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Electrochemical Biosensors and Detectors Based on Silver Solid Amalgam for Analysis in Flow Systems

Summary of the Ph.D. Thesis

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Contents

1. Abstract

In this Ph. D. thesis new possibilities of using amalgam electrodes are presented. First of all, the tubular detector based on silver solid amalgam (TD-AgSA) was designed for determination of reducible compounds in flow systems. It was tested on model solutions of Cd^{2+}, Zn^{2+} and 4-nitrophenol in amperometric mode under conditions of flow injection analysis. Results have shown that developed tubular detector is simple and low cost device suitable for detection of reducible compounds with good sensitivity, repeatability and long-term stability (at least 2 years) with possibility to work at potentials up to −2 V in aqueous solutions. Afterwards, this newly developed detector was successfully used for the determination of an active ingredient lomustine in pharmaceutical preparation CeeNU® Lomustine by non-stop-flow differential pulse voltammetry based on reduction of present nitroso group.

For measurements in the flow system miniature reference electrodes − saturated calomel electrode, mercury-mercurous sulfate, and mercury-mercuric oxide electrode based on paste silver solid amalgam were fabricated and tested for 14 months. The calomel electrode based on paste silver amalgam proved to be the most resistant to polarization and it was used in all experiments in this thesis.

Next, the electrochemical deposition of 11-mercaptoundecanoic acid monolayer on HMDE and on the electrodes based on solid amalgams − the polished silver solid amalgam electrode (p-AgSAE), the mercury film covered silver solid amalgam electrode (MF-AgSAE), the mercury meniscus covered silver solid amalgam electrode (m-AgSAE), the mercury meniscus covered bismuth-silver solid amalgam electrode (m-BiAgSAE), the mercury meniscus covered copper solid amalgam electrode (m-CuSAE) and the mercury meniscus covered cadmium solid amalgam electrode (m-CdSAE) was studied. Statistical results of repeated preparations of thiol monolayer and its subsequent desorption confirm that amalgam electrodes are a suitable instrument to study the electrochemical properties of thiol films. Moreover, two electrodes (MF-AgSAE and m-AgSAE) were used for preparation of impedimetric biosensors for determination biotin and biotin labeled albumin.

Finally tree types of flow amperometric enzymatic biosensors were designed and fabricated. Two of them are based on the enzymatic reactor and the tubular detector mentioned above. In the first case, the enzymatic reactor is based on *porous* silver solid amalgam. The silver amalgam was modified by thiol 11-mercaptoundecanoic acid. The immobilization of enzyme glucose oxidase at thiol layer was carried out using EDC/NHS chemistry. The biosensor was then successfully used for the determination of glucose in commercial honey.

In the second case, the enzymatic reactor contained *powdered* silver solid amalgam. The amalgam powder was modified by 4-aminothiophenol and enzyme was attached via crosslinking agent glutaraldehyde. Five different biosensors with ascorbate oxidase, glucose oxidase, catalase, tyrosinase, and laccase were prepared for the determination of ascorbic acid, glucose, hydrogen peroxide, catechol, pyrogallol, and dopamine. The biosensor with ascorbate oxidase was used for the determination of ascorbic acid in the vitamin tablets Celascon®.

The last biosensor was constructed using polished silver amalgam electrode which was covered by layer of chitosan. Then, the enzyme sarcosine oxidase was immobilized at the surface of the chitosan via crosslinking agent glutaraldehyde. Thus prepared biosensor was used for determination of sarcosine in model samples.

2. Introduction

Flow analytical techniques like flow injection analysis (FIA), electromigration methods or HPLC make an important part of instrumental analysis. As in all techniques, detection plays a crucial role in the flow manifold. Depending on the type of analyte, sensitivity, selectivity, financial aspects and other criteria, detection methods as UV/VIS spectrophotometry, fluorescence spectrometry, mass spectrometry and others are available. However, electrochemical detection comprises a very promising alternative. This type of detection belongs to relatively inexpensive analytical methods and at the same time it is sufficiently sensitive for clinical, pharmaceutical or environmental analysis.

For measurements in flow systems several electrochemical methods are available and undoubtedly, the most frequently investigated, described and applied electrochemical technique in HPLC, FIA and CZE is amperometry. Amperometry has gained its popularity probably because this electrochemical mode (compared to voltammetric techniques) is easily adaptable and simple for using in flow systems. In literature two basic types of cell design predominate. First one is wall-jet arrangement where the flow stream jets perpendicularly towards the electrode surface. The second design is thin-layer where analyzed liquid flows along the electrode surface. For both of them different types of electrode materials are available. In the first place there are various types of carbon electrodes such carbon paste electrodes [1, 2], glassy carbon electrodes [3, 4], carbon fiber electrodes [5], sol-gel carbon composite tubular electrodes [6] or the boron-doped diamond film electrodes [7-12]. Also electrodes of pure metals are still used. A traditional electrode material is platinum. Platinum electrodes in microcylindrical and tubular arrangements are used in conventional and micro-HPLC [13-16]. Above mentioned electrode materials are used mostly for oxidation processes, whereas possibilities of the determinations in cathodic region are significantly limited. Mercury electrodes are the most convenient for analysis of reducible compounds because of their well-known advantages: the high hydrogen overpotential and the easy surface renewal. Some examples of using mercury electrodes for amperometric measurements are given in papers [17, 18]. However, nowadays they are less frequently used probably due to mechanical instability of mercury drop and because of somewhat unsubstantiated fear of mercury toxicity. Fortunately the solid and paste amalgam electrodes represent the adequate substitution. They allow cathodic measurements at potentials up to –2 V in aqueous solutions and to construct electrodes/detectors of necessary shapes and sizes. In our laboratory, electrodes of silver solid amalgam have been previously employed in a batch arrangement [19], in wall-jet, thin layer or in microcylindrical design for HPLC and FIA [20-24] of electrochemically reducible organic compounds. In this thesis the tubular detector based on silver solid amalgam for measurements in flow systems (chapter 4.1., Publication 1-2) is presented. It represents a miniature detector with a simple, robust and inexpensive construction which provides sufficient sensitivity and a good repeatability of measurements even at highly negative potentials. Moreover, it was used as a part of enzymatic biosensors (chapter 4.4. and 4.5., Publication 5-6).

