. Jako pracovní elektrody byly použity uhlíková pastová elektroda (CPE) a borem dopovaná filmová diamantová elektroda (BDDFE). Předběžné testování elektrod bylo prováděno cyklickou voltametrií. Vypracované metody byly zaměřeny na stanovení všech pěti izomerů aminonitrofenolu v barvách na vlasy a na stanovení 2-amino-4-nitrofenolu a 4-amino-2-nitrofenolu v tělních tekutinách po extrakci tuhou fází. Z voltametrických metod se ukázala být dostatečně selektivní pouze metoda využívající pro stanovení v barvách na vlasy BDDFE v katodické oblasti, parametry chromatografických metod byly vyhovující pro stanovení aminonitrofenolů v obou studovaných matricích. Použitelnost vyvinutých metod byla úspěšně potvrzena stanovením analytů v barvách na vlasy a modelových vzorcích moči.

V práci jsou rovněž zahrnuty výsledky předchozích měření, která se zabývala stanovením jiných oxidovatelných sloučenin na bázi fenolu nebo anilinu pomocí BDDFE. Získané informace potvrzují, že v některých případech může BDDFE vykazovat určitou odolnost proti pasivaci ve srovnání s jinými uhlíkovými elektrodami, ale ve většině případů je pasivace elektrody pozorovatelná. K jejich čištění a aktivaci byl úspěšně použit postup, sestávající z vkládání vysokých anodických i katodických potenciálů v kyselém prostředí; v průtokovém uspořádání je rovněž možné pasivaci čelit zvýšením průtokové rychlosti.

ABSTRACT

Methods for the determination of five isomers of aminonitrophenol were developed, based on the technique of differential pulse voltammetry (DPV) and HPLC with amperometric detection. As the working electrodes, boron-doped diamond film electrode (BDDFE) and glassy carvon paste electrode (GCPE) were employed. Preliminary electrode testing was performed by cyclic voltammetry. The methods were aimed to the determination of all five aminonitrophenol isomers in hair dyes and to the determination of 2-amino-4-nitrophenol and 4-amino-2-nitrophenol in body fluids after solid phase extraction. From the voltammetric methods, the one using BDDFE for the cathodic determination in hair dyes exhibits sufficient selectivity. The performance of chromatographic methods was found suitable for the determination of aminonitrophenols in both observed matrices. The applicability of the developed methods was successfully confirmed by the determination of tested analyted in real samples of hair dyes and spiked samples of urine.

Preceding measurements are described, dealing with the determination of other oxidizable compounds, containing phenolic or aniline moieties on BDDFE. The obtained results confirm that in some cases, BDDFE shows more resistance to fouling than other carbon-based electrodes, but in most cases, the passivation of the electrode is observable. The cleaning and activating procedure, consisting of application of high anodic and cathodic potentials in acidic medium, was used successfully; in the flow arrangement, the fouling can also be overcome by application of higher flow rate.

CONTENT

LIST OF ABBREVIATIONS

2A3NP	2-amino-3-nitrophenol
2A4NP	2-amino-4-nitrophenol
2A5NP	2-amino-5-nitrophenol
2D-HPLC	two-dimensional HPLC
4A2NP	4-amino-2-nitrophenol
4A3NP	4-amino-3-nitrophenol
AAS	atomic absorption spectrometry
BDDFE	boron-doped diamond film electrode
BM CPE	biologically modified CPE
C18	octadecyl
CM CPE	chemically modified CPE
CPE	carbon paste electrode
CV	cyclic voltammetry
CVD	chemical vapor deposition
DAD	diode array detector
DPV	differential pulse voltammetry
ED	electrochemical detection
FIA	flow injection analysis
GC	gas chromatography
GC/Ag	Ag particles highly dispersed on glassy carbon
GCE	glassy carbon electrode
GCPE	glassy carbon paste electrode
HPLC	high-performance liquid chromatography
LC	liquid chromatography
MEKC	micellar electrokinetic chromatography
MS	mass spectrometry
MS-MS	tandem mass spectrometry
SPE	solid phase extraction
TLC	thin layer chromatography
UHPLC	ultrahigh performance liquid chromatography
UV	ultraviolet light; spectrophotometric detection using this light

1. INTRODUCTION

This dissertation was elaborated under the framework of a long-term research at UNESCO Laboratory of Environmental Electrochemistry in Prague to develop both highly sensitive and selective electroanalytical methods for determination of nitro, amino, and hydroxy derivatives of polycyclic aromatic hydrocarbons and new electrode materials suitable for this application. The Thesis presented is based on following five scientific publications **[1-5]** which are attached as Appendix parts I – V (Chapters 6 – 10). To distinguish the references referring to these publications in the entire text of this Thesis, corresponding numbers in square brackets are in bold and underlined.

One of the serious problems of the modern world remains the pollution of the environment by undesirable chemical compounds. Both the reasons and results of such contamination vary, but in any case, analytical chemistry is here to give us reliable information about the state of the problem. Techniques of electroanalytical chemistry play in this context the role of reasonably sensitive and selective tool, yet particularly flexible and inexpensive way of determination, and are thus particularly suitable for the screening measurements as well as for the precise determination. The progress in the electrochemistry involves development of new electrode materials in order to improve the possibilities of the determination methods; to involve these advances, electrochemists widen the range of the accessible selective determination methods for various analytes.

With respect to these facts, the aim of the Thesis was the development of sensitive electroanalytical methods for the determination of five common aminonitrophenol isomers, namely 2-amino-3-nitrophenol (2A3NP), 2-amino-4-nitrophenol (2A4NP), 2-amino-5-nitrophenol (2A5NP), 4-amino-2-nitrophenol (4A2NP), and 4-amino-3-nitrophenol (4A3NP). Their occurrence is associated with two main sources: they might be present in cosmetic products as dyes and in body fluids as the products of 2,4-dinitrophenol metabolization. Determination methods for both types of matrix, namely hair dyes and urine, were developed [**1,2**]. For both methods, techniques of differential pulse voltammetry (DPV) and HPLC with amperometric detection were used with glassy carbon paste electrode (GCPE) and boron-doped diamond film electrode (BDDFE) as working electrodes. Also the basic electrochemical characteristics of these electrodes were examined and electrochemical behavior of the compounds was observed using cyclic voltammetry (CV). The parameters of the developed methods were compared together and with the performance of the HPLC method with spectrophotometric detection.

