

**CHARLES UNIVERSITY IN PRAGUE**

**FACULTY OF SCIENCE**

**Department of Analytical Chemistry**



**Voltammetric Determination of Selected Heterocyclic  
Compounds Using Various Amalgam-Based Electrodes**

A Thesis Submitted as the Basis for the Award of the Ph.D. Degree

**Prague 2014**

**Mgr. Dana Fischerová**

I declare that all the results which are used and published in this Ph.D. 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 Ph.D. Thesis, outside the Charles University in Prague is possible only with a written agreement of this university.

I also declare that neither this Ph.D. 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.

In Prague, 28. 1. 2014

.....  
Mgr. Dana Fischerová (born Deýlová)

This Ph.D. Thesis was experimentally carried-out in the period from 2008 till 2013 at the Charles University in Prague, Faculty of Science, Department of Analytical Chemistry, UNESCO Laboratory of Environmental Electrochemistry. During this period, a research visit was realised at National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis, Zografou, 157 71 Athens, Greece supervised by Professor Anastasios Economou. Some experiments were also carried out in cooperation with Ing. Bohdan Josypcuk, Ph.D., from J. Heyrovský Institute of Physical Chemistry, v.v.i. of the Academy of Sciences of the Czech Republic.

## Acknowledgment

I would like to express acknowledgements to all who have supported my research efforts during this time. Especially to **Prof. RNDr. Jiří Barek, CSc.**, at the Department of Analytical Chemistry, Charles University in Prague, my supervisor, for his kind leading my doctoral program and support in organizing research visits, and other members of the Department, particularly RNDr. Vlastimil Vyskočil, Ph.D., RNDr. Aleš Daňhel, Ph.D., RNDr. Hana Dejmková Ph.D. and all colleagues from our research group, for their extensive help and support. Further, I would like to thank to members of the J. Heyrovský Institute of Physical Chemistry, namely Ing. Bohdan Josypcuk, Ph.D. for providing the silver solid amalgam electrodes and his valuable remarks and comments and to Mgr. Věra Mansfeldová for AFM measurements. I thank also to Professor Anastasios Economou from Kapodistrian University of Athens, Laboratory of Analytical Chemistry in Athens in Greece for kind supervising during my research visits and challenging experience not only in chemistry.

Last, but not at least, I thank to my parents, husband RNDr. Jan Fischer, Ph.D. and all my friends for the support, patience and fun during my graduate studies.

This research was financial supported by The Ministry of Education, Youth and Sports of the Czech Republic (Projects LC 06035, MSM 0021620857, and RP 14/63 and KONTAKT (AMVIS) project ME 10004 (NEMVAD)), by the Charles University in Prague (Projects UNCE 2012/44 and SVV 2011-263204, SVV 2012-265201), by The Grant Agency of the Czech Republic (Project P206/12/G151), by The Grant Agency of the Academy of Sciences of the Czech Republic (Project No. IAA400400806), by the project SVV 261204 and by The European Union Lifelong Learning Programme (Erasmus).

## **Abstract**

Development and testing of new non-toxic electrode materials, their preparation and the development of analytical methods applicable for determination of selected substituted nitrobenzimidazoles is the main aim of this Ph.D. Thesis.

New working electrodes based on the modification of surface of polished silver solid amalgam electrode (p-AgSAE) by other electrodes materials which contain mainly mercury (mercury meniscus modified silver solid amalgam electrode (m-AgSAE), mercury film modified silver solid amalgam electrode (MF-AgSAE)), bismuth (bismuth film on silver solid amalgam electrode (BiF-AgSAE)) or carbon (electrode modified by microcrystalline natural graphite-polystyrene composite transducer (CFE)) were developed.

The optimum deposition time for preparation of film electrodes were discover and verified.

Their electrochemical behavior (stable and reproducible responses during measurement) were investigated and further used in voltammetric determination of mutagenic or carcinogenic environmental pollutants 2-amino-6-nitrobenzimidazole (ANBT) and 5-nitrobenzimidazole (5-NBIA). These analytes could be determined by direct current voltammetry (DCV) or differential pulse voltammetry (DPV).

The attempt at increasing the sensitivity using adsorptive stripping DCV or DPV at all tested electrodes with both compounds was not successful. As an alternative method to decrease of the limit of quantification for DPV of 5-NBIA at p-AgSAE addition of cetyltrimethylammonium bromide (CTMAB) was used. Limit of quantification under the optimal conditions decreased about twice by addition of CTMAB in comparison with measurement in the absence of the surfactant with keeping good accuracy.

All newly developed electrodes were found to be suitable alternatives to toxic mercury electrodes and their applicability was verified also on the model samples of deionized, drinking and river waters.

## Contents

<b>Abstract .....</b>	<b>5</b>
<b>List of Symbols and Abbreviations.....</b>	<b>8</b>
<b>1. INTRODUCTION.....</b>	<b>10</b>
1.1 References .....	12
<b>2. SUBSTITUED BENZIMIDAZOLES AND 4-NITROPHENOL.....</b>	<b>13</b>
2.1 Sources, Formation, Occurrence and Biological Effects .....	13
2.1.1 2-Amino-6-nitrobenzothiazole .....	14
2.1.2 5-Nitrobenzimidazole.....	14
2.1.3 4-Nitrophenol .....	15
2.2 Analytical Methods for Determination of Studied Compounds .....	15
2.3 References .....	17
<b>3. Working Electrodes .....</b>	<b>19</b>
3.1 Amalgam Electrode .....	19
3.2 Mercury Modified Amalgam Electrode .....	20
3.3 Bismuth Modified Amalgam Electrode .....	21
3.4 Carbon Modified Amalgam Electrode .....	22
3.5 References.....	23
<b>4. RESULTS AND DISCUSSION .....</b>	<b>24</b>
4.1 Preparation of Electrodes .....	24
4.2 Polarographic and Voltammetric Determination of 5-Nitrobenzimidazole.....	25
4.3 Polarographic and Voltammetric Determination of 2-Amino-6-nitrobenzimidazole .....	28
4.4 References .....	31
<b>5. CONCLUSIONS.....</b>	<b>32</b>
<b>6. APPENDIX I.....</b>	<b>34</b>
<i>Voltammetric determination of 6-nitrobenzimidazole in the presence of     surfactants</i>	
<b>7. APPENDIX II .....</b>	<b>43</b>
<i>Voltammetric determination of 4-nitrophenole and 5-nitrobenzimidazole using     different types of silver solid amalgam electrodes – a comparative study</i>	

<b>8. APPENDIX III.....</b>	<b>51</b>
<i>Voltammetric determination of 2-amino-6-nitrobenzothiazole at two different silver amalgam electrodes</i>	
<b>9. APPENDIX IV .....</b>	<b>57</b>
<i>Bismuth film electrode at silver solid amalgam substrate as a new tool for voltammetric determination of electrochemically reduction organic compounds</i>	
<b>10. APPENDIX V.....</b>	<b>64</b>
<i>New type of large surface bismuth film electrode at a silver solid amalgam substrate and its application for voltammetric determination of 5-nitrobenzimidazole</i>	
<b>11. APPENDIX VI .....</b>	<b>77</b>
<i>Voltammetric determination of 2-amino-6-nitrobenzothiazole and 5-nitrobenzimidazole using a silver solid amalgam electrode modified by a microcrystalline natural graphite-polystyrene composite film</i>	
<b>12. APPENDIX VII CONFIRMATION OF PARTICIPATION.....</b>	<b>83</b>
<b>13. APPENDIX VIII – LIST OF PUBLICATIONS, ORAL AND POSTER PRESENTATIONS.....</b>	<b>85</b>

## List of Symbols and Abbreviations

<b>4-NP</b>	4-nitrophenol
<b>5-NBIA or 6-NBIA</b>	5-nitrobenzimidazole
<b><math>\alpha</math></b>	significance level
<b>AC</b>	alternating current
<b>AdSV</b>	adsorptive stripping voltammetry
<b>AFM</b>	atomic force microscope
<b>AgSAE</b>	silver solid amalgam electrode
<b>ANBT</b>	2-amino-6-nitrobenzothiazole
<b>BiF-AgSAE</b>	bismuth film electrode prepared by electrodeposition of bismuth on a silver solid amalgam substrate
<b>BiF-CPE</b>	bismuth film deposited on a carbon paste substrate
<b>BiF-GCE</b>	bismuth film deposited on a glassy carbon substrate
<b>BR</b>	Britton-Robinson
<b><math>c</math></b>	molar concentration
<b>CAS</b>	chemical Abstract Service
<b>CFE</b>	carbon film electrode
<b>CTMAB</b>	cetyltrimethylammonium bromide
<b>DC</b>	direct current
<b>DCV</b>	direct current voltammetry
<b>DPV</b>	differential pulse voltammetry
<b>DPP</b>	differential pulse polarography
<b>DME</b>	dropping mercury electrode
<b><math>E</math></b>	potential
<b><math>E_{1,reg}</math></b>	more positive regeneration potential
<b><math>E_{2,reg}</math></b>	more negative regeneration potential
<b><math>E_{acc}</math></b>	accumulation potential
<b>EDTA</b>	ethylenediaminetetraacetic acid
<b><math>E_p</math></b>	peak potential
<b>HMDE</b>	hanging mercury drop electrode
<b><math>I</math></b>	current
<b><math>I_p</math></b>	peak current
<b><math>L_Q</math></b>	limit of quantification
<b>m-AgSAE</b>	mercury meniscus modified silver solid amalgam electrode
<b>MF-AgSAE</b>	mercury film modified silver solid amalgam electrode
<b><math>n</math></b>	number of measurements
<b>NPAHs</b>	nitroated polycyclic aromatic hydrocarbons