Application of voltammetric (and amperometric) measurements in flow systems are even more broadly described in analytical literature than potentiometric measurements. One of the reasons is that voltammetry offers several techniques from which we can choose the best one for determination of concrete analyte(s). For example, square-wave and pulse voltammetry is often used in connection with flow-based techniques [25, 26]. The stripping voltammetry allows very sensitive detection especially of trace amount of metals and some organic compounds [27, 28]. In general, flow voltammetric methods offer some significant advantages compared to traditional batch design. Due to the nonzero flow rate of the sample, the convection importantly contributes to the total mass transfer and the signal is higher. Reproducibility can be better because continuous streaming of the solution can facilitate the removal of interfering products of electrode reactions from the working electrode surface. In one part of this thesis (chapter 4.1., Publication 2), the use of non-stop-flow differential pulse voltammetry for determination

of chemotherapeutic drug lomustine is described. Results showed that the signal of analyte considerably increases with increasing flow rate. It confirms the fact that voltammetry under hydrodynamic conditions is more sensitive that stationary one.

Except working electrode, the electrochemical cell also contains a reference electrode. There is no doubt that the reference electrode is the important part of both two- and three electrode system. One of the most frequently used reference electrodes in modern electrochemistry is probably silver–silver chloride–potassium chloride electrode (Ag | AgCl, KCl) due to its simple construction. The most popular mercury reference electrode is a calomel electrode (Hg|Hg_2Cl_2|KCl) [29]. Mercury has properties which are desirable for setting up well-behaved electrode systems. It is noble, liquid metal, easy to purify, and therefore easy to obtain in a standard state with properties quite independent of its chemical, mechanical or thermal history. If it is necessary to avoid the presence of chloride anions in the analyzed solution, mercury–mercurous sulfate electrode (Hg|Hg_2SO_4|K_2SO_4 sat.) or mercury–mercury oxide electrode (Hg|HgO|1 mol dm^{-3} NaOH) can be used. Many other types of reference electrodes are described in monographs [30, 31]. In this Ph.D. thesis, the reference electrodes based on silver and copper paste amalgam are presented. The principal difference between classical mercury reference electrodes and reference electrodes based on paste amalgams is that liquid mercury has been substituted by the paste of non-toxic silver and copper amalgam. The preparation and testing of i) saturated calomel electrode based on silver paste amalgam, ii) silver paste amalgam–mercurous sulfate electrode, iii) silver paste amalgam–mercuric oxide electrode and iv) copper paste amalgam–copper(II) oxide electrode will be discussed in chapter 4.2. and in Publication 3.

Nowadays modification procedures of working electrodes play a very important role. It is partly due to effort to improve the properties of electrodes such as selectivity, sensitivity or resistance to corrosion and partly because it enables the preparation of biosensors which are indispensable in clinical, environmental and others routine analysis requiring high selectivity. Literature offers numerous modification techniques depending on the type of modified surface (mercury, carbon, metals, amalgams, glass, etc.) or on the chemical substances by which the surface will be modified (fatty acids, organosilicon derivatives, thiols, etc.). Substances containing the –SH group (thiols, R–SH) or –S–S– group (disulfides, $R_1S–SR_2$) can spontaneously adsorb on various surfaces and form stable monolayer films (SAM – self-assembled monolayer) [32]. SAMs are typically prepared by immersing carefully cleaned substrate into the solution of thiols of ~ millimolar concentration over night at laboratory temperature. Another way of thiol monolayer formation is electrodeposition using cathodic stripping voltammetry (CSV). In this method, the thiocompound is accumulated at the electrode surface by oxidation of the electrode material at a suitable potential, and the created metal cations bind covalently with sulfur in –SH group(s) [32]. CSV can be used for a deposition of a compact monolayer of thiols on amalgams, gold [33], silver [34] and mercury [35]. Amalgam electrodes of different metals were first used by us to create monolayer thiol films [32]. For these purposes, solid [19, 36] or paste [37-39] amalgams are the most convenient. Electrodes of solid amalgams (MeSAE; where Me is Ag, Au, Cu, Bi, etc.) can be well polished, they do not contain liquid mercury, and they also have similar properties as mercury [19, 40]. Solid amalgams are also well wetted by mercury and hence they can be easily covered by mercury meniscus (m-MeSAE) [19, 41] or by mercury film (MF-MeSAE) [42, 43]. These electrodes have ideally smooth and easily renewable surfaces which ensure preparing a monolayer with a small number of defects. Similar surface is provided by mercury itself, for example in the form of hanging mercury drop electrode (HMDE). Moreover, while mechanical instability of the liquid mercury drop limits the applicability of HMDE [44], the mercury-covered solid amalgams appear to be suitable for the preparation of monolayer-based biosensors. In this Ph.D. thesis we have studied monolayer formation of 11-mercaptoundecanoic acid at polished, mercury meniscus and mercury film covered amalgam electrodes (p-AgSAE, m-AgSAE, MF-AgSAE, m-CuSAE, m-BiAgSAE, m-CdSAE). Then m-AgSAE and MF-AgSAE with thiol

monolayer was used for preparation of biosensors for determination of biotin and biotin-labeled substances (chapter 4.3., Publication 4). Also further biosensors prepared in this Ph. D. thesis are based on immobilization of enzymes at the surfaces of porous and powder silver solid amalgams modified by 11-mercaptoundecanoic acid and 4-amino-thiophenol, respectively (chapter 4.4. and 4.5., Publication 5–6).

