CHARLES UNIVERSITY IN PRAGUE
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Summary of the Ph.D. Thesis

CONTRIBUTION TO THE USE OF NEW TYPES OF CARBON PASTE AND FIBER ELECTRODES FOR VOLTAMMETRIC AND AMPEROMETRIC DETERMINATION OF 5-AMINO-6-NITROQUINOLINE AND RESVERATROL

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ABSTRACT

This dissertation thesis is focused on the study of electrochemical properties of new types of carbon paste electrodes (CPE) and carbon fiber rod electrodes (CFRE), which were used for the development of new highly sensitive and selective voltammetric and amperometric methods for the determination of 5-amino-6-nitroquinoline and *trans*- and *cis*- isomers of resveratrol.

The carbon paste electrodes were compared in terms of size of the microparticles of glassy carbon contained in the paste (diameters 0.4 – 12 μm; 10 – 20 μm; 20 – 50 μm) in comparison with carbon paste electrode consisting of crystalline graphite and solid glassy carbon electrode. The electrochemical properties were tested using potassium hexacyanoferrate in an aqueous supporting electrolyte and 5-amino-6-nitroquinoline (5A6NQ) in a mixed methanol-water supporting electrolyte.

The carbon paste electrode was further used in an amperometric detector of a wall-jet type connected in series with a spectrophotometric detector for the development of a new HPLC method with electrochemical detection and spectrophotometric detection for the determination of *trans*-resveratrol and *cis*-resveratrol in samples of grains, hulls and leaves of common and tartary buckwheat. The method was optimized and used for the determination of resveratrol in samples of six varieties of common buckwheat and two varieties of tartary buckwheat.

The electrochemical properties of carbon fiber rod electrodes were tested for the electrodes of various diameters (0.8 mm; 2 mm, and 3 mm) by comparing the parameters in the determination of selected analytes (potassium hexacyanoferrate and 5-amino-6-nitroquinoline) using voltammetric methods (DC voltammetry (DCV), differential pulse voltammetry (DPV), cyclic voltammetry (CV), adsorptive stripping differential pulse voltammetry (AdSDPV)). Practical use of optimized methods was demonstrated on the determination of 5A6NQ at CFRE in model samples of drinking and pond water.

In the last part of the thesis, a new method of determination of *trans*-resveratrol using voltammetric methods (DCV, DPV and AdSDPV) and flow injection analysis (FIA) with electrochemical detection at CFRE was developed and this detection was compared with electrochemical detection at CPE and in case of FIA also with spectrophotometric detection. Practical applications of

the new methods were tested by determination of *trans*-resveratrol in Evelor pills.

CONTENTS

1. **INTRODUCTION** 5
2. **OBJECTIVES OF THE THESIS** 8
3. **RESULTS AND DISCUSSION** 10
3.1 A voltammetric comparison of the properties of carbon paste electrodes containing glassy carbon microparticles of various size 10
3.2 Determination of resveratrol in grains, hulls and leaves of common and tartary buckwheat by HPLC with electrochemical detection at carbon paste electrode 12
3.3 Electrochemical properties and determination of 5-amino-6-nitroquinoline at carbon fiber rod electrode 13
3.4 Voltammetric and amperometric determination of *trans*-resveratrol at carbon fiber rod electrode and carbon paste electrode 15
4. **CONCLUSION** 18
5. **REFERENCES** 20
6. *CURRICULUM VITAE* 23
7. LIST OF PUBLICATIONS 24

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1. INTRODUCTION

This dissertation thesis contributes to the development and use of new types of carbon paste and fiber electrodes for voltammetric and amperometric determination of 5-amino-6-nitroquinoline (5A6NQ) and both isomers of resveratrol (*cis*- and *trans*-).

Resveratrol (3,5,4´-trihydroxystilbene, Fig. 1 A, B) was first isolated from the roots of white hellebore in 1940[1]. In 1976, resveratrol was detected in the leaves and skins of grapes[2]. Since the 90s of the 20th century, there was an increasing focus on the occurrence and properties due to the so called „French paradox“[3]. Resveratrol exists in two isomeric forms *trans*-resveratrol and *cis*-resveratrol. *Trans*-resveratrol is more stable form, with *trans*- to *cis*-isomerization facilitated by UV light and high pH, while *cis*- to *trans*-conversion is facilitated by visible light, high temperature or low pH[4]. Both isomers can be present in variable amounts in plants, but *trans*-resveratrol usually predominates. Commercially available is only *trans*-resveratrol, *cis*-resveratrol could be prepared from *trans*-form by irradiation by daylight, but the result will always be a mixture of both isomers[5]. Resveratrol belongs to the phytoalexin, secondary metabolites of plants produced by plants in response to stress. Its occurrence is very broad, it is reported, to have been found in more than 72 plant species (grapes, peanuts, red cabbage, cranberries, blueberries and others)[6]. Resveratrol was also identified in buckwheat amongst several other flavonoids[7]. As the content of resveratrol in buckwheat is not yet known part of this thesis is focused on the determination of resveratrol in grains, hulls and leaves in selected varieties of common and tartary buckwheat. At present, a large number of publications demonstrates anti-inflammatory, antifungal, antioxidant, neuroprotective, cardioprotective, anticarcinogenic, hepatoprotective and fytoestrogenic effects[8-10]. The quantitative determination of *trans*-resveratrol is mainly done by HPLC with UV/VIS[11], MS[12], fluorescence[13] and electrochemical detection[14], by GC/MS[15] or electrophoresis[16].