<b>PAHs</b>	polycyclic aromatic hydrocarbons
<b>p-AgSAE</b>	polished silver solid amalgam electrode
<b>pH*</b>	corrected pH in mixed media
<b>PPE</b>	platinum auxiliary electrode
<i>R</i>	correlation coefficient
<b>RAE</b>	reference electrode RAE 113
<i>RSD</i>	relative standard deviation
<b>SDS</b>	sodium dodecylsulphate
<b>SPE</b>	solid phase extraction
<i>t<sub>acc</sub></i>	accumulation time
<i>t<sub>dep</sub></i>	time of deposition on film
<b>UK</b>	United Kingdom
<b>UNESCO</b>	United Nations Educational, Scientific and Cultural Organization
<b>USA</b>	United States of America
<b>v/v</b>	volume ratio

## 1. INTRODUCTION

This diploma Ph.D. thesis has been submitted as a contribution to the ever growing efforts of environmental analysis. It was elaborated under the framework of a long term research at UNESCO Laboratory of Environmental Electrochemistry in Prague in the field of development of highly sensitive and selective methods for the determination of heterocyclic and polycyclic aromatic hydrocarbons (PAHs) their nitro, amino and hydroxy derivatives. **The diploma Ph.D thesis presents four published scientific publications [1-4] and two papers in press [5-6] which are attached as Appendix parts I – VI (Chapters 6 – 11). 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.**

- [1] **Deylova D.**, Yosypchuk B., Vyskocil V., Barek, J.; Voltammetric determination of 4-nitrophenol and 5-nitrobenzimidazole using different types of silver solid amalgam electrodes – A comparative study. *Electroanalysis*, **2011**, 23 (7), 1548-1555.
- [2] **Deylova D.**, Barek J., Vyskocil V.; Voltammetric determination of 6-nitrobenzimidazole in the presence of surfactants. *Collect. Czech. Chem. Commun.*, **2011**, 76 (11), 1317–1325.
- [3] **Deylova D.**, Vyskocil V., Barek, J.; Voltammetric determination of 2-amino-6-nitrobenzothiazole at two different silver amalgam electrodes. *Electrochim. Acta*, **2012**, 62, 335-340.
- [4] **Deylova D.**, Vyskocil V., Barek J., Economou A.; Bismuth film electrode at a silver solid amalgam substrate as a new tool for voltammetric determination of electrochemically reducible organic compounds. *Talanta*, **2012**, 102, 68-74.
- [5] **Deylova D.**, Vyskocil V., Economou A., Mansfeldová V., Barek J.; New tipe of large surface Bismuth film electrode at a silver solid amalgam substrate and its application for voltammetric determination of 5-Nitrobenzimidazole. *Int. J. Electrochem. Sci.*, **2014**, in press.

- [6] **Devlova D.**, Vyskocil V., Barek J.; Voltammetric determination of 2-amino-6-nitrobenzothiazole and 5-nitrobenzimidazole using a silver solid amalgam electrode modified by a microcrystalline natural graphite-polystyrene composite film. *J. Electroanal. Chem.*, **2014**, <http://dx.doi.org/10.1016/j.jelechem.2014.01.017>.

Nitrated polycyclic aromatic hydrocarbons (NPAH) and aromatic heterocyclic hydrocarbons belong among highly dangerous mutagenic compounds. The interest in these substances is obvious, because some of them are directly emitted to the atmosphere by diesel and petrol engines. Diesel engine exhaust belongs to the most important sources of NPAH and in the air (more than 50 was identified [1]). These compounds are responsible for mutagenicity of engine exhausts, many of them even in the absence of exogenous metabolic activation. Great efforts have been made to detect them in different kinds of environmental samples, because they can enter to the body by inhalation, absorption through the skin and through intestinal tract. Attention is also being paid to the analysis of their derivatives, which can be formed during metabolic transformations.

Because of the extremely wide range of matrices and samples, where NPAHs and their derivatives can be present, the often small amounts of individual compounds and a myriad of interfering substances, analytical methods are required which are not only extremely sensitive, but can also provide great selectivity.

Electroanalytical methods have become nowadays more attractive in comparison with dominating separation and spectrometric methods, thanks to development suitable electrode materials, and possibility of their mechanical and/or chemical modifications. Electrochemical sensors represent beneficial tool in environmental, medicinal, pharmaceutical and even toxicological monitoring. A huge advantage is their low cost and simple handling.

**Presented PhD Thesis is focused on the use of the silver amalgam for development of novel sensors by modification of its surface and arrangements further applied in voltammetric determination of selected environmental organic pollutants: 4-nitrophenol (4-NP) [2], 2-amino-6-nitrobenzothiazole (ANBT) [3-5] and 5-nitrobenzimidazole [2, 5-7]. All these compounds are listed as “priority pollutants” by**

United States Environmental Protection Agency (US EPA) and mainly used in agriculture (nitrophenols as fertilizers (plant growth stimulators)), industry (5-NBIA, ANBT), or as an intermediate for preparation of medicaments (5-NBIA). Sensors developed in this Thesis may be obviously used for the determination of other electrochemically reducible or oxidisable organic compounds and they may be used as suitable alternative to mercury electrodes.

## 1.1 References

- [1] J. Arey, B. Zielinska, R. Atkinson, A. M. Winer, T. Ramdahl, J. N. Pitts Jr, *Atmos. Environ.* **1986**, *20*, 2339.
- [2] **D. Deylova**, B. Yosypchuk, V. Vyskocil, J. Barek, *Electroanalysis* **2011**, *23*, 1548.
- [3] **D. Deylova**, V. Vyskocil, J. Barek, A. Economou, *Talanta* **2012**, *102*, 68.
- [4] **D. Deylova**, V. Vyskocil, J. Barek, *Electrochimica Acta* **2012**, *62*, 335.
- [5] **D. Deylova**, V. Vyskocil, J. Barek, *J. Electroanal. Chem.* **2014**,  
<http://dx.doi.org/10.1016/j.jelechem.2014.01.017>.
- [6] **D. Deylova**, J. Barek, V. Vyskocil, *Collect. Czech. Chem. Commun.* **2011**, *76*, 1317.
- [7] **D. Deylova**, V. Vyskocil, A. Economou, V. Mansfeldova, J. Barek, *Int. J. Electrochem. Sci.* **2014**, in press.

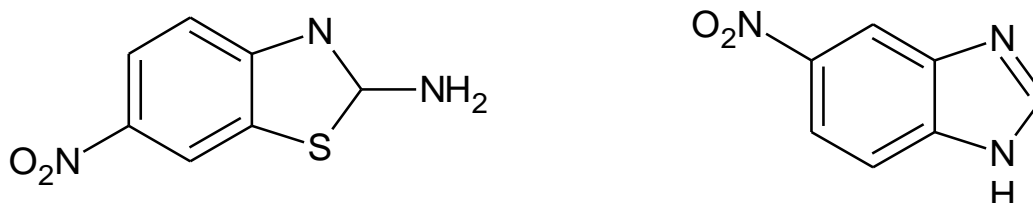
## 2. Substituted benzimidazoles and 4-nitrophenol

### 2.1 Sources, Formation, Occurrence and Biological Effects

Nitrated polycyclic aromatic hydrocarbons (NPAHs) and aromatic heterocyclic hydrocarbons are widespread environmental pollutants. A number of them have been casually connected with increasing risk of cancer [1], many are proven to be mutagenic and carcinogenic. Therefore, many research groups focus to find out the sources of their emission to environment, investigate their mutagenic and carcinogenic activities [2], chemical and physical properties [3] and primarily develop new analytical methods for the identification and quantification of NPAHs in the environment [4-7]. The highest possibility for contact of human beings with NPAHs is in the atmosphere potentially through the inhalation, because the main sources these compounds are emissions from combustion processes (fossil fuels, heavy industry, and car exhaust). Burning processes generates PAHs which can be further converted into NPAH by reaction with  $\text{NO}_x$  [8, 9].