As mentioned above, modification of monolayers allows to prepare various types of biosensors based on a selective interaction between a compound, featuring a biorecognition element and linked to a SAM surface and an analyte. Such compounds can be DNA or RNA [45-47], enzyme [48-50], antibody [51, 52], (strept)avidin or biotin [53], etc. Biosensors can be classified as electrochemical (amperometric, potentiometric, conductimetric or impedimetric), optical (fluorescent, luminescent, refractive), piezoelectric (acoustic, microcantilever) and thermal [54]. As biochemical receptors enzymes, proteins, nucleic acids, antigens/antibodies, whole cells or plant and animal tissues are widely used [55]. Among electrochemical biosensors the amperometric ones are the most widespread [55] and the use of enzymes as biorecognition element is most frequent. The immobilized enzymes provide one very important advantage – depending on enzyme's specificity, they have ability to recognize monitored analyte or group of analytes (substrates) with high selectivity and catalyze their transformation. These unique properties make the enzymes powerful tools to develop analytical devices [56-59]. The amperometric biosensors find employment in various fields. From a practical and commercial point of view four enzymatic biosensors have been widely used [60]: for determination of glucose (diagnosis and treatment of diabetes, food science, biotechnology) [61-63], lactate (sports medicine, critical care, food science, biotechnology) [64-67], urea (clinical applications) [68-71], and glutamate/glutamine (food science, biotechnology) [72-77].

In publications presented in this Ph. D. thesis (chapter 4.4.–4.6., Publication 5-7) we have paid attention to amperometric biosensors with enzymes as biological recognition elements. An example of impedimetric biosensor can be found in chapter 4.3. (Publication 4). The main concept of our first type of enzymatic biosensors consists in the use of two parts – a flow reactor where the enzymatic reaction takes place, and a tubular flow detector where increasing or decreasing concentration of some component of this enzymatic reaction is measured. Both the reactor and the detector are based on silver solid amalgam but of different types. While tubular detector is made of compact smooth amalgam, the consistence of silver solid amalgam of the reactor is porous or powdered. It demonstrates wide possibilities of solid amalgams. It is necessary to note that the large surface of the enzymatic reactor can be provided by various porous or powdered materials, e.g., by porous carbon felt [78], by small particles of organofunctionalized silica [79], by aminoaryl [80] or by aminopropyl [81, 82] controlled pore glass, by the immobilizing agent Eupergit C 250 L (oxirane acrylic beads) [83], by chitosan porous beads [84, 85], by porous agarose beads [86], by nylon membrane coated with a thin layer of gold [87] or by microporous gold [88]. Detailed overview of utilized reactors and biosensors is given in the paper [89]. Two above mentioned biosensors based on porous and powdered amalgams were applied for determination of glucose, sodium ascorbate, hydrogen peroxide, catechol, pyrogallol, and dopamine. Second type of electrochemical flow biosensor consists of classic pen-type silver solid amalgam electrode mechanically covered by layer of chitosan in which the enzyme sarcosine oxidase is immobilized via crosslinking agent glutaraldehyde. It is also intended for analysis in flow systems, namely in flow injection analysis with wall-jet cell.

One group of enzymatic amperometric biosensors are mediatorless ones where concentration of electroactive reagents or products of an enzymatic reaction are directly measured amperometrically at the electrode surface. For example in our work we have used ascorbate oxidase (AscOx), glucose oxidase (GOx), sarcosine oxidase (SOx), catalase (Cat), laccase (Lac) and tyrosinase (Tyr). During enzymatic reaction of AscOx, GOx and SOx the concentration of substrates were determined via decreasing concentration of oxygen, in the case of Cat via increasing concentration of oxygen, and in the case of Lac

and Tyr the concentration of substrates are directly proportional to the concentration of products – quinones which are easily reducible compounds and can be determined by silver solid amalgam detector.

In literature we can find variety of techniques of immobilization of enzymes on the solid surface which are possible to categorize as physical and chemical interactions [58, 90]. The chemical interactions are performed by covalent bonds and offer more stable immobilized product than physical ones. They can be achieved for example by cross-linking. Crosslinking is a process of chemically joining two or more molecules by a covalent bond. Crosslinking agents (or cross-linkers) are molecules that contain two or more reactive ends capable of chemically attaching to specific functional groups on proteins or other molecules. We have used EDC/NHS chemistry to attach avidin and streptavidin (with $-NH_2$ groups) on the monolayer of 11-mercaptoundecanoic acid to prepare impedimetric biosensors based on mercury film modified silver solid amalgam electrode and mercury meniscus modified silver solid amalgam electrode for determination of biotin and biotin-labeled albumin, respectively (chapter 4.3., Publication 4). The similar approach was applied to immobilize enzyme glucose oxidase on porous silver solid amalgam covered with 11-mercaptoundecanoic acid monolayer in reactor of biosensor for determination of glucose (chapter 4.4., Publication 5). Other examples are imidoester cross-linkers, haloacetyls, pyridyl disulfides, hydrazides or diazirines. Among the many available protein crosslinking agents, glutaraldehyde (1,5-pentanedial) has undoubtedly found the widest application in various fields [91, 92] such as cytochemistry [93], enzyme and protein technology [92, 94], immunochemistry [95-97], and many others. Glutaraldehyde is symmetrical linear molecule with two aldehyde groups. It reacts with several functional groups of proteins, such as amino, thio, phenolic and imidazole rings [98].