The second analyte studied in this thesis is 5-amino-6-nitroquinoline (5A6NQ, Fig. 1 C). It belongs to the group of nitro- and aminoderivatives of heterocyclic hydrocarbons. Toxicological properties of 5A6NQ were studied at *Tetrahymena pyriformis* (IGC_{50}-concentration causing 50% decrease in growth), which are comparable to the 3,5-dinitroaniline[17]. The substance has been used for the synthesis of new types of local anesthetics[18] and some radioligands[19] used in positron emission tomography. Generally, the

heterocyclic amines (HCA) are group of harmful substances[20]. They are formed during thermal processing of meat (above 150 °C)[21], the combustion of fossil fuels[22] and can be found also in a cigarette smoke[23]. 5-Amino-6-nitroquinoline was used to test the electrochemical properties of new types of electrodes. The electrochemical properties of 5A6NQ have been studied in previous works[24-26].

It follows from measured cyclic voltammograms in Britton-Robinson (BR) buffer with pH 2; 7 and 11 at CPE (containing microparticles of glassy carbon with diameter 0.4 – 12 μm) that reduction and oxidation of 5A6NQ is irreversible under the given conditions controlled not only by diffusion, and that during the measurement the surface of the working electrode is passivated. The optimum conditions and the limits of detection found for anodic and cathodic DCV, DPV and AdSDPV are shown in Table 1.

Tab. 1 [25,26]
The optimum conditions and the limits of detection for voltammetric methods of determination of 5A6NQ

Voltammetric method for the determination of 5A6NQ	L_D **mol L^{-1}**
Anodic DPV in BR buffer pH 11 with MeOH (1:1, *V*/*V*)	2.0×10^{-6}
Anodic DCV in BR buffer pH 11 with MeOH (1:1, *V*/*V*)	3.1×10^{-6}
Cathodic DPV in 0.1 mol L^{-1} H_3PO_4 pH 1.6 with MeOH (1:1, *V*/*V*)	1.3×10^{-6}
Cathodic DPV in BR buffer pH 11 with MeOH (1:1, *V*/*V*)	1.9×10^{-6}
Cathodic DCV in 0.1 mol L^{-1} H_3PO_4 pH 1.6 with MeOH (1:1, *V*/*V*)	1.5×10^{-6}
Anodic AdSDPV in BR buffer pH 11 with MeOH (95:5, *V*/*V*), E_{accu} = 500 mV, t_{accu} = 5 min	1.4×10^{-6}

Utilizing the knowledge summarized in Tab. 1 the study of 5A6NQ determination in flow systems was performed. RP-HPLC determination of 5A6NQ with electrochemical detection at CPE (containing microparticles of glassy carbon with 0.4 – 12 μm diameter) was developed and compared with spectrophotometric detection (295 nm). In case of electrochemical detection both oxidation and reduction of 5A6NQ at CPE was utilized. The optimum conditions for oxidation of 5A6NQ were found: BR buffer pH 7 with methanol 10:90 (*V*/*V*) at constant potential for amperometric detection at +1.2 V. The limit of detection for spectrophotometric detection ($L_D = 1.1 \times 10^{-7}$ mol L^{-1}) and amperometric detection ($L_D = 1.6 \times 10^{-7}$ mol L^{-1}) are comparable[24]. The optimum conditions for the determination based on the reduction of 5A6NQ were as follows: phosphate buffer pH 2 with methanol 10:90 (*V*/*V*) at constant

potential for amperometric detection at -0.9 V. It follows from the obtained limits of detection for spectrophotometric (L_D = 1.3 × 10^{-7} mol L^{-1}) and amperometric detection (L_D = 4.9 × 10^{-7} mol L^{-1}) that electrochemical detection based on the reduction of 5A6NQ provided worse results than the method based on the 5A6NQ oxidation[25,26].