In this thesis, **2-amino-6-nitrobenzothiazole** (ANBT) and **5-nitrobenzimidazole** (5-NBIA) (see Fig 1) which belongs to similar group of nitrated heterocyclic compounds were selected as model compounds. The other reason why we have studied these compounds is the fact that they pertain to the group used as precursors for synthesis pharmaceuticals.

As a substance with simple structure and voltammetrically known behavior (on similar electrodes) [10] **4-nitrophenol** (4NP, see Fig 2) was used for testing newly developed electrodes.



**Fig 1.** Structural formulae of 2-amino-6-nitrobenzothiazole and 5-nitrobenzimidazole

### 2.1.1. 2-Amino-6-nitrobenzothiazole

2-Amino-6-nitrobenzothiazole (ANBT) is from the group of substances often used as a bright disperse dyes based on production by diazotation [11]. Colors of thus prepared dyes are mainly red and violet and they are used for coloring polyester and textile [12]. ANBT diazotized and coupled to N-phenylmorpholine also gives good dyes for acetate and polyester fibers and it has been used for the preparation of reactive dyes for cellulose [13]. This substance contains a D- $\pi$ -A type of chromophore for the photoconductive measurements [14] and it is also used as a high glass transition chromophore in push-pull nonlinear optical applications [15]. Although this substance is useful from the industrial point of view, ANBT has also been shown to exhibit both genotoxic and mutagenic properties [16]. Nevertheless nitrobenzothiazoles are used as precursors in production of various drugs. For example they can act as inhibitors for Mycobacterium tuberculosis ATP phosphoribosyl transferase [17]. ANBT forms binuclear complexes with copper(II). They have significance in bioinorganic chemistry, magneto chemistry, material science, superconductivity and multi electron redox chemistry. For surface-active properties of complexes of copper(II) with ANBT, these complexes exhibit many applications in agrochemistry as dispersing, foaming and wetting agents [18]. ANBT can be also used for the preparation of fungicides [19] and herbicides [20].

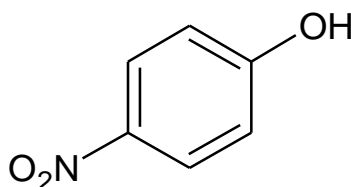
### 2.1.2. 5-Nitrobenzimidazole

5-nitrobenzimidazole (5-NBIA) was common in the past as a part of photographic processing solutions [21]. It is complexation reagent especially for bivalent metal ions [22]. That is the reason why 5-NBIA is used in metallurgic industry for example as primary stabilizer in the electroless deposition of copper [23]. Its adsorptive and inhibitive properties have been studied in the area of metal corrosion protection [24]. This compound is used for processing holographically exposed silver halide sensitized holographic material [25].

The second field of using is milling industry. Moreover, twice-substituted 5-NBIA derivatives have antibacterial, anaesthetic, inflammatory, antihelminthic, and viral effects [26-30]. In a veterinary medicine benzimidazoles are used as anthelmintics for treatment and prevention of acute parasitic diseases. The importance of benzimidazoles in veterinary medicine is documented by the wide spectrum of substances used, as well as the variety of pharmaceuticals and dosage forms [31]. On the other hand the unsubstituted 5-NBIA is proven carcinogen and mutagen [32].

### 2.1.3. 4-Nitrophenol

Nitrophenols are widely used as intermediates for production of explosives (*e.g.* trinitrotoluene, picric acid). They are coming to environment from pesticide degradation products [33, 34], car exhausts, wood preservatives, plastics and industrial wastes [35]. They are listed as priority pollutants by the US Environmental Protection Agency [36, 37]. Pesticides based on simple nitrophenols are generally not allowed but some of them are still used [38]. Because of their toxicity and vast scale distribution in the environment, their determinations have become one of the important goals of environmental analysis.



**Fig 2.** Structural formula of 4-nitrophenol

## 2.2 Analytical Methods for Determination of Tested Compounds

2-amino-6-nitrobenzothiazole contains cathodically reducible nitro group and it was investigated using direct current polarography, oscillopolarography [39], cyclic voltammetry and coulometry [40]. These techniques were used for its determination long time before only Ramman and infrared spectroscopy [41]. For its considerable use as a precursor for production of dyes, more simple and less expensive methods are requested. These method can be for example DC voltammetry (DCV) and differential pulse voltammetry (DPV) at a polished silver solid amalgam electrode (p-AgSAE), or mercury meniscus modified silver solid amalgam electrode (m-AgSAE) [42], Bi film on silver solid amalgam electrode [43] and carbon film electrode deposited on silver solid amalgam electrode [44].

Because 5-NBIA was used in fotoscience, one of typical analytical measurement of this compounds is determination by spectrophotometry in the ultraviolet and visible region [45]. Other popular methods are chromatographic methods (high presure liquid chromatography [46], thin-layer chromatography [47]). Electroanalytical methods represent a useful alternative to more frequent chromatographic methods, because they meet the stringent conditions on sensitivity, in spite of the fact that selectivity is frequently presented as a weak point of

electrochemical methods in analysis of complex matrices. However, the selectivity can be increased by application of preliminary separation step like a solid phase extraction (SPE). Combination of SPE and a voltammetric method is a good way for the determination of these compounds.

As the first electroanalytical determination potentiometric titration [48] was used. Later, TAST polarography and differential pulse polarography (DPP) at the dropping mercury electrode (DME) was proposed. To reach lower limit of quantification newer methods such as direct current voltammetry (DCV) and differential pulse voltammetry (DPV) at the hanging mercury drop electrode (HMDE) were applied [45]. Because of unreasonable fear of mercury toxicity, voltammetric behavior of 5-NBIA on a new electrode material based on a polished silver solid amalgam electrode (p-AgSAE), mercury meniscus modified silver solid amalgam electrode (m-AgSAE), mercury film modified silver solid amalgam electrode (MF-AgSAE) [49], Bi film on silver solid amalgam electrode [50], carbon film electrode deposited on silver solid amalgam electrode [44] and tip amalgam paste electrode [51] was investigated. As an absolutely mercury free electrode glassy carbon, Bi film on glassy carbon and Bi film on gold electrode surface [51] were used. Moreover, the study of polarographic and voltammetric behavior of biologically active compounds can provide information about their electron-transfer reactions. This can be useful in explanation of the mechanism of their interaction with living cells.

Because of ubiquitous presence of above mentioned derivatives in environment and their adverse health effects, new analytical methods are sought for their determination.

## 2.3 References

- [1] J. Jacob, W. Karcher, J. J. Belliaro, R. Dumler, A. Boenke, *Fresenius Journal of Analytical Chemistry* **1991**, 340, 755.
- [2] A. Danhel, J. Barek, *Current Organic Chemistry* **2011**, 15, 2957.
- [3] A. Danhel, V. Mansfeldova, P. Janda, V. Vyskocil, J. Barek, *Analyst* **2011**, 136, 3656.
- [4] A. C. Lewis, R. E. Robinson, K. D. Bartle, M. J. Pilling, *Environ. Sci. Technol.* **1995**, 29, 1977.
- [5] I. S. Kozin, C. Gooijer, N. H. Velthorst, A. C. A. 193., *Anal. Chim. Acta* **1996**, 193.
- [6] M. Murayama, A. C. P.K. Dasgupta, *Anal. Chem.* **1996**, 68, 1226.