Another method of covalent (or non-covalent) attachment is immobilization of the enzyme within the polymeric gel matrix. For these purposes the natural polymers such alginate, collagen, cellulose, pectin or chitosan as supports are commonly used [99]. Chitosan is widely used immobilization matrix. The chitosan layer is not electrochemical active in negative region and it is possible to apply potential up to about –2.0 V. Moreover, the electrolytic permeability of the chitosan hydrogel allows diffusion of components of the enzymatic reaction to the electrode surface. The preparation of the biosensor based on chitosan layer with immobilized of enzyme sarcosine oxidase via glutaraldehyde is described in our last publication (submitted) (chapter 4.6., Publication 7).

The presented Ph. D. thesis is focused on the construction of new type of the detectors/electrodes and enzymatic biosensors based on solid amalgams for detection of biological important compounds in flow systems. For these purposes, different techniques of modification of the electrode surfaces and enzymatic attachment were used. All results were published in following six scientific articles and in one submitted paper which are attached as appendixes:

1. **Yosypchuk, O.**; Barek, J.; Yosypchuk, B.: *Tubular Detector of Silver Solid Amalgam for Electrochemical Measurements in Flow Systems*. Electroanalysis 24, 2230 – 2234 (2012).

2. **Josypčuk O.**, Barek J., Josypčuk B.: *Application of Non-stop-flow Differential Pulse Voltammetry at a Tubular Detector of Silver Solid Amalgam for Electrochemical Determination of Lomustine (CCNU).* Electroanalysis 26, 306–311 (2014).
3. Yosypchuk, B.; Barek, J.; **Yosypchuk, O.**: *Preparation and Properties of Reference Electrodes based on Silver Paste Amalgam*. Electroanalysis 23, 2226–2231 (2011).

4. Yosypchuk, B.; Fojta, M.; **Yosypchuk, O.**: *Thiolate Monolayers Formed on Different Amalgam Electrodes. Part II: Properties and Application*. Journal of Electroanalytical Chemistry 694, 84–93 (2013).

5. Josypčuk B., Barek J., **Josypčuk O.**: *Flow Electrochemical Biosensors Based on Enzymatic Porous Reactor and Tubular Detector of Silver Solid Amalgam.* Analytica Chimica Acta 778, 24–30 (2013).

6. **Josypčuk O.**, Barek J., Josypčuk B.: *Electrochemical Biosensors Based on Enzymatic Reactor of Silver Solid Amalgam Powder for Measurements in Flow Systems.* Electroanalysis 26, 1729–1738 (2014).

7. **Josypčuk O.**, Barek J., Josypčuk B.: *Construction and Application of Flow Enzymatic Biosensor Based of Silver Solid Amalgam Electrode for Determination of Sarcosine.* Electroanalysis (submitted).

3. Aims of the Work

The main aim of this Ph. D. thesis was a development and fabrication of electrochemical flow enzymatic biosensors based on silver solid amalgam. To meet the target it was necessary to realize the following steps:

- Design and preparation of the tubular detector based on silver solid amalgam
- Preparation and construction of minireactors filled by porous and powdered silver solid amalgam where enzymes would be attached
- Development of a methodology for a) covalent modification the silver amalgam surface by thiols; b) covalent immobilization of enzymes at the thiol layer in the reactors and at the surface of the pen-type electrode
- Connection of enzymatic reactor with tubular detector to create a first type of biosensors
- Preparation of the second type of biosensors based on polished silver solid amalgam electrode covered by chitosan with covalently immobilized enzyme
- The application of biosensors for the determination of biological active species in model and real samples in flow systems (flow injection analysis and voltammetry in flow)
- Design and construction of all flow cells and miniaturized reference electrode based on paste silver amalgam.

4. Results and Discussion

4.1. Tubular Detector of Silver Solid Amalgam

The electrochemical detection plays important role in combination with flow systems as FIA, HPLC or electromigration methods. For this purposes silver solid amalgam electrode in a new tubular shape is presented just for electrochemical measurements in flow (Fig. 1, Publication 1).

The tubular detector was tested under flow injection analysis conditions in amperometric mode. For this purpose model samples of Zn^{2+} and Cd^{2+} were selected as representatives of inorganic reducible analytes and 4-nitrophenol (4-NPh) as an organic one. Following parameters of this method were optimized with respect to voltammetric response: the composition of the mobile phase, the detection potential, and the flow rate of the mobile phase.

Zn^{2+} and Cd^{2+} were determined in 0.02 mol dm^{-3} acetate buffer (pH 4.8) and 4-NPh in 0.01 mol dm^{-3} HCl. The interesting parameter and in the same time the main advantages of tubular detector is potential of detection. The optimal values for Zn^{2+}, Cd^{2+} and 4-NPh were −1.500 V, −1.700 V, and −1.200 V, respectively. It can be seen that the tubular detector can operate at relatively high negative potential without any problems. That was proved by good repeatability of the peak heights of 10 consecutive injections of each analyte. The RSD for Zn^{2+} and Cd^{2+} and 4-NPh were 0.8 %, 0.9 %, and 0.8 % ($c_{Zn} = 7.7 \cdot 10^{-5}$ mol dm^{-3}, $c_{Cd} = 4.5 \cdot 10^{-5}$ mol dm^{-3} and $c_{4\text{-NPh}} = 3.6 \cdot 10^{-5}$ mol dm^{-3} (i.e. 5 ppm for all substances)). The optimal flow rate of the mobile phase was found to be 0.10 ml min^{-1}.