Besides, influence of the addition of bismuth powder in the GCPE to the parameters of the determination was investigated on the example of 4A3NP [**3**].

Included in the Thesis are also two papers, which deal with the application of BDDFE for the determination of phenolic compounds and amino derivatives of aromatic hydrocarbons, concerning particularly the problem of the passivation of the electrode and the influence of the surface pretreatment on the electroanalytical response. These papers served as the basis for the handling of BDDFE during the determination of ANP derivatives [**4,5**].

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2. PROPERTIES AND DETERMINATION METHODS OF AMINONITROPHENOLS

2.1 Occurrence and biological effects of aminonitrophenols

Compounds of the main interest in this Thesis are five commonly occurring isomers of aminonitrophenol, namely 2-amino-3-nitrophenol (2A3NP), 2-amino-4-nitrophenol (2A4NP), 2-amino-5-nitrophenol (2A5NP), 4-amino-2-nitrophenol (4A2NP), and 4-amino-3-nitrophenol (4A3NP).

The occurrence of these compounds is, as well as in the case of many other aniline derivatives, mainly connected with the dye industry. They can be found as intermediates in the manufacturing of some polymers and azo dyes and as final products, they can serve as dyes for leather or fur [1]. Nevertheless, the most important source of their exposition is their utilization in cosmetics as the active component of permanent and semipermanent hair dyes, responsible for obtaining yellow and red tones [2,3]. The close contact with the cosmetics during the application allows their transdermal delivery [4].

The utilization of and exposition to amononitrophenols is closely observed due to the fact that they are suspected of adverse health effects including mutagenity and carcinogenity. The hazard level varies according to the isomer. 2A4NP, 2A5NP, and 4A2NP exhibit some level of mutagenic [5-7] and carcinogenic effects [3,8-10] while such behavior was not observed in the case of 2A3NP and 4A3NP [5]. Besides, dermatitis was observed after the exposition to aminonitrophenols, both in the laboratory studies [11,12] and on the people using aminonitrophenol containing cosmetics [13,14]. The adverse effects of aminonitrophenols resulted in the ban of these compounds in cosmetics in EU [15]. Nevertheless, there are opinions evaluating the hazardousness of aminonitrophenols as less serious [16].

Another point causing interest in aminonitrophenols is the fact that two of them, namely 2A4NP and 4A2NP, are the main metabolites of 2,4-dinitrophenol [17]. 2,4-Dinitrophenol is compound used as intermediate in chemical industry and as a component in agrochemicals [18]; nevertheless, an important source of the exposition is its direct intentional consumption.

In exposed organism, it uncouples oxidative phosphorylation by increasing the basal proton conductance through mitochondria membrane, which results in the increased metabolic rate, oxygen consumption and body temperature, finally leading to the loss of body fat [19]. During 1930s, it was medically utilized as a weight-reduction drug [20]. Adverse side effects, including skin lesions and eye cataract [21] and also the dosage problems [18] changed its application from official to unofficial. Non-professional approach contributed to the causes of poisoning, some of them fatal [22-24]. The danger is mainly connected with the fact that the high metabolic rate and related weight loss as well as body temperature increase with the increasing dose until the thermoregulation is not possible any more and the hyperthermia causes death. Besides, the personal tolerance varies greatly and the dosage needs to be set individually [25]. Causes of poisoning connected with the industrial applications are also described [26].

2.2 Analytical methods for the determination of aminonitrophenols

Structure of aminonitrophenols makes them accessible for various determination techniques. Overview of the described methods is given in Tab. 1.

The most frequent technique is HPLC, usually in combination with spectrophotometric detection; mass spectrometric detection and amperometric detection are also described. Other separation techniques are represented by thin layer chromatography (TLC), gas chromatography (GC) and micellar electrokinetic chromatography (MEKC). The only spectrometric method described is indirect atomic absorption spectrometry (AAS). From the batch electrochemical methods, potentiometric titration and differential pulse voltammetry (DPV) were employed.

The majority of the chromatographic determination methods is related to the matrices of the analytes. Most often, the analytes are determined in hair dyes; in this matrix, number of similar compounds can occur, as the coloring effect is usually achieved by a mixture of dyeing agents. Frequent determination matrices are also various biological samples, due to the toxicological importance of the analytes. Less selective methods employ aminonitrophenols only in the role of model compounds, without a particular analytical aim.

The sensitivity of the methods is not considered as a subject of interest in some works. If sought for, determination limits usually reach submicromolar concentration. The most sensitive method is HPLC with electrochemical detection; in this case, detection limit is 25 nmol L^{-1}.

Tab. 1 – Overview of the described methods for aminonitrophenol determination.

Determined aminonitrophenol	**Technique**	**Detection limit**	**Matrix**	**Cit.**
2A3NP, 2A4NP, 2A5NP, 4A2NP	UHPLC-MS-MS	0,32 μmol L^{-1}	Hair dyes	[27]
2A3NP, 2A4NP, 2A5NP, 4A2NP, 4A3NP	HPLC-DAD	0.6-1.2 μmol L^{-1}	Hair dyes	[28]
4A3NP	HPLC-DAD	<65 μmol L^{-1}	Hair dyes	[29]
2A4NP, 4A2NP	LC-MS-MS	3 μmol L^{-1}	Biological fluids	[17]
2A4NP	HPLC-UV	---	Model mixture (column efficiency testing)	[30]
2A4NP, 2A5NP	HPLC-ED (GCE)	25 nmol L^{-1}	Hair dyes	[31]
2A4NP	DPV (GC/Ag)	approx. 0.5 μmol L^{-1}	Model mixture (electrode testing)	[32]
2A3NP, 4A3NP	HPLC-DAD	---	Hair dyes	[33]
2A4NP	2D-HPLC-UV	---	Model mixture (exploring efficiency of the separation system)	[34]
2A5NP	HPLC-UV	---	Model mixture (stationary phase modificator testing)	[35]
2A4NP	TLC	---	Biological tissues	[36]
2A4NP, 2A5NP, 4A2NP	MEKC	1.2-2.5 μmol L^{-1}	Hair dyes	[37]
2A4NP	GC-MS	---	Coal	[38]
2A4NP, 2A5NP	HPLC-DAD	---	Hair dyes	[39]
2A4NP	HPLC-DAD	---	Hair dyes	[40]
2A4NP	GC	---	Hair dyes	[41]
4A3NP	HPLC-DAD	0.6 μmol L^{-1}	Hair dyes	[42]
2A4NP	HPLC-UV	0.7 μmol L^{-1}	Industrial effluents	[43]
2A5NP	Potentiom. titr.	1 μmol L^{-1}	Model mixture	[44]
4A3NP	TLC	---	Hair dyes	[45]
2A4NP	LC-imunoassay	15 μmol L^{-1}	Soil extr., plant extr.	[46]
2A4NP, 2A5NP	HPLC-DAD (photochemical derivatization)	---	Hair dyes	[47]
2A4NP	Indirect AAS	1 μmol L^{-1}	---	[48]