- [7] H. Kazuichi, M. Tsuyoshi, B. Mizuka, M. Motoichi, *Environ.Sci. Technol.* **1995**, 29, 928.
- [8] R. Atkinson, *Atmos. Environ. Part A. General Topics*, **1990**, 24, 1.
- [9] J. Arey, B. Zielinska, R. Atkinson, A. M. Winer, T. Ramdahl, J. N. Pitts Jr, *Atmos. Environ.* **1986**, 20, 2339.
- [10] A. Danhel, *Ph.D. Thesis Carles University, Prague* **2012**.
- [11] J. Sokolowskagajda, H. S. Freeman, *Dyes Pigment.* **1992**, 20, 137.
- [12] A. D. Towns, *Dyes Pigment.* **1999**, 42, 3.
- [13] *MCI European Patent Application 121825; Chem. Abstr.* **1985**, 102, 47330x.
- [14] K. Diduch, M. Wubbenhorst, S. Kucharski, *Synthetic Metals* **2003**, 139, 515.
- [15] K. Van den Broeck, T. Verbiest, J. Degryse, M. Van Beylen, A. Persoons, H. Samyn, *Polymer* **2001**, 42, 3315.
- [16] M. O. Kenyon, J. R. Cheung, K. L. Dobo, W. W. Ku, *Regulatory Toxicology and Pharmacology* **2007**, 48, 75.
- [17] Y. Cho, T. R. Ioerger, J. C. Sacchettini, *J. Med. Chem.* **2008**, 51, 5984.
- [18] N. Mathur, L. C. Heda, V. K. Mathur, P. Saxena, *Tenside Surfactants Deterg.* **2011**, 48, 23.
- [19] S. V. Mikulasek S., Odlerova V., Konecny V, Mikulasek S., Sutoris V., Odlerova V, Konecny V, , *Czechoslovakian Patent 187860; Chem. Abstr.* **1982**, 96, 181273s.
- [20] M. Lacova, Gvozdjakovi, A., Chovancovi, J. & Volna, F., *Chem. Papers* **1984**, 38, 693.
- [21] D. R. Canterford, *Journal of Photographic Science* **1978**, 26, 65.
- [22] E. K. Larisa, H. Sigel, *Eur. J. Inorg. Chem.* **1999**, 1999, 1781.
- [23] R. C. V. Piatti, J. J. Podestá, *Surf. Coat. Technol.* **1989**, 37, 193.
- [24] A. Popova, M. Christov, S. Raicheva, E. Sokolova, *Corrosion Science* **2004**, 46, 1333.
- [25] T. N. Briggs, T. J. Matemaghan, Method of processing holographic material, Application No. EP19860810321 19860717 **1987**.
- [26] A. E. Püttün, G. Bereket, E. Keskinö, *J. Chem. Eng. Data* **1995**, 40, 22.
- [27] T. Khrizanova, I. Torlakov, L. Zhelyazko, *Chemotherapy* **1972**, 449, 173.
- [28] V. J. Theodorides, R. J. Gyurik, W. D. Kingsbury, *Experienta* **1967**, 32, 702.
- [29] J. W. Sharp, R. B. Wescott, *Lab. anim. Sci.* **1976**, 26, 222.
- [30] K. Otaky, J. Inoje, Z. Yamazaki, , *Yukuguhu Zusshi* **1965**, 12, 926.
- [31] J. Velík, V. Baliharová, J. Fink-Gremmels, S. Bull, J. Lamka, L. Skálová, *Res. Vet. Sci.* **2004**, 76, 95.

- [32] H. S. Rosenkranz, M. H. Karol, *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis* **1999**, 431, 81.
- [33] D. H. Han, D. G. Jung, H. S. Shin, *Bull. Korean Chem. Soc.* **2008**, 29, 985.
- [34] G. S. Garbellini, G. R. Salazar-Banda, L. A. Avaca, *Food Chem.* **2009**, 116, 1029.
- [35] D. T. Sponza, O. S. Kuscu, *J. Hazard. Mater.* **2011**, 185, 1187.
- [36] *U.S. Environmental Protection Agency, Federal Register* **1989**, 52, 131.
- [37] J. Luttkke, V. Scheer, K. Levsen, G. Wunsch, J. N. Cape, K. J. Hargreaves, R. L. StoretonWest, K. Acker, W. Wieprecht, B. Jones, *Atmos. Environ.* **1997**, 21, 2637.
- [38] SRS, ed., List of the Registered Plant Protection Products, Vol. 3, The state phytosanitary administration, Brno, Czech republic **2006**.
- [39] V. I. Gorokhovskaya, *Zhurnal Obshchei Khimii* **1962**, 32, 3853.
- [40] V. K. Saraswati K., Prameela P. , *Transaction of the SAEST* **31** **1996**, 96.
- [41] S. Mohan, A. R. Prabakaran, F. Payami, *Journal of Raman Spectroscopy* **1989**, 20, 455.
- [42] **D. Deylova**, V. Vyskocil, J. Barek, *Electrochimica Acta* **2012**, 62, 335.
- [43] **D. Deylova**, V. Vyskocil, J. Barek, A. Economou, *Talanta* **2012**, 102, 68.
- [44] **D. Deýlová**, V. Vyskocil, J. Barek, *J. Electroanal. Chem.* **2014**,  
<http://dx.doi.org/10.1016/j.jelechem.2014.01.017>.
- [45] **D. Deylova**, *M. Sc. Thesis Carles University, Prague* **2008**.
- [46] M. M. Rhemrev-Boom, *J. Photogr. Sci.* **1988**, 36, 53.
- [47] K.-T. W. Wang, Iris S. Y.; Lin, Alice L., *J. Chin. Chem. Soc. (Taipei)* **1966**, 13, 77.
- [48] W. Selig, *Mikrochim. Acta* **1979**, 1, 453.
- [49] **D. Deylova**, B. Yosypchuk, V. Vyskocil, J. Barek, *Electroanalysis* **2011**, 23, 1548.
- [50] **D. Deylova**, V. Vyskocil, A. Economou, V. Mansfeldova, J. Barek, *Int. J. Electrochem. Sci.* **2014**, in press.
- [51] B. Chladkova, *M. Sc. Thesis Carles University, Prague* **2012**.

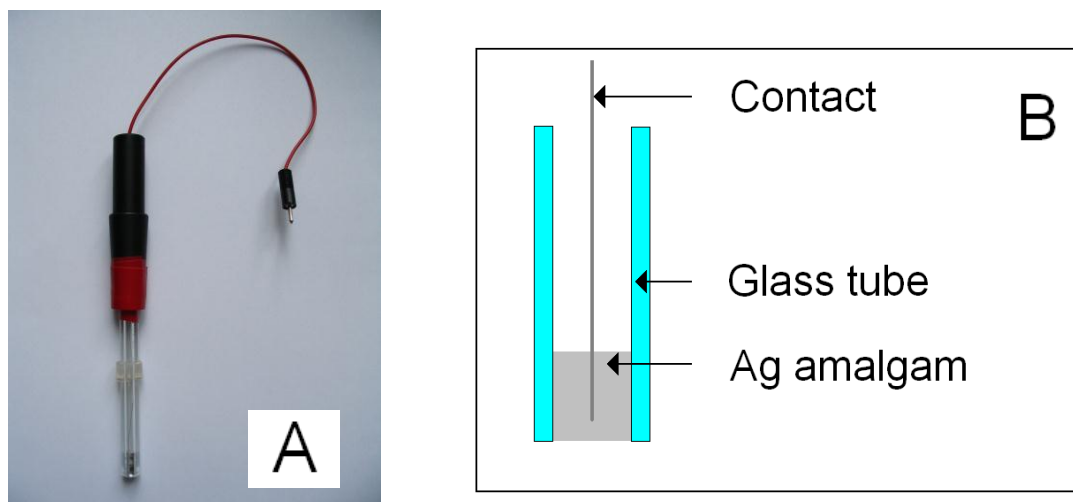
### 3. Working Electrodes

#### 3.1 Amalgam Electrodes

From discovery of polarography by Professor Heyrovský, as a working electrode material is used liquid mercury [1]. His colleagues and followers tried to find out other new electrode materials with similar or better qualities than mercury. Mercury is unique electrochemical material because its surface is atomically smooth, have large cathodic potential window and its surface is simply renewable. But in the recent time there is an increased fear of mercury toxicity (which is not so well-founded because toxic are first of all mercurio-organic compounds [2]). However, this is the main reason to try finding out new electrode materials. On the other hand, for over 170 years people used in mouth mercury compound. It is dental amalgam, which consists from mercury and silver in defined ratio. Electrodes based on this type of material represent a transition between solid metal surfaces and liquid mercury saturated by dissolved metals. Due to similar electrochemical properties, there is the reason use this type of material for electrodes to substitute mercury.

The non-toxic silver solid amalgam electrode (AgSAE) [3] have good electrochemical quality and it can be easily prepared. The construction scheme of typical amalgam electrode is depicted on fig. 3 Surface is not so smooth like mercury, but it is solid. This is big advantage for many applications. This type of electrode can be used in flow systems without problems [4] linked to liquid mercury based electrodes. But it has one of the huge disadvantages similar to all solid electrodes – the passivation of the surface [5]. This can be eliminated by suitable preliminary treatment e.g. an electrochemical cleaning of surface or mechanical cleaning on alumina. Polished surface electrode (p-AgSAE) provides potential window similar to HMDE, but electrode surface is more liable to passivation. These electrodes have found a lot of electroanalytical applications reviewed by B. Yosypchuk. Metals forming amalgams may be more electrochemically sensitive (*e.g.* Cu, Bi, Cd, Pb) or less sensitive (*e.g.* Ag, Au, Pt) [6] than pure mercury electrodes.

Some electrochemical or mechanical qualities of amalgam electrodes may be also improved by a modification of the electrode material or its surface [7, 8].