The calibration dependences were obtained over the concentration range $1.5 \cdot 10^{-6}$–$1.5 \cdot 10^{-4}$ mol dm^{-3} (0.10–10.0 ppm) for Zn^{2+}, $8.9 \cdot 10^{-7}$–$8.9 \cdot 10^{-5}$ mol dm^{-3} (0.10–10.0 ppm) for Cd^{2+}, and $1.8 \cdot 10^{-6}$–$3.6 \cdot 10^{-5}$ mol dm^{-3} (0.25–10.0 ppm) for 4-NPh. The dependences were linear for Zn^{2+} and Cd^{2+} in the whole concentration range with high correlation coefficients. In the case of 4-NPh, the narrower linear range was obtained (only up to the concentration of 5.00 ppm ($3.6 \cdot 10^{-5}$ mol dm^{-3}). The calculated limits of detection were $1.4 \cdot 10^{-6}$ mol dm^{-3} (0.09 ppm) for Zn^{2+}, $7.0 \cdot 10^{-7}$ mol dm^{-3} (0.08 ppm) for Cd^{2+}, and $5.0 \cdot 10^{-7}$ mol dm^{-3} (0.07 ppm) for 4-NPh, respectively.

The effect of a length of the amalgam column on the signal was also investigated. Zinc cations were measured by tubular detector of three different lengths – 6.0, 7.5, and 13.5 mm. RSD of 10 consecutive measurements using tubular detector of different length were 0.3 % (6.0 mm), 0.6 % (7.5 mm), and 0.6 % (13.5 mm), respectively. Average values for all detectors indicate that the detector length does not have any significant effect on the peak height in the length range 6.0–13.5 mm.

For all substances degrees of conversion at the highest and at the lowest flow rates were calculated. From results it was concluded that the tubular detector operated in the amperometric mode because in contrast to coulometric detectors ($\gamma \sim 100$ %), only relatively low fraction of the analyte was electrochemically converted (max. 19.8% in Cd^{2+} at flow rate of 0.04 ml min^{-1}).

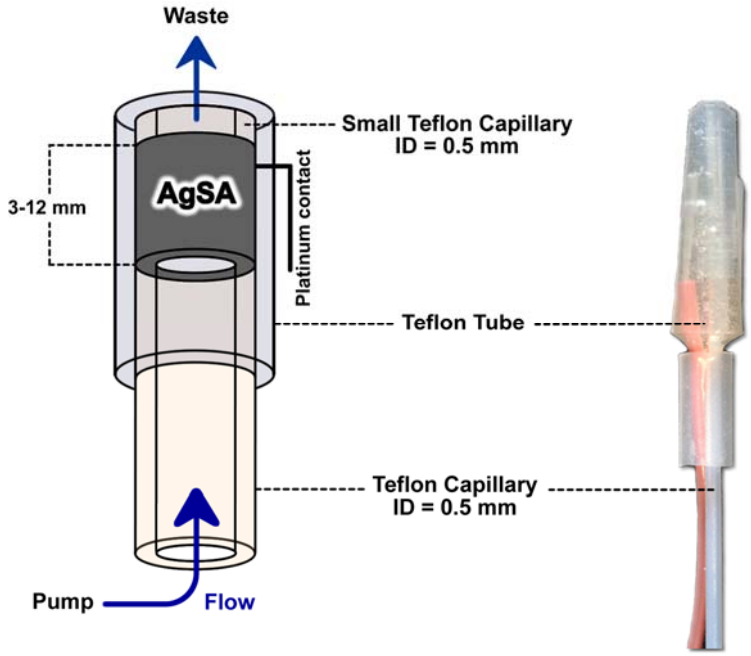

Fig. 1. Scheme and photo of the tubular detector of silver solid amalgam (AgSA).

After previous experiments confirming that the prepared tubular detector provides a reliable and well repeatable signal, it was used for control of pharmaceutical preparation CeeNU® Lomustine (Publication 2) containing electrochemically reducible nitroso group. In this case the voltammetric technique – non-stop-flow differential pulse voltammetry under conditions of flow injection analysis was used (Flow-DPV). During measurements the sample flows with constant rate without stopping and DP-voltammograms are recorded. In the search for optimum conditions for determination of lomustine, attention was paid to the dependence of electrochemical signal on composition of the supporting electrolyte (pH of the buffer, concentration of NaCl, content of ethanol), flow rate of the sample solution and the modulation amplitude.

The best results were obtained with the mobile phase [0.10 mol dm^{-3} MES; 2.00 mol dm^{-3} NaCl; pH 6.0]:EtOH 9:1, the flow rate 0.10 ml min^{-1}, and the magnitude of the modulation amplitude –0.070 V. In the whole studied pH range (6.0–8.0) only one reductive peak was observed which presumably corresponds to the two-electron reduction of N-nitroso group to hydroxylamino group [100]:

$$R–NO + 2\ e^- + 2\ H^+ \rightarrow R–NHOH$$

The mobile phase as well as the sample contained a high concentration of NaCl to ensure a constant ionic strength of measured sample. Flow rate of the mobile phase/sample seemed to be very important parameter which has significant influence on the signal. When the flow rate was increased from 0.10 ml min^{-1} to 1.00 ml min^{-1} the peak height increased by 78 %. It is assumed that the signal increase is caused by the increase of the convection as a consequence of the increased flow rate. It considerable increases a sensitivity of the determination of this analyte. It is one of the main advantages of voltammetry in flow systems in comparison with voltammetry in batch arrangement.

The RSD of 25 consecutive measurements of model samples of lomustine ($c = 1 \cdot 10^{-6}$ mol dm^{-3}) was 1.6 %. Before each Flow-DPV recording, the regeneration program (E_1 = –1.300 V, t_1 = 60 s; E_2 = –0.100 V, t_2 = 10 s) was applied.

The calibration dependences were obtained over the concentration range $1 \cdot 10^{-6}$–$1 \cdot 10^{-4}$ mol dm^{-3} at three different combinations of the modulation amplitude (*Amp*) and the flow rate. Concentration dependences are linear in the whole range with high correlation coefficients. Predictably, the most

sensitive is combination of higher modulation amplitude and higher flow rate: –0.070 V and 0.50 ml min^{-1} (LOD = $1.5 \cdot 10^{-7}$ mol dm^{-3}). But for controlling of amount of lomustine in drugs, when the concentration of lomustine is relatively high (it depends how the sample will be diluted), the flow rate about 0.1 ml min^{-1} is absolutely sufficient and high value of modulation amplitude (–0.070 V) can be used (LOD = $1.9 \cdot 10^{-7}$ mol dm^{-3}).