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3. PROPERTIES OF THE USED ELECTRODES

3.1 Carbon paste electrodes

Paste electrodes form, due to their distinct properties, a specific kind of electrodes. They consist of conductive particles mixed with liquid binder, resulting in the plastic paste, which situates them to the border of solid and liquid electrodes [1]. This was actually the idea standing behind the first preparation of this electrode type – to combine the advantages of liquid and solid electrodes. The most frequent conductors in the pastes are various forms of carbon, hence we mostly talk about carbon paste electrodes (CPE), and in this way they will be addressed in this Thesis; nevertheless, paste electrodes made of other materials, such as metals [2] or solid silver amalgam [3], are also described and their properties are analogous.

The structure and physical properties of carbon paste electrodes determine their properties. Their plasticity allows simple renewal of their surface – plain wiping by the filtration paper replaces polishing necessary for solid electrodes, while keeping reasonable repeatability [4].

Both of the components also determine the features of the electrode surface; the surface reminds the mosaic of the conductive and insulating areas, whose proportion depends on the proportion and properties of the both constituents. It was proposed that this structure, resembling the random array of microelectrodes, is responsible for the particularly low background current and wide potential window of CPE [5]. On the other hand, this heterogeneity of the surface also limits the chemical stability of the electrode. Addition of organic solvents into the aqueous measured solution can cause the dissolution of the pasting liquid and hence the disintegration of the electrode [6]. Besides, partly liquid-based surface enables the diffusion of oxygen to the electrode surface and consequent difficult suppression of the oxygen cathodic response [7,8]. For this reason, CPE are seldom employed for measurements in cathodic potential region. Seriousness of these both problems depends also on the electrode composition and arrangement.

Last, but not least, carbon powder used for the paste preparation is less expensive than blocks of carbon for the manufacturing of solid electrodes, at least if we do not need a paste prepared from special components.

Indeed, the number of components for the paste preparation offers an enormous range of combinations, with a big variability of properties; the scope for choice of the main paste components is further widened by the possibility of third component addition in order to enhance the electrode performance, to increase the selectivity, and to make it suitable for a particular purpose [5].

As the conductive particles, graphite powder is the most common material, utilizable for the majority of applications. Some other, less defined carbon forms, such as coal [9], carbon black [10] etc. were employed, with varying results. Good results were obtained with the microscopic particles of glassy carbon [11,12]. Recently, more sophisticated materials were tested: we should name particularly diamond powder [13] and carbon nanostructures (nanotubes [14,15], fullerenes [16], and graphene [17]).

Even wider range of possibilities is offered when choosing the pasting liquid. Virtually any non-volatile, non-conductive and water-immiscible liquid can be used, which involves many aliphatic and aromatic hydrocarbons and their derivatives. Nevertheless, paraffin and silicone oil are clearly prevalent, because besides the above mentioned requirements, they are also non-toxic and inexpensive. Interesting results are obtained using ionic liquids, particularly in combination with carbon nanostructures [18].

So-called “bare” CPE (i.e. consisting only of the two above-mentioned components) present only the minority of CPE mixtures. Modification of the electrodes by addition of another constituent(s) usually aims either to the preconcentration of the analyte on the electrode surface prior the measurement itself or to the catalysis or mediation of the electrochemical reaction in order to increase response of the electrode and decrease the redox potential; modification leading to the change in physico-chemical properties of the electrodes is less frequent. The range of possible modifiers involves the widest range of substances or their combinations: metal powders, inorganic compounds, small organic molecules, inorganic and organic polymers, enzymes or enzyme-containing biological tissues and many others. Traditionally, the group using simple organic or inorganic modifiers is addressed as chemically modified (CM) CPE and the group employing enzymes or other biomolecules are called biologically modified (BM) CPE.

To make the possibilities of the modified electrodes more apparent, we can name several types of modified electrodes. Electrodeposition of metals is improved by the presence of metals or metal-based modifiers, such as bismuth [19]. For the accumulation of organic as well as some inorganic compounds, supramolecular interactions are used, particularly sorption of the analyte in a hollow of an inorganic structure (such as zeolite) or organic molecule (such as cyclodextrine), adsorption, or ion-pair formation. Successful modification allows to decrease the determination limit into the subnanomolar range [20]. The application of electrocatalytical modifiers or enzymes enables the determination of compounds with otherwise overlapping signals [21,22] or of compounds inactive in direct determination [23]. However, the quoted possibilities are just an example, because the number of modifications is truly eternal.

Besides the preparation of the paste, attention is also paid to the electrode construction. A very simple, yet less convenient approach is the filling of the paste in a suitable cavity in an insulating material, usually glass or plastic. Construction with screw and piston allows easier surface renewal [24]. Several attempts to connect easy manipulation with small dimensions of the electrode were made. The most common way is the utilization of a suitable capillary as the electrode body; simplicity of such electrode body can be advantageous for certain applications [25,26]. Other constructions employing pipette tips [27] or some more sophisticated structures [28] are still infrequent variations.

The works concerning carbon paste electrodes were reviewed several times [5,29-31]; monograph [32] is the most recent and thorough compendium for obtaining further information about this topic.