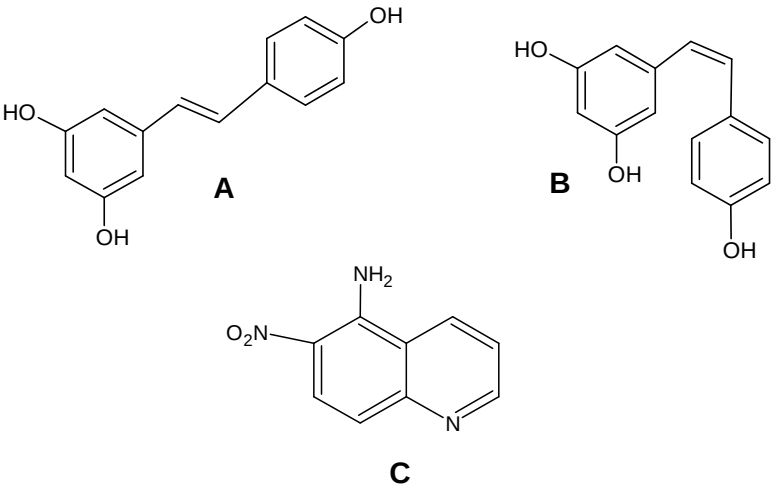

Fig. 1 Structural formulas of studied compounds: *trans*-resveratrol (A), *cis*-resveratrol (B) a 5-amino-6-nitroquinoline (C).

2. OBJECTIVES OF THE THESIS

This dissertation has been submitted as a contribution to the ever growing efforts in environmental analysis. It was elaborated in the framework of a long term research at the UNESCO Laboratory of Environmental Electrochemistry in Prague to study and compare electrochemical properties of new types of carbon paste and fiber electrodes, which were simultaneously used for the development of highly sensitive and selective voltammetric methods for the determination of 5-amino-6-nitroquinoline and both isomers of resveratrol (*cis*- and *trans*-).

The thesis is based on following four scientific publications[27-30], for differentiation quoted in bold format, underlined and in superscript.

1. **L. Němcová**, J. Barek, J. Zima, A Voltammetric Comparison of the Properties of Carbon Paste Electrodes Containing Glassy Carbon Microparticles of Various Sizes, *J. Electroanal. Chem.*, 675 (2012): 18-24.
2. **L. Němcová**, J. Zima, J. Barek, D. Janovská, Determination of resveratrol in grains, hulls and leaves of common and tartary buckwheat by HPLC with electrochemical detection at carbon paste electrode, *Food Chem.*, 126 (2011): 374-378.
3. **L. Němcová**, H. Dejmková, J. Barek, J. Zima, Voltammetric determination of 5-amino-6-nitroquinoline at a carbon fiber rod electrode, *Int. J. Electrochem. Sci*., 6 (2011): 6373-6384.
4. **L. Němcová**, J. Barek, J. Zima, Determination of *trans*-resveratrol using voltammetric and amperometric methods at carbon fiber rod electrode and carbon paste electrode, *Int. J. Electrochem. Sci.*, 7 (2012): 9221-9231.

The main aim of the thesis was to develop electrochemical methods for the determination of *cis*- and *trans*-resveratrol at carbon paste electrode (CPE) and carbon fiber rod electrode (CFRE) and to compare the electrochemical properties of both types of electrodes.

Because both isomers of resveratrol are sensitive to light, potassium ferrocyanide and 5-amino-6-nitroquinoline (5A6NQ) were used for studying the electrochemical properties of studied electrodes. The aim of the thesis was to compare the properties of carbon paste electrodes based on various microparticles of glassy carbon contained in the paste (diameters: 0.4 – 12 μm; 10 – 20 μm; 20 – 50 μm). The aim was also to compare the carbon fiber rod electrode of different diameters (diameters: 0.8 mm; 2 mm, and 3 mm) with the

determination of the above mentioned analytes using voltammetric methods (DC voltammetry (DCV), differential pulse voltammetry (DPV), cyclic voltammetry (CV), adsorptive stripping differential pulse voltammetry (AdSDPV)) and flow injection analysis (FIA), with comparison of detection using CPE and spectrophotometry in the UV/VIS. Newly developed voltammetric and amperometric methods for determination of *cis*- and *trans*-resveratrol were applied to the real samples of tartary and common buckwheat (determination of resveratrol in samples of grains, hulls and leaves) and pharmaceutical preparation Evelor.