Finally, optimized method was successfully applied for the determination of lomustine in chemotherapy drug CeeNU® Lomustine 40 mg. The measured amount of lomustine found by our method was 103.2 % ± 2.4 % ($\tilde{x}$ ± 1 SD) with RSD 2.3 % (10 tablets) which corresponded to the value declared by manufacturer.

In summary, it was demonstrated that tubular detector based on silver solid amalgam used in presented works is an effective and low cost device for monitoring reducible organic and inorganic analytes in an amperometric mode with possibility of measurement at highly negative potentials (up to –2 V in aqueous solutions). It is also suitable for rapid and sensitive control of the amount of the active ingredient in drugs, in this case lomustine, by DPV in flow system without separation steps. Tubular detector has good measurements repeatability and a long-term stability at least 2 years. Moreover, as will be described in chapter 4.4. and 4.5., the tubular detector plays important role as an inseparable part of two types of flow enzymatic biosensors.

4.2. Reference Electrodes Based on Silver Paste Amalgam

The principle of these electrodes is the substitution of metallic mercury in three commonly used reference electrodes 1) calomel ($Hg|Hg_2Cl_2|KCl$ sat.), 2) mercury-mercurous sulfate ($Hg|Hg_2SO_4|K_2SO_4$ sat.), and 3) mercury-mercuric oxide ($Hg|HgO|1$ mol dm^{-3} NaOH)] by silver paste amalgam (AgPA, weight ratio: 13 % Ag and 87 % Hg) (Publication 3).

The amalgam paste is not liquid; it is soft but a long-term stable and dense enough to keep the allocated shape. This enables to prepare the reference electrode of small size. The body of prepared reference electrode is made of 10 µl pipette tip and it is about 2 cm long.

From an electrochemical point of view, AgPA is very similar to pure mercury. Experimental confirmation of the electrochemical similarity of metallic mercury and silver paste amalgam is given by the close values of potentials of electrodes prepared from these two materials (Table 1).

Table 1. Potentials of prepared reference electrodes in comparison with commonly used ones. Electrolyte: 0.1 mol dm^{-3} KCl; *x*-AgPA were connected with the negative and classical SCE with the positive connector of the digital millivoltmeter.

Prepared reference electrode	E vs. SCE, mV	E vs. SHE, mV	Commonly used reference electrode	E vs. SHE, mV
SCE-AgPA saturated, ~ 22–25 °C	–0.56	+244.44	SCE saturated, 25 °C	+ 244
MMSE-AgPA saturated, ~ 22–25 °C	+391.5	+635.5	MMSE saturated, 25 °C	+ 640
MMOE-AgPA 1 mol dm^{-3} NaOH, ~ 22–25 °C	–104.9	+139.1	MMOE 1 mol dm^{-3} NaOH, 25 °C	+ 140

SHE – standard hydrogen electrode
SCE-AgPA – silver paste amalgam-calomel electrode
MMSE-AgPA – silver paste amalgam-mercurous sulfate electrode
MMOE-AgPA – silver paste amalgam-mercury oxide electrode

Prepared reference electrodes were tested with respect to possible polarization in a two-electrode mode when each tested reference electrode was connected as working electrode and a large classic SCE was connected as a reference one (it was assumed that SCE does not get polarized). The cyclic voltammograms (CV) were recorded from –2.0 V to +2.0 V. The value of the current flowing through the circuit was regulated by connecting optional standard resistors. If tested reference electrode does not polarize, the cathodic and anodic branches of CV are identical and the CV has the shape of two overlapping lines (in fact it is a record of Ohm's law). On the other side, the electrode polarization results in the separation of both curves of CV. Results of this experiment showed the calomel reference electrode based on silver paste amalgam has proved to be the most resistant to polarization and thus its miniaturized version (in a pipette tip) was used in all further experiments in this thesis.

4.3. Electrochemical Modifications of Solid Amalgam Electrodes

Properties of thiol 11-mercaptoundecanoic acid (MUA) monolayer were investigated at the polished silver solid amalgam electrode (p-AgSAE), the mercury film covered AgSAE (MF-AgSAE), the mercury meniscus covered AgSAE (m-AgSAE), the mercury meniscus covered bismuth-silver SAE (m-BiAgSAE), the mercury meniscus covered copper SAE (m-CuSAE), and the mercury meniscus covered cadmium SAE (m-CdSAE) (Publication 4).

Procedure of forming monolayer of MUA at the electrodes includes creation of the mercury meniscus or the mercury film at the surface of polished AgSAE, the electrochemical accumulation of thiol for a given time at the optimal potential for each concrete working electrode in the thiol solution [0.5 mol dm^{-3} NaOH, 48 % EtOH, $1 \cdot 10^{-3}$ mol dm^{-3} $HS(CH_2)_{10}COOH$], and registration and evaluation of cyclic voltammogram(s). The principle of thiol layer formation is that metals of the electrode material are electrochemically oxidized during the potential application and create covalent bonds with sulfur. Since the thiol deposition is potential-controlled, this process on mercury and amalgam electrodes is much faster than self-assembly method of the monolayer formation.

The dependence of the amount of the chemisorbed compound on the deposition time (1–300 s) was investigated. A monolayer was prepared in $1.0 \cdot 10^{-3}$ mol dm^{-3} thiol solution at an optimal accumulation potential at all electrodes within 1−2 min. Areas of reductive desorption peaks were practically unchanged with longer deposition time and hence, short accumulation times (up to 120 s) were used in our experiments.