3.2 Diamond electrodes

Diamond is, due to its structure, an exceptional material, particularly notable for extreme hardness and chemical stability. Electrochemistry is one of the areas that are trying to benefit from these properties. [33]

The expansion of this electrode material is connected with the invention of diamond fabrication by chemical vapor deposition (CVD) [34]. This method takes advantage of the difference between chemical stability of diamond and graphite in hydrogen atmosphere. Diamond film is grown on the substrate of metal or silicon; for the beginning of the crystallization, seeds of diamond are placed on the substrate. The gaseous reaction mixture contains a volatile carbon compound (typically methane) and hydrogen under sub-atmospheric pressure and is activated thermally (heated filament) or electrically (microwave method or arc or glow discharge). Under this temperature and pressure, deposition of graphitic form of carbon is actually preferred, but the atomic hydrogen present reacts with graphitic carbon, while diamond modification remains unchanged. As a result, diamond deposition is observed. To maintain the necessary conductivity, diamonds are doped by other atoms, particularly with boron. For that purpose, volatile boron-containing compounds are added to the reaction mixture. [35]

Resulting product is a compact layer of aggregated diamond crystals of micrometer range size or smaller, depending on the exact conditions of deposition, called boron-doped diamond film electrodes (BDDFE). The resistivity of this material can drop, with respect to the doping level, to 10^3 Ω cm. [36]

The electrochemical properties of diamond are connected particularly with its chemical and physical inertness. On one hand, the corrosion resistance and hardness allows the application of the wide range of measurement conditions, including measurement in strongly acidic or alkaline media, in organic solvents, under ultrasonication etc. without the changes in the micro- or macrostructure of the surface. On the other hand, weak interaction of certain analytes with the diamond surface can result in high overpotential of their electrochemical reaction. The reversibility of the redox reaction usually increases with increasing degree of boron doping. However, even on the surface of metal-like, highly doped diamond, the electrode reactions do not reach the reversibility on platinum or similar metals. The same cause is probably responsible for the low background current of diamond electrodes, related to low detection limit obtainable by methods employing this electrode [37-39].

The topic of the diamond electrode surface and its termination is widely discussed, as it contributes significantly to the physical and chemical properties of BDDFE and it is also connected with the electrode history, pretreatment and cleaning. The surface of the electrode is terminated by hydrogen after the fabrication; it is hydrophobic and rather inactive. For some applications, it might be advantageous to change the surface so that oxygen-carrying terminal groups prevail, usually by anodic polarization, but other oxidative techniques (chemical reaction, oxygen plasma) are also possible [40]. The effect of cathodic reduction treatment was also observed [41]. It does not transform the electrode surface back to its original state, as the electrochemical reduction does not decrease the surface oxygen content [42]. Nevertheless, cathodically treated electrode is used in the place of true hydrogen-terminated electrode, due to its similar behavior and experimental accessibility [39]; the hydrogen-terminated surface is changed by anodic polarization connected with the measurement and it can be restored only by treatment with hydrogen plasma [42].

The difference in interaction of the oxygen-terminated and hydrogen-terminated surface with various compounds results in the change concerning the electrode response, electrode reaction reversibility and the electrode surface passivation. Generally, positively charged species are attracted to the oxygen-terminated electrode surface and repulsed from hydrogen-terminated electrode surface, and *vice versa* [43]. This effect causes increased electrode response and shift of the anodic peak potential to less positive values [44-46].

Already mentioned low chemical activity of the diamond surface is the reason of weak passivation of the diamond electrode surface, which is a feature particularly important for the utilization in anodic potential range due to the frequent formation of polymeric reaction products [47]. The dark side of the low passivation of diamond electrodes is that once passivated, mechanical cleaning stronger than mild wiping is virtually impossible. The most frequent electrochemical cleaning influences the surface termination, as was already mentioned. The problem of keeping the electrode surface in the optimum and stable state can hence present the key role in the method development.

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4. RESULTS

4.1 BDDFE surface pretreatment, cleaning and activation

As mentioned in Chapter 3.2, measurement with BDDFE requires attention to the stability of the electrode concerning the electrode surface fouling and termination. In this Thesis, BDDFE was used for the determination of three different compounds or classes of compounds: polyphenolic antioxidants, pharmaceutical metoclopramide, and aminonitrophenols. From the electrochemical point of view, these compounds contain phenolic structure in the first case and aniline structure in the latter two cases. Electrochemical oxidation of both structures follows a complicated mechanism; after the initial formation of a cation radical, consecutive dimerization or polymerization follows, with the orientation of the original molecules depending on the substitution of the aromatic ring, electrode material, supporting electrolyte, current density, and other conditions. Polymeric products tend to adhere to the electrode surface, causing its fouling. In the same time, evolution of oxygen on the electrode surface can disintegrate the undesired layer. Besides, the termination of the diamond structure influences the electrochemical processes, as the electrode surface itself can be oxidized or reduced under sufficient potential, which means that every potential application during measurement or electrochemical cleaning can alter the conditions for the following scan. All these influences have to be considered when dealing with the repeatability of the electrochemical determination.

In this Thesis, the first work dealing with the fouling of BDDFE was aimed to the determination of polyphenolic compounds present in beverages, such as tea, fruit juice and wine. Such a complex matrix naturally did not allow the determination of individual compounds, but it was not the aim of the work; instead, total phenol content as a value related to overall antioxidant capacity was determined [1]. Preliminary testing has indeed shown passivation of BDDFE as well as of GCE, which was used for the comparison purpose. Electrochemical cleaning of BDDFE was tested, consisting of application of highly cathodic and/or anodic potentials in 1M nitric acid, in an attempt to clean the surface by the electrochemical oxidation of the passivating layer [2] and to adjust the electrode surface to the state suitable for the electrochemical reaction [3]. Results show that when only anodic cleaning is applied, the peak potential moves toward anodic values, suggesting slower electron transfer kinetic at the electrode, and when the cathodic cleaning step is applied, the peak current decreases, indicating the failure of the cleaning process. Cleaning consisting of two steps, first cathodic one followed by second anodic one, ensured good repeatability of both the peak current and potential. The total phenol content was determined as the charge passed during the oxidation of the sample; comparison of the obtained results with the standard Folin-Ciocalteu method proved the suitability of the proposed method. [**4**]