3. RESULTS AND DISCUSSION

3.1 A voltammetric comparison of the properties of carbon paste electrodes containing glassy carbon microparticles of various size[27]

The carbon paste electrodes containing glassy carbon microparticles are commonly known and used mainly in supporting electrolytes with higher content of organic modifier (methanol, acetonitrile) [1,2]. As the electrodes were not yet systematically compared the electrochemical properties of electrodes prepared from variously sized glassy carbon microparticles were the focus of this part. Carbon paste containing 250 mg of glassy carbon microparticles with diameters 0.4 – 12 μm; 10 – 20 μm; 20 – 50 μm or crystalline graphite with diameter of particle 2 μm and in all cases with the mineral oil used as pasting liquid were tested. The electrochemical properties were tested using potassium ferrocyanide in aqueous medium and 5-amino-6-nitroquinoline in a methanol-water medium.

Electrochemical characterization of CPEs

The surfaces of prepared pastes were photographed under a microscope. It follows from obtained images, that the bigger are the particles the rougher is the resulting paste surface. During the preparation of pastes it was also found that the preparation of paste from the smallest microparticles is much easier and faster than from bigger microparticles. The first step was to measure the potential windows of all used electrodes in supporting electrolytes NaOH, KCl, $HClO_4$, H_2SO_4 (all 0.1 mol L^{-1}) and in Britton-Robinson (BR) buffer pH 2; 7 and 12. The potential windows of all three pastes containing glassy carbon microparticles were narrower in alkaline medium than in case of glassy carbon electrode. Potential window of the electrode containing crystalline graphite was the narrowest of all the electrodes. The electrochemical properties of the electrodes were tested using cyclic voltammetry (CV), DC voltammetry (DCV), differential pulse voltammetry (DPV) in a solution of potassium ferrocyanide of various concentrations in a 1 mol L^{-1} KCl. It was found by cyclic voltammetry that the oxidation process of $K_4[Fe(CN)_6]$ at all electrodes is controlled by diffusion. The signal stability was tested by 20 consecutive DP voltammograms in a solution of 1×10^{-3} mol L^{-1} $K_4[Fe(CN)_6]$ in 1 mol L^{-1} KCl. The paste with crystalline carbon had the worst stability of signal (RSD 53 %). All three electrodes made of glassy carbon microparticles had stable signal with RSD about 4.5 %. The calibration curves were measured for potassium ferrocyanide in the concentration range from 6×10^{-6} to 1×10^{-3} mol L^{-1} in 1

mol L^{-1} KCl supporting electrolyte. The limits of detection for tested electrodes (for DPV L_D = 9.3 to10.5 μmol L^{-1}, for DCV L_D = 5.7 to 9.9 μmol L^{-1}) are comparable with limits of detection obtained at the glassy carbon electrode (DPV L_D = 6.1 μmol L^{-1}, DCV L_D = 9.2 μmol L^{-1}). We can observe weak downward trend in the values of limit of detection with decreasing the size of microparticles of glassy carbon in used carbon paste electrodes. All measured calibration curves were linear.

The determination of 5-amino-6-nitroquinoline at CPEs

The analyte 5A6NQ was used for testing electrodes in a supporting electrolyte containing 50% of methanol and 50% of water. At first, the influence of pH of BR buffer (2–12) on voltammetric behavior of the analyte was investigated. It follows from voltammograms of 5A6NQ (1×10^{-4} mol L^{-1}), that independently of pH the peak heights decrease with increasing size of glassy carbon microparticles in carbon paste. The worst results were obtained at the electrode from the crystalline carbon, thereby confirming its unsuitability in a supporting electrolyte with a higher content of organic components. The optimum conditions for DCV and DPV were BR buffer pH 10 with methanol 1:1 (*V*/*V*). The cyclic voltammograms for 5A6NQ (1×10^{-4} mol L^{-1}) in the potential range from 0 to +1.2 V at scan rates from 2 to 1000 mV s^{-1} were recorded under the optimum conditions. It follows from the results that the oxidation process of 5A6NQ is irreversible at all tested electrodes; it is controlled by diffusion for electrodes of glassy carbon microparticles and both by diffusion and adsorption at crystalline graphite. Due to the effect of passivation it is necessary for all tested electrodes to use the renewal of electrode surface after each measurement. The repeatability was evaluated using 1×10^{-4} mol L^{-1} 5A6NQ and the results show that the lowest relative standard deviation (RSD 4.6 %) has the electrode with the smallest glassy carbon microparticles and it is comparable to that of a glassy carbon electrode (RSD 4.5 %). The calibration curves were measured under the optimum conditions in the concentration range from 1×10^{-6} to 1×10^{-4} mol L^{-1}. We can observe decreasing trend in limits of detection with decreasing the size of glassy carbon microparticles in used CPE. Measured calibration curves for all tested electrodes were linear.