The concentration dependence of MUA was studied in the range $2.0 \cdot 10^{-6}$–$1.1 \cdot 10^{-3}$ mol dm^{-3}. The obtained experimental data were fitted much better to Langmuir isotherm then to Frumkin one. It is assumed that the lateral interactions among MUA molecules are compensated by a repulsion of the negatively charged carboxyl groups and the system therefore behaves as if the molecules at the electrode surface do not interact among themselves. The lowest saturated surface coverage was observed for HMDE ($8.5 \cdot 10^{-10}$ mol cm^{-2}) and the highest one for MF-AgSAE ($9.8 \cdot 10^{-10}$ mol cm^{-2}).

The dependence of the peak current (I_P) on the scan rate (v) was investigated from 20 to 1000 mV s^{-1}. The nonlinearity of this dependence in our experiments showed that among the adsorbed molecules lateral interactions exist. Moreover, dependence $I_P \sim v^{0.60}$ indicated that MUA forms a two-dimensional monolayer.

Finally, the biosensors based on MF-AgSAE and m-AgSAE which use the avidin-biotin and streptavidin-biotin labeled albumin interactions, respectively, were prepared. First, monolayer of MUA was electrochemically formed at both electrodes. Then, avidin was bonded at MF-AgSAE and streptavidin at m-AgSAE via EDC/NHS chemistry. Thus prepared biosensors were successfully tested for the determination of biotin and biotin labeled albumin, respectively, by electrochemical impedance spectroscopy. Under optimal conditions, the response of the prepared biosensors with avidin or streptavidin to additions of biotin or biotin labeled albumin was linear in the logarithmic scale in the range 0.58–4.03 μg ml^{-1} of biotin and 1.96–20.0 μg ml^{-1} of biotin labeled albumin.

A good reproducibility of repeated formation and desorption of thiol monolayers attests that the different types of amalgam are convenient electrochemical materials for modification by thiols as well as for preparation of biosensors based on thiol monolayers.

4.4. Flow Electrochemical Biosensor Based on Enzymatic Porous Reactor of Silver Solid Amalgam

A flow amperometric enzymatic biosensor for the determination of glucose was constructed (Publication 5). The biosensor consists of a flow reactor based on porous silver solid amalgam and a flow tubular detector described in chapter 4.1 (Fig. 2). Firstly, the porous silver amalgam was modified by a thiol (11-mercaptoundecanoic acid) by self-assembled method. Then, an enzyme was covalently immobilized at the modified surface of porous amalgam via EDC/NHS chemistry. For our experiment we have selected the glucose oxidase (GOx) as one of the most studied and stable enzymes. Whole immobilization process took about 1 hour and 40 minutes. In general, one reactor could be used for repetitive preparation of the biosensor, where many other enzymes could be attached. Finally, the porous reactor with attached GOx was connected with the tubular detector and placed into the miniature lab-made glass cell.

During enzymatic reaction oxygen (dissolved in the mobile phase) is consumed. Decrease of O_2 concentration is directly proportional to the concentration of glucose and it is measured amperometrically by the tubular detector. The following parameters of glucose determination were optimized with respect to amperometric response: composition of the mobile phase, concentration of base electrolyte, the potential of detection, and the flow rate. The optimal conditions were: mobile phase [0.10 mol dm^{-3} acetic buffer; 0.001 mol dm^{-3} Na_2EDTA, pH 6.5], the injected volume 50 µl, the potential of the detection −1.100 V and the flow rate 0.10 ml min^{-1}.

The biosensor had a good repeatability of 11 consecutive injections of glucose ($c = 4.0 \cdot 10^{-4}$ mol dm^{-3}) with RSD 1.83 %. When the reactor with the immobilized enzyme was not used, it was stored in mobile phase at 4 °C. The result of monitoring of enzyme stability during 35 days indicated that 77% of the current response of glucose was retained.

The calibration dependence of model sample of glucose was linear in the range $2.0 \cdot 10^{-5}$–$8.0 \cdot 10^{-4}$ mol dm^{-3} with limit of detection of $1.0 \cdot 10^{-5}$ mol dm^{-3}. Finally, the biosensor was used for the determination of glucose in real sample – commercial honey with declared glucose content 32–37 mass %. Using our method glucose concentration 35.5±1.0 mass % (n = 7, RSD = 3.2 %) was found which agrees well with the declared and literature values.

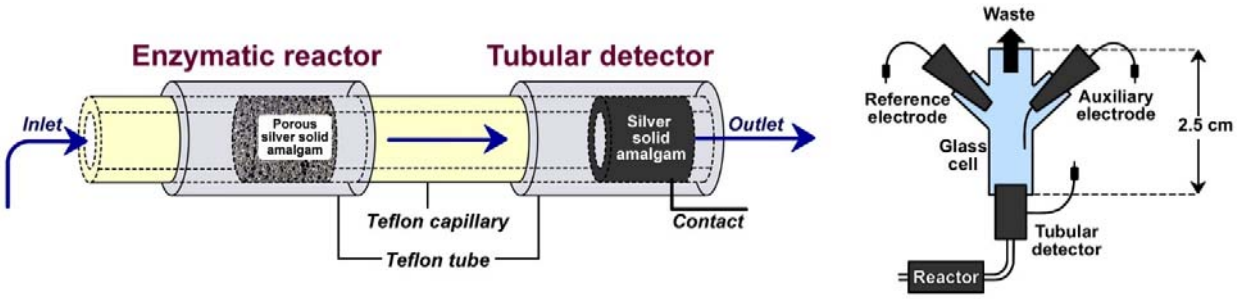

Fig. 2. Scheme of experimental arrangement for amperometric measurements under conditions of flow injection analysis.