The same cleaning procedure was used during the determination of metoclopramide, pharmaceutical with antiemetic and gastroprokinetic effect; however, it was possible to use milder cleaning conditions under the lower pH, probably due to the change in the electrochemical reaction. Determination method using flow injection analysis (FIA) with amperometric detection was also tested; expectations were that the electrode fouling is usually less pronounced in the flow arrangement [5]. However, electrode fouling was evident at lower flow rates, which confirms both strong passivating properties of metoclopramide and low passivation resistance of oxygen-terminated diamond electrode. High flow rate ensured the stable response of amperometric detection, suppressed electrode passivation and enabled the repeated measurement without the necessity of a demanding cleaning step. Method based on this optimization proved to be applicable for the determination of metoclopramide in simple matrices, namely pharmaceutical formulations. DPV method was also developed, but its lower accuracy and sensitivity and particularly the time-consuming electrode treatment made it less advantageous possibility. [**6**]

The last group of analytes, whose behavior was observed, comprised of five isomers of aminonitrophenol. In this case, BDDFE proved its superiority to GCE and GCPE concerning passivation resistance, as the first approximately ten measurements provide stable signal and even after that, the decrease is rather slow. Again, similar activation and cleaning procedure was used; in the case of these compounds, the cathodic potential was less important. During the experiments in the flow arrangement, no electrode fouling or necessity of activation was observed. [**7**]

We can sum up that all analytes of interest tended in some extent to the electrode fouling during anodic oxidation, but the lower impact to the electrode performance can be expected in comparison with other carbon-based electrodes. Strong electrochemical oxidation proved to be an accessible way for the removal of the passivating layer; determination of the negatively charged or neutral molecules was successfully performed on the hydrogen-terminated electrode surface, which is in compliance with previous experience [8].

4.2 Preliminary characterization of the used electrodes

Cyclic voltammetry (CV) was used for the characterization of BDDFE and GCPE; GCE was used for comparison. Observed parameters were background current, potential window width, and reversibility of the electrode reaction of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$. Prior the measurements, the surface of GCPE was wiped off with wet filtration paper, GCE was polished with 0.05 μm alumina slurry and BDDFE was activated by application of high anodic and cathodic potential, as described in the previous chapter.

The parameters of the BDDFE were found superior concerning the background current and potential window width, but the performance of GCPE was almost as good, in contrary to the expectations, and the properties of GCE were the worst. The main drawback of the GCPE is the oxygen signal in cathodic potential region, making the determination in this range experimentally difficult [7].

Concerning the reversibility of the electrode reaction of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ system, however, the reverse order was found; neither of the electrodes was able to reach the theoretical peak potential separation, but GCE was close to this value, at GCPE the peak potential separation was higher than the theoretical expectation, and the difference at BDDFE was markedly higher. The dependence of the peak height on the scan rate of the cyclic voltammograms for BDDFE also suggested mixed diffusion and adsorption controlled process [7]. The same pattern was observed later during the determination of aminonitrophenols (see part 4.2) and it was the reason of problems during the determination of analyte mixtures.

4.3 Voltammetric methods for aminonitrophenols determination

As was already mentioned, voltammetric methods for the determination of aminonitrophenols were aimed to the two main fields of interest concerning these compounds. The first one is their determination in cosmetics, particularly hair dyes; their presence in these products is restricted because of their toxicological properties (see Chapter 2.1). The other is the determination of 2A4NP and 4A2NP in body fluids, in our case in urine, as the metabolites of 2,4-dinitrophenol. During the optimization measurements, GCPE and BDDFE were employed; for the specific applications, the more advantageous of these two electrodes was selected.

As the initial measurement, cyclic voltammetry was used for studying the electrochemical behavior of aminonitrophenols [7]. Nitro moiety, present in the molecule, is electrochemically reduced; the probable product of this reaction is amino-hydroxylaminophenol, whose quasi-reversible oxidation to nitroso derivative gives rise to the pair of peaks at slightly positive potentials [9]. Besides, two oxidative peaks are present, corresponding to the oxidation of amino and possibly also hydroxy moiety. The reactions are almost irreversible, with only a small indication of one cathodic peak. This behavior is common for this kind of compounds due to the fast chemical transformation of the reaction products [10]. The only observed difference between GCPE and BDDFE was the overpotential of the reactions on BDDFE, reaching in some cases almost 100 mV.

Dependence of the DP voltammetric behavior of aminonitrophenol isomers on pH of the supporting electrolyte was observed and under the optimum conditions for the particular compound and electrode, concentration dependences were measured. Certain overlay of the oxygen and analyte signals appeared in case of cathodic measurements on CPE, which caused low sensitivity of the determination. BDDFE, on the other hand, suffered from the peak broadening due to the overpotential of the electrochemical reactions [7].

It was proposed earlier that CPE with addition of bismuth powder is less sensitive to the oxygen influence [11]. For that reason, series of electrodes was prepared with part of carbon powder replaced by bismuth powder and their behavior was observed using 4A3NP as the representative of the analytes. While the graduating suppression of the oxygen signal was observed with increasing proportion of the bismuth powder, as expected, decrease of the analyte peak height, high noise and narrow potential window were the disadvantages of such electrodes. Therefore, these electrodes were not utilized in further measurements [**12**].

In the case of the determination in cosmetics, conditions for the determination in the real samples were selected according to the maximum overall peak height and the separation of the peaks, particularly in more populated anodic potential range. Therefore, pH 5 was selected for DPV on CPE in anodic potential region and pH 6 for DPV on BDDFE in cathodic potential region. Successful determination of 4A3NP in the real samples of hair dyes proved the suitability of these conditions, although some interferences with another compounds present in the sample were observed [7].

In the case of the determination of 2,4-dinitrophenol metabolites, suitable resolution of the two analyte peaks was priority; only CPE in anodic potential range was able to provide it. Medium of pH 3 was selected. Under these conditions, it was proved that the peak heights of both analytes are independent and that their individual determination is thus possible. Nevertheless, the determination in the urine sample was not successful due to the interference of matrix components in spite of the preliminary separation by solid phase extraction (SPE) [**13**].

4.4 HPLC with amperometric detection

Selectivity of the voltammetric methods described in the previous chapter is suitable for simple mixtures, but in the case of a complex sample or in the presence of other oxidisable/reducible compounds, it might be unsatisfactory. To enhance the selectivity, electrochemistry was combined with the chromatographic separation; this combination naturally requires more complex instrumentation.