**Fig 3.** Silver solid amalgam electrode which can be further modified (A), and (B) scheme of electrode

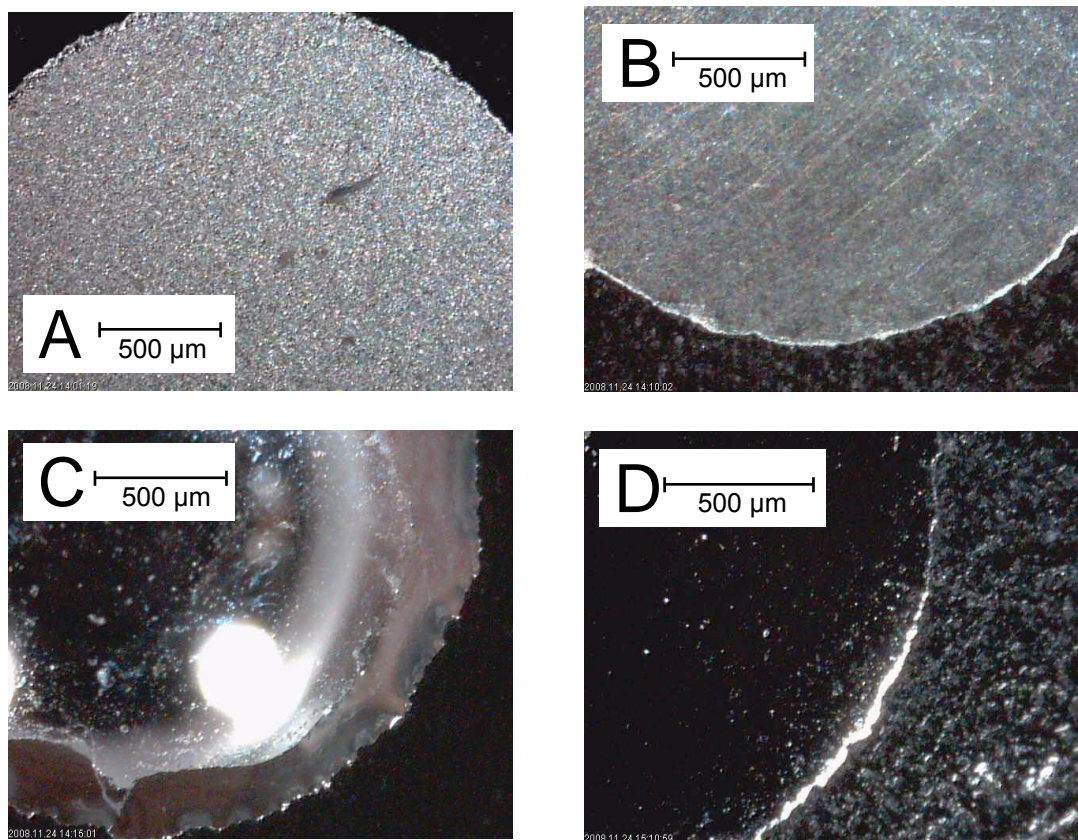
### 3.2 Mercury Modified Amalgam Electrode

In the previous chapter amalgam was mentioned as an electrode material with its good and negative properties. Simple way to improve its qualities is modification of electrodes [7, 8]. For modification other electrode materials, which are expensive or not easy available in solid state [9].

Into the second group belongs mercury modified silver solid amalgam electrode (m-AgSAE) which is prepared from p-AgSAE by immersing it for certain time into mercury. Thus prepared electrode combine advantages of HMDE (the DPV peaks are almost the same) [10] and solid electrode, e.g simple handling, good mechanical stability, simple surface regeneration including an electrochemical pretreatment of electrode surface and its mechanical renewing is simple and quick [8, 11]. Electrode is stable for several weeks, but with longer time the reproducibility and sensitivity declines. The wide field of analytical applications of these electrodes have been reviewed [6, 12]. After some time it is necessary to renew the meniscus surface. There is one of disadvantages of this electrode; preparation of new meniscus is not completely reproducible. We tried to overcome this by immersing m-AgSAE on exactly the same time into mercury, but it had no effect on reproducibility. One possibility how to remove this disadvantage is to use different method of modification by mercury.

This different method is based on electrochemical deposition of mercury from solution for selected time. The thickness of film is easy to calculate from Faradays law [13]. This electrode is denoted as mercury film modified silver solid amalgam electrode (MF-AgSAE),

which have repeatable thickness of mercury layer. On figure 4 surfaces of AgSAE, p-AgSAE, m-AgSAE and MF-AgSAE are shown.



**Fig 4.** Surface of the AgSAE (A), p-AgSAE (B), m-AgSAE (C) and MF-AgSAE (D).

### 3.3 Bismuth Modified Amalgam Electrode

Mercury is the best electrode material for reduction, but here is a pressure to replace mercury. Therefore, there is growing demand for materials with similar electrochemical qualities as mercury. We have confirmed that many suitable materials can be deposited on amalgam electrode as a support [14]. The main advantage of these electrodes is the fact that only a small quantity of chosen metal can be used. There are two ways how to prepare this electrode “ex situ” and “in situ”. First possibility is to deposit the metal from solution of its salt on the surface of an electrode and then to immerse the electrode into the measured solution and record the voltammogram. The second possibility is to add metal salt solution into the measured solution and then by electrochemical processes deposit the film on the electrode and carried out the measurement in one solution [7]. Both possibilities have their advantages and disadvantages. First method is useful in the case of danger of bare surface

contamination by impurities from measured solution and there is a possibility of simple visual control of deposited film quality and presence of defects. Electrode could be used for several hours. The second method can be better in the moment when it is necessary to be sure about the size of deposited area, but this method is available only for one measurement and afterwards the old surface must be removed and the new one formed [15]. Both of these methods are common for deposition of metals on the carbon type of electrodes [16] as a common surface material or on p-AgSAE as a suitable alternative.

### 3.4 Carbon Modified Amalgam Electrode

Mercury and amalgams are the good choice for electrochemical reduction, but oxidation is complicated or impossible on this type of surface, because the potential window is very narrow in positive potential region. Therefore, it is necessary to use other materials for electrochemical oxidation. Here, many forms of carbon can be used.

One of modern material favored for determination of large group of substances is boron doped diamond. This material has many advantages as a large cathodic and also anodic potential window, chemical stability, but sometime it can be poisoned by products of an electrochemical processes or analyte itself and must be intricately cleaned [17]. If we are lucky, electrode need only electrochemical cleaning, but in the case of stronger passivation it is necessary to use rougher cleaning procedures such as solutions of concentrated mineral acid [18].

This disadvantage is eliminated by the use of a carbon paste electrode. The problem with passivated surface is simple solved by wiping off electrode surface on a wet filter-paper. A disadvantage of this type of electrode is first of all lower reproducibility of the electrode surface because each wiping changes the surface and a manually skillful analyst is needed to get well reproducible results. Another problem is connected with work at negative potentials [19]. High amount of oxygen is adsorbed or dissolved in the structure of the paste when it is prepared, and broad signal of oxygen could collide with the signal of analyte [20].

For these reason it could be useful to prepare electrode from carbon based ink by covering a suitable electrode which serve just as electric contact. As support electrodes are most often used glassy carbon electrodes or metals disks. The amalgam as a surface can be suitable choice, too. These electrodes were used until recently mainly for determination of metals, but it was only a matter of time when the determination of organic compounds will be

investigated as well. One disadvantages of this type of electrode is small oxidation peak near +100 mV, which is most likely connected with not completely covered amalgam surface and probability of oxidation of certain part of mercury in the amalgam [21]. We have also observed, when the organic medium is used as a solvent, with increasing number of scans there is an increase in the background current. This effect is probably connected with damage of an adhesive between the glass edge and carbon film.

### 3.5 References

- [1] M. Heyrovsky, *J. Solid State Electrochem* **2011**, *15* (7-8), 1799.
- [2] R. Kalvoda, *Chemia Analityczna* **2007**, *52*, 869.
- [3] B. Yosypchuk, L. Novotny, *Electroanalysis* **2002**, *14*, 1733.
- [4] P. W. Alexander, U. Akapongkul, *Anal. Chim. Acta* **1984**, *166*(DEC), 119.
- [5] B. Yosypchuk, J. Barek, *Crit. Rev. Anal. Chem.* **2009**, *39*, 189.
- [6] A. Danhel, J. Barek, *Current Organic Chemistry* **2011**, *15*, 2957.
- [7] **D. Deylova**, V. Vyskocil, J. Barek, A. Economou, *Talanta* **2012**, *102*, 68.
- [8] **D. Deylova**, B. Yosypchuk, V. Vyskocil, J. Barek, *Electroanalysis* **2011**, *23*, 1548.
- [9] F. Lucarelli, A. Kicela, I. Palchetti, G. Marrazza, M. Mascin, -. *Bioelectrochemistry, Bioelectrochemistry* **2002**, *58*, 113.
- [10] J. Fischer, L. Vaňourková, A. Daňhel, V. Vyskočil, K. Čížek, J. Barek, K. Pecková, B. Yosypchuk, T. Navrátil, -. , *Int. J. Electrochem. Sci* **2007**, *2*, 226.
- [11] V. Vyskocil, T. Navratil, P. Polaskova, J. Barek, *Electroanalysis* **2011**, *22*, 2034.
- [12] V. Vyskocil, A. Danhel, J. Fischer, V. Novotny, **D. Deylova**, J. Musilova-Karaova, L. Maixnerova, K. Peckova, J. Barek, *Chemické Listy* **2010**, *104*, 1181.
- [13] B. Yosypchuk, M. Fojta, J. Barek, *Electroanalysis* **2011**, *22*, 1967.
- [14] B. Yosypchuk, J. Barek, *Chem. Listy* **2009**, *103* 284.
- [15] A. Krolicka, A. Bobrowski, *Electrochemistry Communications* **2004**, *6*, 99.
- [16] A. Economou, *Trac-Trends in Analytical Chemistry* **2005**, *24*, 334.
- [17] K. Peckova, J. Barek, *Current Organic Chemistry* **2011**, *15*, 3014.
- [18] J. Musilová, J. Barek, K. Pecková, *Electroanalysis* **2011**, *23*, 1236.
- [19] J. Zima, I. Svancara, J. Barek, K. Vytras, *Crit. Rev. Anal. Chem.* **2009**, *39*, 204.
- [20] **D. Deylova**, J. Barek, V. Vyskocil, *Collect. Czech. Chem. Commun.* **2011**, *76*, 1317.
- [21] V. Vyskočil, J. Barek, *Procedia Chemistry* **2012**, *6*, 52.