3.2 Determination of resveratrol in grains, hulls and leaves of common and tartary buckwheat by HPLC with electrochemical detection at carbon paste electrode[28]

The advantages of carbon paste electrodes were utilized in amperometric detection in flow methods (HPLC), in wall-jet arrangement in three-electrode system. The amperometric detector was connected in series with the spectrophotometric detector, which served as a control and comparison with electrochemical detector. The capillary outlet was directed against the surface of carbon paste electrode, which was placed in the mobile phase with the auxiliary and reference electrode. This arrangement has the advantages of continuous washing the surface of the working electrode by the mobile phase, which enables to measure without the renewal of the electrode surface, as is usually required in batch voltammetric methods.

The aim of this part of thesis was to develop a new HPLC method with electrochemical detection and spectrophotometric detection for the determination of *trans*-resveratrol and *cis*-resveratrol in samples of grains, hulls and leaves of tartary and common buckwheat. Resveratrol was among other flavonoids so far only identified in flour of common buckwheat[4], therefore, this part of thesis is devoted not only to develop new method for its determination, but also to determine the actual contents of both isomers of resveratrol in samples of six varieties of common buckwheat and two varieties of tartary buckwheat.

Method optimization

At first, the optimum conditions were found for the determination of standards of *trans*-resveratrol and *cis*-resveratrol (standard was prepared from *trans*-resveratrol using isomerization by daylight for 48 hours with 85% conversion[5]). The mobile phase containing acetonitrile and diluted BR buffer 1:1 (*V*/*V*) allowed us to separate two isomers of resveratrol at column (Kromasil C-18 (7 μm), 125 × 4 mm) in 4.5 minutes with a resolution of 1.6. Because the separation of the isomers was independent of pH of BR buffer in pH range from 3 to 7, the conditions were optimized only using hydrodynamic voltammograms. The hydrodynamic voltammograms were measured in BR buffer pH 3; 5 and 7 with acetonitrile (1:1, *V*/*V*) in the potential range from 0 to +1.4 V. The optimum conditions chosen were BR buffer pH 7 with acetonitrile (1:1, *V*/*V*) at the potential of +1.2 V. The optimum wavelength chosen for spectrophotometric detector was 306 nm for *trans*-resveratrol and 286 nm for *cis*-resveratrol. The calibration curves were measured in the concentration

range from 4×10^{-8} to 1×10^{-4} mol L^{-1} under optimum conditions. The limits of detection were for *trans*-resveratrol $L_D = 3.5 \times 10^{-8}$ mol L^{-1} for electrochemical detection, $L_D = 3.2 \times 10^{-8}$ mol L^{-1} for spectrophotometric detection (306 nm) and for *cis*-resveratrol $L_D = 1.8 \times 10^{-8}$ mol L^{-1} for electrochemical detection, $L_D = 6.5 \times 10^{-8}$ mol L^{-1} for spectrophotometric detection (286 nm). It follows from the results that both detections are comparable for *trans*-resveratrol and for *cis*-resveratrol while electrochemical detection is slightly better than spectrophotometric detection.

The real samples of tartary and common buckwheat

The first step was the extraction of resveratrol by ethanol. The sample of buckwheat grains (12 g), hulls (4 g) or leaves (12 g) was always weighted and extracted in 300 mL of ethanol under the reflux for 2 hours, filtered and pre-concentrated under the reduced pressure to 5 mL of concentrated extract. The optimization of separation of *cis*- and *trans*-resveratrol in real samples was done by changing the composition of the mobile phase (50:50; 40:60; 35:65; 30:70 and 20:80 acetonitrile: diluted BR buffer, *V*/*V*) in isocratic mode. The optimum conditions chosen were mobile phase containing acetonitrile: diluted BR buffer pH 7, 30:70 (*V*/*V*), for samples of grains and hulls or 20:80 (*V*/*V*) for samples of leaves, with electrochemical detection at +1.2 V. *Trans*-resveratrol was determined in all real samples, but the second isomer *cis*-resveratrol was not found in any analyzed sample (the presence and the content of *trans*-resveratrol and *cis*-resveratrol was determined by standard addition of the analyte). The content of *trans*-resveratrol in the grains of buckwheat was similar in individual varieties (0.98 – 1.72 mg kg^{-1}), but a large difference was observed in grains of tartary buckwheat (3.43 – 3.50 mg kg^{-1}), which was almost three times higher. On the other hand, content of *trans*-resveratrol in leaves of common buckwheat (1.81 mg kg^{-1}) was almost ten times higher than in the leaves of tartary buckwheat (0.19 mg kg^{-1}). The lowest level of *trans*-resveratrol was found in hulls of common buckwheat (0.16 – 0.66 mg kg^{-1}).