For the determination, amperometric detection in anodic potential range was selected in order to avoid the experimental problems connected with the removal of oxygen from the chromatographic system for the application of cathodic potentials. As in voltammetric measurements, GCPE and BDDFE were employed; RP-HPLC on column with chemically bonded C18 phase was used in both cases. The determination conditions, namely composition of the mobile phase, pH of the aqueous part of the mobile phase, and detection potential were optimized for a suitable determination of the analytes. The behavior of the analytes regarding pH corresponds with their acidobasic properties: changes in the retention occur in pH lower than 3 and higher then 7, where protonization or deprotonization takes place. Acidic pH was employed, as it is more compatible with the working pH range of the column. In the second step, methanol content was adjusted to provide suitable resolution of the peaks. Detailed description of the optimized determination conditions can be found in Appendices and the original works [**7,13**]. For the selection of detection potential, hydrodynamic voltammograms were measured under the optimum separation conditions. Utilization of CPE allowed the use of lower potentials than when using BDDFE, hence compensating for the lower range of applicable potentials; this behavior reflects the trend already observed during the voltammetric measurements. No problems with the electrode passivation were observed.

Solid phase extraction (SPE) method was developed for the preconcentration and preliminary separation of the analytes from urine samples using EN extraction columns (Merck). It was necessary to use different elution solvent for each of the analytes and not even this combination ensured the stable recovery of 4A2NP, which caused high interception of the 4A2NP concentration dependence and hence the limited applicable concentration range [**13**]. The developed methods are suitable for the determination of the compounds of interest in the selected samples, i.e. of all studied aminonitrophenol isomers in hair dyes and of 2A4NP and 4A2NP in urine samples. In comparison with the spectrophotometric detection, higher selectivity of the electrochemical detection provided better separation of analytes responses from the signals of matrix components and interfering compounds.

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5. CONCLUSION

Several electrochemically oxidizable compounds containing phenolic or aniline moieties were investigated on BDDFE. The obtained results confirm that in some cases, BDDFE exhibits more resistance to fouling than other carbon-based electrodes, but in most cases, the passivation of the electrode is observable. The cleaning and activating procedure, consisting of application of high anodic and cathodic potentials in acidic medium, was developed. In the flow arrangement, the fouling can be overcome by application of higher flow rate.

Electrochemical methods for the determination of aminonitrophenols were developed; the methods are based on the technique of DPV and HPLC with amperometric detection and they employed BDDFE and GCPE as working electrodes. Preliminary electrode testing was performed by cyclic voltammetry. Modification of the GCPE by the addition of bismuth did not bring any substantial advantage. The applicability of the developed methods was verified by the determination of tested analytes in real samples of hair dyes and in the case of 2A4NP and 4A2NP also in the model samples of urine after the solid phase extraction. From the voltammetric methods, the one using BDDFE for the cathodic determination in hair dyes showed sufficient selectivity. The performance of chromatographic methods was found suitable for the determination of aminonitrophenols in the observed matrices.

11. APPENDIX VI

CONFIRMATION OF PARTICIPATION

1) **Dejmkova, H.;** Zima, J.; Barek, J., Application of carbon paste electrodes with admixed bismuth powder for the determination of 4-amino-3-nitrophenol. In *Sensing in Electroanalysis*; (Eds: Vytřas, K.; Kalcher, K.; Švancara, I.) Vol. 3, University of Pardubice: Pardubice, **2008**; pp. 83-89 (ISBN 978-80-7395-087-3).

   Percentage of participation of Mgr. H. Dejmkova ~ **75 %**.

2) **Dejmkova, H.**; Barek, J.; Zima, J. Determination of Aminonitrophenols in Hair Dyes Using a Carbon Paste Electrode and a Boron-Doped Diamond Film Electrode – A Comparative Study. *International Journal of Electrochemical Science*, **2011**, *6*, 3550-3563.

   Impact Factor (2011): **3.729**; percentage of participation of Mgr. H. Dejmkova ~ **75 %**.

3) **Dejmkova, H.**; Stoica, A.-I.; Barek, J.; Zima, J. Voltammetric and amperometric determination of 2,4-dinitrophenol metabolites. *Talanta*, **2011**, *85*, 2594-2598.

   Impact Factor (2011): **3.794**; percentage of participation of Mgr. H. Dejmkova ~ **70 %**.

4) **Dejmkova, H.**; Scampicchio, M.; Zima, J.; Barek, J.; Mannino, S. Determination of Total Phenols in Foods by Boron Doped Diamond Electrode. *Electroanalysis*, **2009**, *21*, 1014–1018.

   Impact Factor (2009): **2.630**; percentage of participation of Mgr. H. Dejmkova ~ **65 %**.

5) **Dejmkova, H.**; Dag, C.; Barek, J.; Zima, J. Voltammetric and amperometric determination of metoclopramide on boron-doped diamond film electrode. *Central European Journal of Chemistry*, **2012**, *10*, 1310-1317.

   Impact Factor (2011): **1.073**; percentage of participation of Mgr. H. Dejmkova ~ **65 %**.

I declare that the percentage of participation of Mgr. Hana Dejmkova at the above given papers corresponds to above given numbers.

Prague, 20. 5. 2013 Prof. RNDr. Jiří Zima, CSc.

12. APPENDIX VII

LIST OF PUBLICATIONS, ORAL AND POSTER PRESENTATIONS

Journal articles

[1] Zima, J.; **Dejmkova, H.**; Barek, J. HPLC Determination of Naphthalene Amino Derivatives Using Electrochemical Detection at Carbon Paste Electrodes. *Electroanalysis*, **2007**, *19*, 185-190.

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[6] Fischer, J.; **Dejmkova, H.**; Barek, J. Electrochemistry of Pesticides and its Analytical Applications. *Current Organic Chemistry*, **2011**, *15*, 2923-2935.

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[9] **Dejmkova, H.**; Dag, C.; Barek, J.; Zima, J. Voltammetric and amperometric determination of metoclopramide on boron-doped diamond film electrode. *Central European Journal of Chemistry*, **2012**, *10*, 1310-1317.

[10] **Dejmkova, H.**; Mika, J.; Barek, J.; Zima, J. Behavior of Glassy Carbon Paste Electrode in Flowing Methanolic Solutions. *Electroanalysis*, **2012**, *24*, 1766–1770.

[11] **Dejmkova, H.**; Mikes, M.; Barek, J.; Zima, J. Determination of Sulfamethizole Using Voltammetry and Amperometry on Carbon Paste Electrode. *Electroanalysis*, **2013**, *25*, 189-194.