## 4. Results and discussion

### 4.1 Preparation of Electrodes

Before starting a voltammetric determination of selected compound it was necessary to prepare requested electrode.

Preparation of **p-AgSAE** is simple. The p-AgSAE consisted of a drawn-out glass tube, whose tip was packed with a fine silver powder (2.0 – 3.5  $\mu\text{m}$ ), amalgamated by liquid mercury and connected to an electric contact. Everything what is needed before starting measurement is polishing the surface to mirror shine on the polishing set by alumina powder (1.1  $\mu\text{m}$  particle) [1].

The **m-AgSAE** was prepared from p-AgSAE by immersing this electrode into a small volume of liquid mercury and agitating for 15 s [2].

The **MF-AgSAE** was prepared using a special device which consists of a plastic tube filled with 0.2 mL of 0.01 mol L<sup>-1</sup> HgCl<sub>2</sub> and 1 mol L<sup>-1</sup> KI, containing liquid mercury at the bottom with carbon contact. The MF-AgSAE must be prepared every day because the lifetime of 1 – 2 nm thick mercury film is about 2 hours. After the optimization of film thickness, which depends on time of deposition (300 – 3600 s) and repeatability of deposited film, the time of deposition 3600 s was selected as an optimum [3].

The **BiF-AgSAE** was prepared *ex situ* in a plating solution (after deaeration of the solution for 5 min with pure nitrogen) containing bismuth standard solution (1000 mg L<sup>-1</sup> Bi(III)) and 1.0 mol L<sup>-1</sup> acetate buffer pH 4.75 (5:95, v/v) by the deposition of the bismuth film on the p-AgSAE at constant potential -1.2 V (vs Ag/AgCl) for selected time ( $t_{\text{dep}}$ ) under stirred conditions [4, 5]. The BiF-AgSAE had to be prepared every day because a lifetime of the electrode surface is relatively short (similar as for mercury film). Even in this case it is necessary to optimize deposition time of Bi film. For electrode with disc diameter 2.64 mm (using for determination of 5-NBIA) investigated time range was 300 – 3600 s. As an optimal deposition time was selected 1800 s. This film was stable for long time and repeatability of film preparation (calculated from measurements on 10 films) was good (12 %) and 20 measurements on one freshly prepared film have  $RSD = 3 \%$ . For electrode with disc diameter 0.5 mm investigated time range was 60 – 600 s. As an optimal deposition time was selected 300 s. This film was stable for long time and repeatability of film preparation (calculated from



measurements on 10 films) was good (16 %) and 20 measurements on one freshly prepared film have  $RSD = 5\%$  [6].

The CFE was prepared by immersing p-AgSAE surface in the conductive ink containing 1,2-dichloroethane, crystalline graphite 2  $\mu\text{m}$  and polystyrene. The active part of the electrode just touched the surface of the ink. Two minutes after immersing, 1,2-dichloroethane evaporates and the film, resp. the electrode surface, is ready to use. When it is necessary to renew the old film (for example because of the passivation or mechanical damages), it can be easily removed by wiping it off with a filter paper [7].

## 4.2 Polarographic and Voltammetric Determination of 5-Nitrobenzimidazole

Determination of 5-NBIA at DME and HMDE have been investigated recently [8], but in this Thesis we have used newly developed electrodes and methods of determination of this compound. Therefore, these results can be compared with one another.

The optimum conditions for polarographic and voltammetric determination of 5-NBIA at DME and HMDE have been investigated in BR buffered aqueous solutions. Optimum conditions for the determination of 5-NBIA were found in BR buffer pH 2.0, 7.0 and 12.0 and for TAST and DPP at DME, BR buffer pH 4.0 or 5.0 were used [8]. The optimum conditions for voltammetric determination of 5-NBIA at p-AgSAE, m-AgSAE, MF-AgSAE were pH 6.0 and 8.0 for solution modified by surfactants for increase of sensitivity of DCV and DPV. For measurement on BiF-AgSAE and CFE was optimal pH 7.0. An attempt to increase the sensitivity using adsorptive stripping voltammetry (AdSV) at every kind of using electrode was not successful. Under optimal conditions, the calibration dependences of 5-NBIA were measured.

For measurement of calibration dependences on p-AgSAE, m-AgSAE, MF-AgSAE electrochemical activation before starting a daily measurement was used in 0.2 mol L<sup>-1</sup> KCl at -2.2 V under stirring for 300 s. Before each measurement regeneration of the electrode was carried out. It was based on application of 300 polarization cycles switching the electrode potential from  $E_{1,\text{reg}}$  to  $E_{2,\text{reg}}$  for 50 ms.  $E_{1,\text{reg}}$  was about 50 to 100 mV more negative than the potential of the anodic dissolution of the electrode,  $E_{2,\text{reg}}$  was about 50 to 100 mV more positive than the potential of the hydrogen evolution in the given supporting electrolyte. Under these conditions, eventual oxides of mercury or silver are reduced and adsorbed

molecules are desorbed [9]. For all electrodes were  $E_{1,\text{reg}} = -100$  mV and  $E_{2,\text{reg}} = -600$  mV. Under these conditions the calibration dependences of 5-NBIA were measured within concentration ranges of  $2 \times 10^{-7} - 1 \times 10^{-5}$  mol L<sup>-1</sup> for DCV and DPV at p-AgSAE, m-AgSAE, MF-AgSAE. Limits of quantification ( $L_Q$ ) were at p-AgSAE for DCV  $L_Q = 8 \times 10^{-7}$  mol L<sup>-1</sup>, for DPV  $L_Q = 5 \times 10^{-7}$  mol L<sup>-1</sup>; at m-AgSAE for DCV  $L_Q = 3 \times 10^{-7}$  mol L<sup>-1</sup>, for DPV  $L_Q = 2 \times 10^{-7}$  mol L<sup>-1</sup>; at MF-AgSAE for DCV  $L_Q = 1 \times 10^{-6}$  mol L<sup>-1</sup>, for DPV  $L_Q = 7 \times 10^{-7}$  mol L<sup>-1</sup> [10].

The optimum conditions found above for DCV and DPV at p-AgSAE, m-AgSAE, MF-AgSAE determination of 5-NBIA were used for direct determination of the substance in model samples of drinking water from the public water line in Prague. The BR buffer was replaced by 0.01 mol L<sup>-1</sup> NaOH (9:1) for simplification. It was found that the calibration curves are linear (in the concentration range  $2 \times 10^{-7} - 1 \times 10^{-6}$  mol L<sup>-1</sup>). Limits of quantification were at p-AgSAE for DCV  $L_Q = 1 \times 10^{-6}$  mol L<sup>-1</sup>, for DPV  $L_Q = 4 \times 10^{-7}$  mol L<sup>-1</sup>; at m-AgSAE for DCV and DPV  $L_Q = 3 \times 10^{-7}$  mol L<sup>-1</sup>; at MF-AgSAE for DCV  $L_Q = 9 \times 10^{-7}$  mol L<sup>-1</sup>, for DPV  $L_Q = 8 \times 10^{-7}$  mol L<sup>-1</sup>) [10].

These results confirm the possible application of the proposed methods for drinking water monitoring.