3.3 Electrochemical properties and determination of 5-amino-6-nitroquinoline at carbon fiber rod electrode[29]

Carbon fiber rod electrode (CFRE) is a composite electrode. The electrode is made of carbon fiber rods, which combine identically oriented carbon fiber and epoxy resin by manufacturing process called pultrusion. Although this is not a new electrode, electrochemical studies on its properties exist just few[7-10]. For this reason, this part of the thesis is focused on the study

of the electrochemical properties of CFRE (electrodes with diameters of 0.8 mm; 2 mm, and 3 mm) using potassium ferrocyanide in aqueous medium and following electrochemical determination of 5A6NQ in the anodic and cathodic potentials. The practical application was demonstrated by the determination of 5A6NQ at CFRE in model samples of drinking and pond water.

Electrochemical characterization of CFREs

At first, the surfaces of CFRE were photographed under a microscope. The obtained figures[11] show that epoxy resin is located between the fibers in cross-section of carbon fiber rod, and also along the walls covering carbon fiber rod with a thin layer. From subsequent measurement of $K_4[Fe(CN)_6]$ (1×10^{-3} mol L^{-1}) peak heights measured by DPV in 1 mol L^{-1} KCl on the depth of immersion (1 – 20 mm) of CFRE (diameter 0.8 mm) in the analyzed solution, that only the end of electrode is electrochemically active, walls of electrode are not participating in the electrochemical reaction probably because they are well coated by non-conductive epoxy resin. The potential range was measured in hand-made CFRE in solution of NaOH, KCl, $HClO_4$, H_2SO_4 (all 0.1 mol L^{-1}) in Britton-Robinson (BR) buffer pH 2; 7 and 12. Obtained voltammograms were compared with the results at the GCE (2 mm diameter). The width of potential windows of CFRE is almost comparable to GCE, except cathodic areas in alkaline supporting electrolytes, where the potential windows of CFRE are narrower. The peak potential of anodic and cathodic system of $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ in 1 mol L^{-1} KCl is almost independent on potential scan rate. The electrochemical oxidation process is reversible and controlled by diffusion. This was confirmed by the dependence of the peak current on square root of the scan rate from 2 to 1000 mV s^{-1}.

The determination of 5-amino-6-nitroquinoline at CFREs

The development of method for the determination of 5A6NQ was started by optimizing conditions of measurements. The influence of pH of the BR buffer was measured in the range from 2 to 12 in a mixture with methanol (1:1, *V*/*V*) for DPV and DCV in the anodic and cathodic potentials at CFRE with diameters of 0.8 mm; 2 mm, and 3 mm. All tested electrodes showed similar behavior. Optimal conditions were BR buffer pH 12 with methanol (1:1, *V*/*V*) for DPV and DCV in the anodic and cathodic potentials. The electrode passivation effect was studied by DPV for 5A6NQ (1×10^{-4} mol L^{-1}) under the optimum conditions. It follows from the results (20 consecutive voltammograms measured without renewal) that passivation is significant in the

anodic potentials (height of peak decreased by 28 %). Various methods of surface renewal were tested (fine sandpaper, alumina, electrochemical activation), but the best results were obtained at CFRE regenerated by alumina suspension (20 consecutive measurements – anodic potentials, RSD = 5.5 %, cathodic potentials, RSD = 2.2 %), therefore, this way of regeneration was used in all other measurements. The cyclic voltammograms were measured under the optimum conditions with scan rates from 2 to 1000 mV s^{-1}. The oxidation and reduction of 5A6NQ at CFRE was irreversible, controlled by both diffusion and adsorption. The calibration curves were measured in the concentration range from 4×10^{-7} to 1×10^{-4} mol L^{-1}. The lowest limit of detection was obtained at CFRE with diameter of 2 mm $L_D = 4.3 \times 10^{-7}$ mol L^{-1} for cathodic DPV. The best signal to noise ratio was obtained also with CFRE with 2 mm diameter.

The real application

The practical application of CFRE was demonstrated by the determination of 5A6NQ in model samples of drinking and pond water at CFRE with the diameter of 2 mm. The obtained calibration curves were comparable with the ones obtained in deionized water. The limits of detection are slightly higher in drinking and pond water, which could be due to higher noise in model samples.

3.4 Voltammetric and amperometric determination of *trans*-resveratrol at carbon fiber rod electrode and carbon paste electrode[30]

In this part of the thesis new methods for the determination of *trans*-resveratrol using voltammetric methods (DCV, DPV, and AdSDPV) and flow injection analysis (FIA) with electrochemical detection at CFRE were developed, the last method was compared with the determination at CPE and FIA also with spectrophotometric detection. The practical application of the developed methods was demonstrated by the determination of *trans*-resveratrol in Evelor pastilles.