[12] Zavazalova, J.; **Dejmkova, H.**; Barek, J.; Peckova, K. Voltammetric and Amperometric Determination of Mixtures of Aminobiphenyls and Aminonaphthalenes Using Boron Doped Diamond Electrode. *Electroanalysis*, **2013**, *25*, 253-262.

[13] **Dejmkova, H.**; Houskova, L.; Barek, J.; Zima, J. Utilization of carbon paste electrodes for the voltammetric determination of chlortoluron. In press, *Electroanalysis*, **2013**.

Chapters in book

[1] **Dejmkova, H.**; Zima, J.; Barek, J., Application of carbon paste electrodes with admixed bismuth powder for the determination of 4-amino-3-nitrophenol. In *Sensing in Electroanalysis*; Vytřas, K.; Kalcher, K.; Švancara, I., Eds. University of Pardubice: Pardubice, **2008**; Vol. 3, pp. 83-89.

[2] Zavazalova, J.; **Dejmkova, H.**; Ramesova, S.; Barek, J.; Peckova, K., Amperometric and Spectrophotometric Detection of Aminobiphenyls and Aminonaphthalenes in HPLC. In *Sensing in Electroanalysis*; Vytřas, K.; Kalcher, K.; Švancara, I., Eds. University of Pardubice: Pardubice, **2010**; Vol. 5, pp. 163-173.

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[5] Libansky, M.; Zima, J.; Barek, J.; **Dejmkova, H.**, Voltammetric Determination of Arbutin on Carbon Paste Electrode. In *Sensing in Electroanalysis*; Kalcher, K.; Metelka, R.; Švancara, I.; Vytřas, K., Eds. University of Pardubice: Pardubice, **2011**; Vol. 6, pp. 281-287.

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[5] **Dejmkova, H.**; Cienciala, M.; Cerna, P.; Svecova, M.; Vokalova, V.; Zima, J.; Barek, J. *Použití HPLC-ED ke stanovení léčiv*, XXVIII. Modern Electrochemical Methods, Jetrichovice, 2008; Barek, J.; Navratil, T., Eds. BEST Servis, Ústí nad Labem: Jetrichovice, 2008; pp 8-11.

[6] **Dejmkova, H.**; Zima, J.; Barek, J. *Reduction of 4-amino-3-nitrophenol on bismuth-modified electrodes*, 4th International Students Conference "Modern Analytical Chemistry", Prague, 2008; Opekar, F.; Svobodova, E., Eds. Prof. Ing. Jiri G.K.Sevcik, DrSc. - CONSULTANCY: Prague, 2008; pp 89-94.

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[9] **Dejmkova, H.**; Zima, J.; Barek, J. *HPLC of Aminonitrophenols Using Electrochemical Detection on Boron-Doped Diamond Electrode*, 5th International Students Conference "Modern Analytical Chemistry", Prague, 2009; Nesmerak, K., Ed. Charles University in Prague, Faculty of Science: Prague, 2009; pp 11-14.

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[15] Barek, J.; **Dejmkova, H.**; Fischer, J.; Peckova, K.; Vyskocil, V.; Zima, J. *Vývoj nových elektrodových materiálů na Katedře analytické chemie Přírodovědecké fakulty Univerzity Karlovy v Praze*, Pokroky v analytickej chémii, Kočovce, Slovakia, 2011; Ústav analytickej chémie Fakulty chemickej a potravinárskej technológie STU v Bratislave: Kočovce, Slovakia, 2011.

[16] Barek, J.; **Dejmkova, H.**; Peckova, K.; Zima, J. *Non-Traditional Electrode Materials for Voltammetry and Amperometry*, Prague-Dresden Electrochemical Seminar 2011, Dresden, Germany, 2011; Dresden, Germany, 2011; p 4.

[17] Dedik, J.; Janovcova, M.; **Dejmkova, H.**; Barek, J.; Peckova, K. *Využití tištěných uhlíkových elektrod v elektroanalýze aminoderivátů polyaromatických uhlovodíků*, 13. študentská vedecká konferencia, Bratislava, Slovakia, 2011; Čopan, P., Ed. Bratislava, Slovakia, 2011; pp 57-58.

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[19] **Dejmkova, H.**; Barek, J.; Zima, J. *Determination of 2,4-Dinitrophenol Metabolites in Urine Using HPLC with Amperometric Detection*, XXXI. Modern Electrochemical Methods, Jetrichovice, 2011; Navratil, T.; Barek, J., Eds. BEST Servis, Ústí nad Labem: Jetrichovice, 2011; pp 33-37.

[20] Libansky, M.; Zima, J.; Barek, J.; **Dejmkova, H.** *Stanovení arbutinu na uhlíkové pastové elektrodě*, 13. študentská vedecká konferencia, Bratislava, Slovakia, 2011; Čopan, P., Ed. Bratislava, Slovakia, 2011; pp 59-60.

[21] Vlachova, K.; Barek, J.; Zima, J.; **Dejmkova, H.** *Determination of Aminoglutethimide Using HPLC-ED on CPE*, XXXI. Modern Electrochemical Methods, Jetrichovice, 2011; Navratil, T.; Barek, J., Eds. BEST Servis, Ústí nad Labem: Jetrichovice, 2011; pp 181-184.

[22] Zavazalova, J.; **Dejmkova, H.**; Barek, J.; Peckova, K. *Ampérometrická a spektrofotometrická detekce aminobifenylů a aminonaftalenů v HPLC*, 14. ročník soutěže "O cenu firmy Merck 2011", Usti nad Labem, Czech Republic, 2011; Česká společnost chemická - Chemické listy 105: Usti nad Labem, Czech Republic, 2011; pp s87-s89.

[23] Zima, J.; Barek, J.; **Dejmkova, H.** *Electrochemical determination of biologically active compounds*, XI. Workshop of Physical Chemists and Electrochemists, Brno, Czech Republic, 2011; Trnkova, L., Ed. Mendelova univerzita v Brně: Brno, Czech Republic, 2011; pp 317-321.

[24] Zima, J.; **Dejmkova, H.**; Vyskocil, V.; Danhel, A.; Barek, J. *Non-traditional Electrodes in Analysis of Organic Pollutants*, 7th International Conference on Instrumental Methods of Analysis, Modern Trends and Applications, Chania, Crete, Greece, 2011; Chania, Crete, Greece, 2011; p OP27.