For the decrease of limit of quantification at HMDE, p-AgSAE and m-AgSAE was tested addition of surfactants into the solution. The selected surfactant was cationactive cetyltrimethylammonium bromide (CTMAB), non-ionic Triton X-100 and anionactive sodium dodecyl sulfate (SDS). Before measurement electrochemical activation was carried out in 0.2 mol L<sup>-1</sup> KCl at  $-2.2$  V under stirring for 300 s and electrochemical regeneration  $E_{1,\text{reg}} = 0$  mV,  $E_{2,\text{reg}} = -1200$  mV for p-AgSAE, and  $E_{1,\text{reg}} = -100$  mV,  $E_{2,\text{reg}} = -900$  mV for m-AgSAE. The calibration dependences at 5-NBIA were measured within concentration ranges of  $2 \times 10^{-7} - 1 \times 10^{-5}$  mol L<sup>-1</sup> for DPV. The addition of SDS and Tritone X-100 does not significantly influence the DPV determination of 5-NBIA on HMDE, p-AgSAE and m-AgSAE. Just the addition of CTMAB significantly increases the height of DPV peaks only at the p-AgSAE. This signal increasment did not have great influence to the method sensitivity (DPV at p-AgSAE, which were without CTMAB  $L_Q = 4 \times 10^{-7}$  mol L<sup>-1</sup>, and with CTMAB  $L_Q = 3 \times 10^{-7}$  mol L<sup>-1</sup>) [11].

For measurement of calibration dependences on BiF-AgSAE no activation or regeneration of electrode was used since such procedures damage the bismuth film formed. The calibration dependences of 5-NBIA were measured within concentration ranges for DCV

$2 \times 10^{-7} - 1 \times 10^{-5} \text{ mol L}^{-1}$  and for DPV of  $2 \times 10^{-8} - 1 \times 10^{-5} \text{ mol L}^{-1}$  with limits of quantification for DCV at  $L_Q = 4 \times 10^{-7} \text{ mol L}^{-1}$ , and for DPV at  $L_Q = 7 \times 10^{-8} \text{ mol L}^{-1}$ .

The optimum conditions found above for DCV and DPV at BiF-AgSAE determination of 5-NBIA were used for direct determination of the substance in model samples of drinking public water line in Athens and mineral water Spring Olympos. Both types of water were treated with EDTA (1g per 50 mL of water) for masking of cations present in solution or without EDTA to compare if this addition necessary. It was shown that measurement with EDTA is necessary in mixture of 9 mL spiked water and 1 mL of proper buffer (without EDTA is impossible to obtain calibration dependences). If we measured mixture of 5 mL spiked water and 5 mL of proper buffer, it was not necessary to add EDTA. It was found that the calibration curves are linear (in the concentration range  $2 \times 10^{-7} - 1 \times 10^{-5} \text{ mol L}^{-1}$ ). Limits of quantification was at BiF-AgSAE in model sample of drinking water for DCV  $L_Q = 7 \times 10^{-7} \text{ mol L}^{-1}$  (5:5) and  $L_Q = 3 \times 10^{-7} \text{ mol L}^{-1}$  (9:1), for DPV  $L_Q = 1 \times 10^{-6} \text{ mol L}^{-1}$  (5:5) and  $L_Q = 7 \times 10^{-7} \text{ mol L}^{-1}$  (9:1); for model sample of mineral water for DCV  $L_Q = 8 \times 10^{-7} \text{ mol L}^{-1}$  (5:5) and  $L_Q = 5 \times 10^{-7} \text{ mol L}^{-1}$  (9:1), for DPV  $L_Q = 4 \times 10^{-6} \text{ mol L}^{-1}$  (5:5) and  $L_Q = 3 \times 10^{-7} \text{ mol L}^{-1}$  (9:1) [12].

These results confirm the possible application of the proposed methods for both drinking and mineral water.

For measurement of calibration dependences on CFE it was necessary to use regeneration potentials  $E_{1,\text{reg}} = -400 \text{ mV}$  and  $E_{2,\text{reg}} = -900 \text{ mV}$ . The calibration dependences of 5-NBIA were measured within concentration ranges for DCV and DPV  $2 \times 10^{-7} - 1 \times 10^{-5} \text{ mol L}^{-1}$  (for DCV was  $L_Q = 6 \times 10^{-7} \text{ mol L}^{-1}$ , for DPV was  $L_Q = 3 \times 10^{-7} \text{ mol L}^{-1}$ ) [13].

These conditions were used for measurement of calibration curves using a mixture of 9.0 mL of a spiked model water sample (drinking water from the public water line in Prague or the river water from the Vltava river in Prague) and 1.0 mL of a BR buffer of appropriate pH in concentration range  $2 \times 10^{-7} - 1 \times 10^{-5} \text{ mol L}^{-1}$ . Reached  $L_Q$  determination in model samples of drinking water were for DCV  $L_Q = 3 \times 10^{-7} \text{ mol L}^{-1}$ , for DPV  $L_Q = 1 \times 10^{-7} \text{ mol L}^{-1}$  and in model samples of river water were for DCV  $L_Q = 2 \times 10^{-7} \text{ mol L}^{-1}$ , DPV  $L_Q = 1 \times 10^{-7} \text{ mol L}^{-1}$  [13]. Again, these results confirm the possible application of the proposed methods for both drinking and river water.

### 4.3 Polarographic and Voltammetric Determination of 2-Amino-6-nitro-benzothiazole

Determination of ANBT at DME and HMDE have been described long time ago [14]. In this Thesis a newly developed methods and new electrodes materials for determination of this compound were used and the results can be compared with results obtained at DME and HMDE.

The optimum conditions for polarographic and voltammetric determination of ANBT measured in this Thesis at p-AgSAE and m-AgSAE were BR buffer pH 4.0 – methanol (9:1), pH 10.0 – methanol (9:1) for DCV and pH 3.0 – methanol (9:1), pH 10.0 – methanol (9:1) for DPV. For BiF-AgSAE BR buffer pH 10.0 – methanol (9:1) was selected for both DCV and DPV. For CFE BR buffer pH 4.0 – methanol (9:1) was selected for both DCV and DPV [15].

An attempt to increase the sensitivity using adsorptive stripping voltammetry (AdSV) at any of the used electrodes was not successful. Under these conditions, the calibration dependences of ANBT were measured.

For measurement of calibration dependences on p-AgSAE and m-AgSAE an electrochemical activation before starting daily measurement was carried out in  $0.2 \text{ mol L}^{-1}$  KCl at  $-2.2 \text{ V}$  under stirring for 300 s. Before each measurement regeneration electrode was carried out based on application of 300 polarization cycles switching the electrode potential from  $E_{1,\text{reg}}$  to  $E_{2,\text{reg}}$  for 50 ms. For p-AgSAE were  $E_{1,\text{reg}} = -200 \text{ mV}$  and  $E_{2,\text{reg}} = -600 \text{ mV}$ . For m-AgSAE were  $E_{1,\text{reg}} = -400 \text{ mV}$  and  $E_{2,\text{reg}} = -900 \text{ mV}$ . Under this condition the calibration dependences of ANBT were measured on selected electrodes (see the Table 1) within concentration ranges of  $2 \times 10^{-6} - 1 \times 10^{-5} \text{ mol L}^{-1}$  for DCV and DPV at p-AgSAE and  $2 \times 10^{-7} - 1 \times 10^{-5} \text{ mol L}^{-1}$  for DCV and DPV at m-AgSAE.

Table 1

Summary of used methods and optimum medium for measured of ANBT at p-AgSAE and m-AgSAE and  $L_Q$  for this measurements.

Electrode	Optimum medium	$L_Q$ for DCV $10^{-7}$ mol L <sup>-1</sup>	for DPV $10^{-7}$ mol L <sup>-1</sup>
p-AgSAE	BR buffer pH 4.0 – methanol (9:1)	30	–
	BR buffer pH 3.0 – methanol (9:1)	–	30
	BR buffer pH 10.0 – methanol (9:1)	1	2
m-AgSAE	BR buffer pH 4.0 – methanol (9:1)	7	–
	BR buffer pH 3.0 – methanol (9:1)	–	4
	BR buffer pH 10.0 – methanol (9:1)	2	1

The optimum conditions found above for determination of ANBT using DCV and DPV at p-AgSAE and m-AgSAE were used for direct determination of the substance in model samples of drinking water from the public water line in Prague and river water in Prague. For both electrodes 9.0 mL of spiked sample of waters were diluted to 10 mL by BR buffer pH 10.0. It was found that the calibration curves are linear (in the concentration range  $2 \times 10^{-7} - 1 \times 10^{-6}$  mol l<sup>-1</sup>). Limits of quantification are summarized in Table 2.

Table 2

Summary of used methods for determination of ANBT and their  $L_Q$  at p-AgSAE and m-AgSAE in spiked samples of waters.

Electrode	Matrix	$L_Q$ for DCV $10^{-7}$ mol L <sup>-1</sup>	for DPV $10^{-7}$ mol L <sup>-1</sup>
p-AgSAE	Drinking water	1	1
	River water	4	1
m-AgSAE	Drinking water	4	1
	River water	2	1

These results confirm the possible application of the proposed methods for drinking and river water.