The voltammetric methods

The effect of pH of BR buffer was tested in the range from 2 to 12 with methanol (1:1, *V*/*V*) for DCV, DPV and CV at CFRE and CPE. The optimum conditions chosen were BR buffer pH 2 with methanol (1:1, *V*/*V*) for all tested electrodes. The cyclic voltammograms were measured in solution of *trans*-resveratrol (1×10^{-4} mol L^{-1}) using scan rates from 2 to 1000 mV s^{-1}, in the potential range from 0 to +1.5 V. The oxidation process of *trans*-resveratrol is

irreversible and controlled by diffusion at both electrodes. The calibration curves were measured in the concentration range from 6×10^{-7} to 1×10^{-4} mol L^{-1} at CFRE and CPE (both 2 mm diameter). The measured calibration curves are linear over the whole measured range, with similar limits of detection in the range from 7.3×10^{-7} to 9.7×10^{-7} mol L^{-1}.

The accumulation of the analyte on the surface of CFRE or CPE was observed due possibility to increase the sensitivity of the method. The accumulation was measured in solution of *trans*-resveratrol (8×10^{-6} mol L^{-1}) at potential range from 0 to 0.6 V with accumulation time from 30 s to 10 min in the supporting electrolyte containing BR buffer pH 2; 7; 10 or 12 always mixed with methanol in the ratio (95:5, *V*/*V*). The significant accumulation was not observed at CPE, but at CFRE the accumulation was significant and as the optimum conditions were chosen: BR buffer pH 10 with methanol (95:5, *V*/*V*) at constant potential of 100 mV and accumulation time of 10 min. The calibration curves were measured by AdSDPV in the concentration range from 1×10^{-7} to 1×10^{-5} mol L^{-1} under the optimum conditions at CFRE. The limit of detection was 2.2×10^{-7} mol L^{-1}.

Flow injection analysis

The optimum conditions for the determination of *trans*-resveratrol by FIA at CFRE or CPE (both with diameter 3 mm) were chosen from the measured hydrodynamic voltammograms measured in the potential range from +0.1 to +1.6 V in supporting electrolyte containing BR buffer pH from 2 to 12 with methanol (1:1, *V*/*V*). The highest and best developed signal was obtained in the supporting electrolyte containing BR buffer pH 10 with methanol (1:1, *V*/*V*) at the potential of +1.0 V at CFRE or CPE. The influence of flow rate was measured in the range from 1 to 7 mL min^{-1}. The optimal flow rate chosen was 3 mL min^{-1}. The stability of signal and repeatability were tested by ten consecutive injections of *trans*-resveratrol (1×10^{-4} mol L^{-1}) under the optimum conditions. The relative standard deviation was 4.5 % for CFRE, 5.4 % for CPE and 2.3 % for the spectrophotometric detection (306 nm).

The calibration curves were measured in the concentration range from 8×10^{-8} to 1×10^{-4} mol L^{-1} at both electrodes. The obtained limits of detection for FIA were for electrochemical detection $L_D = 9.5 \times 10^{-8}$ mol L^{-1} for CFRE, $L_D = 5.2 \times 10^{-7}$ mol L^{-1} for CPE and $L_D = 8.3 \times 10^{-8}$ mol L^{-1} for spectrophotometric detection.

The real samples

The practical application of developed methods was tested by pastilles of Evelor with declared content of *trans*-resveratrol 50 mg in each pastille. Determinations of *trans*-resveratrol were performed by the standard additions method (three additions) under the optimum conditions for developed methods (DPV, FIA at CFRE) and obtained results were compared with results using spectrophotometry, voltammetry at CPE, and FIA with electrochemical detection at CPE, and spectrophotometric detection (306 nm). All obtained results from the analysis of three tablets of Evelor correspond to the declared 50 mg content of *trans*-resveratrol.

4. CONCLUSION

This dissertation thesis is focused on the development of new method for voltammetric and amperometric determination of *cis*- and *trans*-resveratrol at carbon paste electrode and carbon fiber rod electrode and on the comparison of electrochemical properties of both electrodes. Because both isomers of resveratrol are sensitive to light, potassium ferrocyanide and 5-amino-6-nitroquinoline (which allows the test electrodes in cathodic potentials) were used to test the electrochemical properties of the studied electrodes. The voltammetric methods (DCV, DPV, CV, AdSDPV), FIA and HPLC with electrochemical and spectrophotometric detection were used. The thesis is based on four scientific publications[27-30].