[25] Zima, J.; Nemcova, L.; **Dejmkova, H.** *Carbon paste based sensors for monitoring of biologically active organic compounds*, 43rd IUPAC World Chemistry Congress, San Juan, Puerto Rico, 2011; San Juan, Puerto Rico, 2011; pp 415-416.

[26] Dedik, J.; Janovcova, M.; **Dejmkova, H.**; Barek, J.; Peckova, K. *Voltametrická a ampérometrická detekce amino derivátů bifenylu a naftalenu na sítotiskové uhlíkové elektrodě*, 15. ročník soutěže "O cenu firmy Merck 2012", Praha, Czech Republic, 2012; Česká společnost chemická - Chemické listy 106: Praha, Czech Republic, 2012; pp s7-s10.

[27] **Dejmkova, H.**; Barek, J.; Zima, J. *Využití uhlíkových pastových elektrod v analýze léčiv a jejich metabolitů*, ACP 2012 – Súčasný stav a perspektívy analytickej chémie v praxi, Bratislava, Slovakia, 2012; Česká společnost chemická - Chemické listy 106: Bratislava, Slovakia, 2012; pp s154-s156.

[28] **Dejmkova, H.**; Houskova, L.; Barek, J.; Zima, J. *Electrochemical Determination of Chlortoluron on Carbon Paste Electrode*, XXXII. Modern Electrochemical Methods, Jetrichovice, 2012; Navratil, T.; Fojta, M., Eds. BEST Servis, Ústí nad Labem: Jetrichovice, 2012; pp 23-26.

[29] Jarosova, R.; Barek, J.; Zima, J.; **Dejmkova, H.** *Voltametrické a amperometrické stanovení pesticidu carboxinu na uhlíkové pastové elektrodě*, 14. študentská vedecká konferencia, Bratislava, Slovakia, 2012; Horňáček, M., Ed. Bratislava, Slovakia, 2012; pp 44-45.

[30] Mika, J.; Zima, J.; Barek, J.; **Dejmkova, H.** *Změny povrchové struktury pastové elektrody z mikročástic skelného uhlíku v methanolickém roztoku*, 15. ročník soutěže "O cenu firmy Merck 2012", Praha, Czech Republic, 2012; Česká společnost chemická - Chemické listy 106: Praha, Czech Republic, 2012; pp s67-s71.

[31] Zavazalova, J.; **Dejmkova, H.**; Barek, J.; Peckova, K. *Voltammetric Determination of Amino Derivatives of Naphthalenes Using Boron-Doped Diamond Film Electrode*, XXXII. Modern Electrochemical Methods, Jetrichovice, 2012; Navratil, T.; Fojta, M., Eds. BEST Servis, Ústí nad Labem: Jetrichovice, 2012; pp 159-162.

[32] Zavazalova, J.; **Dejmkova, H.**; Barek, J.; Peckova, K. *Využití elektrod na bázi bórem dopovaného diamantu a platiny v ampérometrické detekci aminoderivátů naftalenu a bifenylu v HPLC*, 64. sjezd chemických společností, Olomouc, Czech Republic, 2012; Česká společnost chemická - Chemické listy 106: Olomouc, Czech Republic, 2012.

[33] Zima, J.; Houskova, L.; Mikes, M.; Barek, J.; **Dejmkova, H.** *New applications of carbon paste electrodes in environmental and clinical analysis*, 14th International Conference on Electroanalysis, Portoroz, Slovenia, 2012; Nationa Institute of Chemistry, Ljubljana, Slovenia: Portoroz, Slovenia, 2012; p 107.

Poster presentations

[1] Zima, J.; **Dejmkova, H.**; Barek, J. *HPLC stanovení aminoderivátů naftalenu pomocí uhlíkových pastových elektrod*, 57. Zjazd chemikov, Tatry, Slovakia, 2005; Slovenská chemická spolocnost - ChemZi 1: Tatry, Slovakia, 2005; p 159.

[2] **Dejmkova, H.**; Zima, J.; Ersoz, M.; Barek, J. *Voltametrické stanovení aminonitrofenolů na uhlíkové pastové elektrodě*, 58. sjezd chemických společností, Usti nad Labem, Czech Republic, 2006; Česká společnost chemická - Chemické listy 100: Usti nad Labem, Czech Republic, 2006; p 586.

[3] Zima, J.; **Dejmkova, H.**; Jemelkova, Z.; Cerna, P.; Barek, J. *Carbon Paste Electrodes as Detectors in HPLC-ED of Biologically Active Organic Compounds*, 11th International Conference on Electroanalysis, Bordeaux, France, 2006; Bordeaux, France, 2006; p P1 097.

[4] **Dejmkova, H.**; Zima, J.; Barek, J. *Redukce aminonitrofenolů na bismutem modifikovaných elektrodách*, 59. Zjazd chemikov, Tatry, Slovakia, 2007; Slovenská chemická spolocnost - ChemZi 3: Tatry, Slovakia, 2007; p 166.

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[6] Zima, J.; Kocourkova, M.; **Dejmkova, H.**; Jemelkova, Z.; Barek, J.; Moreira, J. C. *Voltammetric determination of Benserazide using carbon paste electrodes*, 6th Spring Meeting of the International Society of Electrochemistry, Foz do Iguacu, Brazil, 2008; International Society of Electrochemistry: Foz do Iguacu, Brazil, 2008; p 134.

[7] **Dejmkova, H.**; Fischer, J.; Zima, J.; Barek, J. *Voltammetric determination of aminonitrophenols using boron-doped diamond electrode*, 47th Meeting of the Serbian Chemical Society, Belgrade, 2009; Markovic, R.; Dunjic, B.; Dekanski, A., Eds. Serbian Chemical Society: Belgrade, 2009; pp 15-18.

[8] **Dejmkova, H.**; Hranicka, Z.; Navratilova, Z.; Barek, J. *Voltametric determination of nitrophenols using clay-modified carbon paste electrodes*, ACP 2010 – Súčasný stav a perspektívy analytickej chémie v praxi, Bratislava, Slovakia, 2010; Česká společnost chemická - Chemické listy 104: Bratislava, Slovakia, 2010; pp s563-s566.

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