For measured calibration dependences on BiF-AgSAE no activation or regeneration of electrode was used since such procedures damage the bismuth film formed. The calibration dependences of ANBT were measured within concentration ranges for DCV and DPV in  $2 \times 10^{-7} - 1 \times 10^{-5} \text{ mol L}^{-1}$  (for DCV and DPV were  $L_Q = 2 \times 10^{-7} \text{ mol L}^{-1}$ ) [6].

The optimum conditions found above for DCV and DPV at BiF-AgSAE determination of ANBT were used for direct determination of the substance in model samples of drinking public water line in Athens and mineral water Spring Olympos. Both types of water were purified with EDTA (1g per 50 mL of water) for masking of cations. For both electrodes 9.0 mL of spiked sample of waters were diluted to 10 mL by BR buffer pH 10.0 in the concentration range  $2 \times 10^{-7} - 1 \times 10^{-6} \text{ mol L}^{-1}$ . Limit of quantification was at BiF-AgSAE in model sample of drinking water without EDTA DCV  $L_Q = 5 \times 10^{-7} \text{ mol L}^{-1}$ , DPV  $L_Q = 4 \times 10^{-7} \text{ mol L}^{-1}$ ; for drinking water with EDTA DCV and DPV  $L_Q = 3 \times 10^{-6} \text{ mol L}^{-1}$ ; for mineral water with EDTA DCV and DPV  $L_Q = 4 \times 10^{-6} \text{ mol L}^{-1}$  [6].

These results confirm the possible application of the proposed methods for both drinking and mineral water.

For measurement of calibration dependences based on reduction at CFE it was necessary to use regeneration potentials  $E_{1,\text{reg}} = -400 \text{ mV}$  and  $E_{2,\text{reg}} = -900 \text{ mV}$ . The calibration dependences of reduction of ANBT were measured within concentration ranges for DCV and DPV  $2 \times 10^{-7} - 1 \times 10^{-5} \text{ mol L}^{-1}$  (for DCV  $L_Q = 9 \times 10^{-7} \text{ mol L}^{-1}$ , for DPV  $L_Q = 8 \times 10^{-7} \text{ mol L}^{-1}$ ). The calibration dependences based on oxidation of ANBT were measured within concentration ranges for DCV and DPV  $2 \times 10^{-6} - 1 \times 10^{-6} \text{ mol L}^{-1}$  (for DCV  $L_Q = 5 \times 10^{-6} \text{ mol L}^{-1}$ , for DPV  $L_Q = 4 \times 10^{-6} \text{ mol L}^{-1}$ ) [13].

These conditions were used for measurement of calibration curves using a mixture of 9.0 mL of a spiked model water sample (drinking water from the public water line in Prague or the river water from the Vltava river in Prague) and 1.0 mL of a BR buffer of pH 4.0 in the concentration range  $2 \times 10^{-7} - 1 \times 10^{-6} \text{ mol L}^{-1}$  for reduction and  $2 \times 10^{-6} - 1 \times 10^{-6} \text{ mol L}^{-1}$  for oxidation. In model samples of drinking water for reduction were for DCV  $L_Q = 5 \times 10^{-7} \text{ mol L}^{-1}$ , for DPV  $L_Q = 2 \times 10^{-7} \text{ mol L}^{-1}$ ; in model samples of river water for reduction were for DCV  $L_Q = 2 \times 10^{-7} \text{ mol L}^{-1}$ , for DPV  $L_Q = 5 \times 10^{-7} \text{ mol L}^{-1}$ ; in model samples of drinking and river water for oxidation were for DCV  $L_Q = 3 \times 10^{-7} \text{ mol L}^{-1}$ , DPV  $L_Q = 2 \times 10^{-7} \text{ mol L}^{-1}$  [13].

These results confirm the possible application of the proposed methods for both drinking and river water.

An attempt to increase the sensitivity using adsorptive stripping voltammetry (AdSV) at any of the used electrodes was not successful.

#### 4.4 References

- [1] B. Yosypchuk, J. Barek, *Crit. Rev. Anal. Chem.* **2009**, 39, 189.
- [2] J. Barek, J. Fischer, T. Navratil, K. Peckova, B. Yosypchuk, *Sensors* **2006**, 6, 445.
- [3] B. Yosypchuk, M. Fojta, J. Barek, *Electroanalysis* **2011**, 22, 1967.
- [4] A. Economou, *Trac-Trends in Analytical Chemistry* **2005**, 24, 334.
- [5] I. Svancara, C. Prior, S. B. Hocevar, J. Wang, *Electroanalysis* **2010**, 22, 1405.
- [6] **D. Deylova**, V. Vyskocil, J. Barek, A. Economou, *Talanta* **2012**, 102, 68.
- [7] I. Jiranek, T. Rumlova, J. Barek, in *Modern Electrochemical Methods Xxx* **2011**.
- [8] **D. Deylova**, J. Barek, V. Vyskocil, *Collect. Czech. Chem. Commun.* **2009**, 74, 1443.
- [9] J. Barek, J. Fischer, T. Navratil, K. Peckova, B. Yosypchuk, J. Zima, *Electroanalysis* **2007**, 19, 2003.
- [10] **D. Deylova**, B. Yosypchuk, V. Vyskocil, J. Barek, *Electroanalysis* **2011**, 23, 1548.
- [11] **D. Deylova**, J. Barek, V. Vyskocil, *Collect. Czech. Chem. Commun.* **2011**, 76, 1317.
- [12] **D. Deylova**, V. Vyskocil, A. Economou, V. Mansfeldova, J. Barek, *Int. J. Electrochem. Sci.* **2014**, in press.
- [13] **D. Deylová**, V. Vyskocil, J. Barek, *J. Electroanal. Chem.* **2014**,  
<http://dx.doi.org/10.1016/j.jelechem.2014.01.017>.
- [14] V. I. Gorokhovskaya, *Zhurnal Obshchei Khimii* **1962**, 32, 3853.
- [15] **D. Deylova**, V. Vyskocil, J. Barek, *Electrochimica Acta* **2012**, 62, 335.

## 5. CONCLUSION

The presented Thesis describes development of new electroanalytical methods for the determination of derivatives of nitrobenzimidazole using new electrode materials which are based on amalgam and its modified surface. Tested analytes belong to the group of heterocyclic compounds, which represent a considerable health risk to humans by their genotoxic and/or carcinogenic properties. Nitro-derivatives of benzimidazole are used as raw materials and intermediates in the manufacture of pharmaceuticals, dyes, photochemical science, industry and they are contaminants of industrial wastewaters.

This Thesis represents a contribution to the search for new analytical methods applicable in environmental analysis. The current state-of-art concerning the sources formation, properties, occurrence, and biological impact of nitrobenzimidazole derivatives is summarized in Chapter 2.

Experimental work was focused on assessment of new approaches in the detection of the studied compounds. Attention was paid to the 2-Amino-6-nitrobenzothiazole and 5-nitrobenzimidazole and the possibilities of their determination using polarography and voltammetry. This substance was used as testing compounds for newly developed modified amalgam electrodes. First of all several types of films were deposited on the silver amalgam electrode substrate as described in Chapter 3.

The obtained results can be summarized as follows:

- p-AgSAE, m-AgSAE, MF-AgSAE, BiF-AgSAE and CFE electrode using silver solid amalgam electrode as substrate can be successfully used for the determination of trace amounts of tested compounds as suitable non-toxic and environmentally friendly alternatives to mercury electrodes. They provide stable and reproducible responses during measurement.
- The attempt at increasing the sensitivity using adsorptive stripping DCV or DPV at all tested electrodes with both compounds was not successful.
- The applicability of all tested electrodes for DCV and DPV determination of selected compounds in model samples of drinking and river waters has also been verified. On the bismuth film electrodes it was necessary to use for both waters further pretreatment/purification by solid EDTA which was added for masking cations present in solution.



- 
- It has been proved that the addition of SDS and Triton X-100 does not significantly influence the DPV determination of 6-NBIA at HMDE, p-AgSAE and m-AgSAE. However, under the optimal conditions, the limit of quantification for DPV of 6-NBIA at p-AgSAE decreased about twice by addition of CTMAB in comparison with measurement in the absence of the surfactant while keeping good accuracy.
  - Bismuth and mercury film electrode on a silver solid amalgam support can be used as a working electrode for cathodic voltammetry. Both DC voltammetry (DCV) and differential pulse voltammetry (DPV) can be used for the determination of 6-NBIA and in the case of bismuth film also for the determination of ANBT. The optimum conditions of determination of these compounds were found.
  - Surface topography of bismuth film was characterized by AFM. The image clearly shows that bismuth film was not grown uniformly over the supporting surface, but created three-dimensional formations which cover only a fraction of the surface.
  - It has been shown that CFE is a good sensor for reduction reactions of selected compound. However, it is not too suitable for oxidation reaction of 2-amino-6-nitrobenzothiazole.