All obtained results can be summarized in the following points:

- The electrode with the best electrochemical properties from all tested carbon paste electrodes containing glassy carbon microparticles with diameters of 0.4 – 12 μm; 10 – 20 μm, and 20 – 50 μm is the electrode with the smallest glassy carbon microparticles (the lowest limit of detection, repeatability 4.6 %).
- HPLC method for the determination of resveratrol was optimized. The optimum mobile phase was BR buffer pH 7 with acetonitrile (1:1, *V*/*V*) at +1.2 V using electrochemical detection at CPE. The optimum wavelength for spectrophotometric detection was 306 nm for *trans*-resveratrol and 286 nm for *cis*-resveratrol. The limits of detection were for *trans*-resveratrol $L_D = 3.5 \times 10^{-8}$ mol L^{-1} for electrochemical detection, $L_D = 3.2 \times 10^{-8}$ mol L^{-1} for spectrophotometric detection (306 nm) and for *cis*-resveratrol $L_D = 1.8 \times 10^{-8}$ mol L^{-1} for electrochemical detection, $L_D = 6.5 \times 10^{-8}$ mol L^{-1} for spectrophotometric detection (286 nm).
- The new HPLC method for the determination of resveratrol (*trans*-, *cis*-) in grains, hulls and leaves of tartary and common buckwheat using electrochemical detection at CPE was developed. The optimum conditions were: the mobile phase containing acetonitrile and diluted BR buffer pH 7 (30:70, *V*/*V*) for samples of grains and hulls or the one of 20:80 (*V*/*V*) for samples of leaves with electrochemical detection at +1.2 V (CPE).
- The best results were obtained using CFRE with diameter of 2 mm (DPV $L_D = 4.3 \times 10^{-7}$ mol L^{-1}) for the determination of 5A6NQ from

tested CFRE (0.8 mm; 2 mm, and 3 mm). The oxidation and reduction process of 5A6NQ at CFRE is irreversible, controlled by both diffusion and adsorption. The practical application was successfully demonstrated on the determination of 5A6NQ at CFRE with a diameter of 2 mm in model samples of drinking and pond water.

- The simple and sensitive voltammetric and FIA methods for the determination of *trans*-resveratrol at CFRE were developed. The optimum conditions were BR buffer pH 2 with methanol (1:1, *V*/*V*) for voltammetric methods (except AdSDPV) and BR buffer pH 10 with methanol (1:1, *V*/*V*) at +1.0 V or 306 nm for spectrophotometric detector for FIA.
- Significant accumulation of *trans*-resveratrol was not observed unlike at CFRE, which were the optimum conditions of accumulation: BR buffer pH 10 with methanol (95:5, *V*/*V*) at the accumulation potential of 100 mV for 10 min ($L_D = 2.2 \times 10^{-7}$ mol L^{-1}).
- The developed voltammetric and FIA methods for the determination of *trans*-resveratrol were successfully applied to the pharmaceutical preparation of Evelor (declared content of *trans*-resveratrol 50 mg). The content of *trans*-resveratrol was in ratio from 49.0 to 50.9 mg for all used methods (DPV at CPE and CFRE, spectrometry, FIA with electrochemical detection at CPE and CFRE and spectrophotometric detection).

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2003 – 2006	**Charles University in Prague**, Faculty of Science -BCHPV, Bc.
1999 – 2003	**Masaryk Secondary School of Chemistry**, Prague - Analytical chemistry

Practice

- Advanced course in analytical chemistry
- Project Open Science (Otevřená věda) II (Voltammetric determination of dapsone at the electrode based on carbon, 2010 - 2012)
- Consultant of bachelor thesis (Barbora Fähnrichová, Jarošová Romana)
- Part time job in Energocentrum plus s.r.o. – laboratory analysis of boiler water in buildings of VFN (2009 - now)
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7. LIST OF PUBLICATION

A) Publication in journals

1. **L. Němcová**, J. Barek, J. Zima: Determination of *trans*-resveratrol using voltammetric and FIA methods at carbon fiber rod electrode and carbon paste electrode, *Int. J. Electrochem. Sci.*, **7**, 9221-9231, 2012. (IF = 2.808)

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B) Oral presentations

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2. J. Zima, **L. Němcová**, H. Dejmková: Carbon paste based sensors for monitoring of biologically active organic compounds, 43rd World Chemistry Congress; Chemistry Bridging Innovation Among the Americans and the World, San Juan 2011, Portoriko USA, 415-416, ISBN: 978-0-615-52557-0.

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4. **L. Němcová**, B. Fähnrichová, R. Jarošová, J. Zima, J. Barek: Determination of natural antioxidants at a carbon paste electrode, XXX. Moderní Elektroanalytické metody - Proceedings, p. 124-128, BEST Servis, Jetřichovice 2010, ČR, ISBN: 978-80-254-6710-7.

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