4.5. Flow Electrochemical Biosensor Based on Enzymatic Reactor of Silver Solid Amalgam Powder

While in the previous chapters we have prepared and tested the biosensor with reactor of porous silver solid amalgam, in this chapter the reactor filled by silver solid amalgam powder will be introduced (Publication 6). The preparation of the powder reactor included next several steps. Firstly, silver solid amalgam powder was prepared from the metals with mercury/silver powder ratio 70/30 (w/ w) in a dental amalgamator and then it was pulverized to the fine powder in an agate mortar. Then, at the surface of amalgam powder particles a self-assembled monolayer of 4-aminothiophenol was formed. The immobilization of enzyme was carried out via crosslinking agent glutaraldehyde. Glutaraldehyde is symmetrical linear molecule with two aldehyde groups. One group reacts with NH_2-group of 4-aminothiophenol, and second one reacts with NH_2-group of enzyme. Thus glutaraldehyde serves as a connection between thiol and enzyme. The amalgam powder could be regenerated and used for repetitive preparation of the biosensor, in which many other enzymes could be immobilized. Thus prepared powder reactor can be used immediately. In this way, a few reactors containing enzyme ascorbate oxidase (AscOx), glucose oxidase (GOx), catalase (Cat), tyrosinase (Tyr) and laccase (Lac) were prepared. The complete flow enzymatic biosensor consists of this powder reactor and the tubular detector. Concentration of substrates in the model/real samples was measured amperometrically by the tubular detector under flow injection analysis conditions from the change of oxygen concentration during enzymatic reaction or from measuring of the reduction of an appropriate product (Table 2).

Table 2. Statistical parameters of repeated measurements of the substrates with various enzymatic biosensors. Experimental conditions: amperometry; mobile phase [0.10 mol dm^{-3} acetic buffer; 0.001 mol dm^{-3} Na_2EDTA; pH 6.5]; injected volume 50 µl; flow rate 0.1–0.2 ml min^{-1}; c(NaAsc) = c(glucose) = $5.0 \cdot 10^{-4}$ mol dm^{-3}; $c(H_2O_2)$ = $2.0 \cdot 10^{-4}$ mol dm^{-3}; c(catechol) = $2.0 \cdot 10^{-5}$ mol dm^{-3}; c(pyrogallol) = c(dopamine) = $1.0 \cdot 10^{-4}$ mol dm^{-3}; N = 11. RSD – relative standard deviation.

Enzyme	Substrate	Reducing compound	Detection potential, mV	Detection limit, µM	RSD, %
Ascorbate oxidase	Na-ascorbate	O_2	–1300	12.0	0.81
Glucose oxidase	glucose	O_2	–1100	14.0	0.95
Catalase	H_2O_2	O_2	–900	11.0	1.81
Tyrosinase	catechol	quinone	–50	0.80	1.37
	pyrogallol	quinone	–50	5.10	1.68
Laccase	catechol	quinone	–50	0.51	0.85
	dopamine	quinone	–50	6.01	2.12
	pyrogallol	quinone	–50	4.10	1.37

The current response of each biosensor was optimized with respect to the detection potential, flow rate of the mobile phase, injection volume of the sample and the reactor volume.

Concentration dependence of Asc–biosensor was linear from 0.02 to $0.6 \cdot 10^{-3}$ mol dm^{-3} with limit of detection $12 \cdot 10^{-6}$ mol dm^{-3}. This linear part was used as basic information for preparing the solution of vitamin tablet Celaskon® for determination of ascorbic acid in these tablets. The content of ascorbic acid found in one tablet was 104.9 ± 2.2 mg (N = 9; SD = 2.9 mg; RSD = 2.8 %) which corresponds well with the manufacturer´s declared value (95–105 mg per 1 tablet). The limit of detection of other analytes were in the range $0.51–14.10^{-6}$ mol dm^{-3}.

A volume of the enzymatic reactor was the last investigated parameter. For this study the amalgam powder with the enzyme laccase was used. Parallel measurements with catechol solution were carried out with Lac–reactors of different volumes (length): 5.6 µl (2.8 mm), 10.3 µl (5.2 mm), 13.5 µl (6.8 mm),

18.1 µl (9.1mm), 21.4 µl (10.8 mm), and 29.8 µl (15.0 mm). The reduction peak current of quinone (which was generated during the enzymatic reaction) depended on the volume of the reactor linearly while peak height increased 2.2-times when the reactor volume was changed from 5.6 µl to 29.8 µl (5.4-times). It is important that the peak width at half-height was almost unchanged. So, the choice of optimal volume should be in agreement with required sensitivity and with the accessible amount of the enzyme, and thus for most of our experiments the reactor volume of about 20 µl have been used.

The repeatability of each biosensor was determined by evaluation of 11 consecutive measurements of model samples of the studied substrates based on peak height under optimal conditions. The values of RSD ranged from 0.8 to 2.1 % what indicates a good repeatability of measurements. It is necessarily to note that tubular detector was electrochemically cleaned before each measurement by imposing an appropriate cleaning potential(s) for 10–20 s.

According to our experience, it can be generally said that the working temperature have the biggest influence on the progressive decrease of the enzyme activity. Therefore, enzymatic reactors should be stored in the refrigerator at 4 °C. The amalgam powder covered by enzyme should be stored at −18 °C. Such powder after unfreeze is active even after storing for several months.

The proposed powder reactor is an improved version of porous silver amalgam reactor described in the previous chapter. We did a comparative experiment when in both the porous and powder reactors enzyme glucose oxidase was immobilized. It was found that sensitivity of powder reactor was 2–3 times higher than the porous one.

4.6. Flow Electrochemical Enzymatic Biosensor Based on Polished Silver Solid Amalgam Electrode

In this chapter a different type of biosensor which is more suitable for enzymes with lower activity in comparison with previously described biosensors is presented. It consists of a classical pen-type electrode of polished compact silver solid amalgam covered by layer of chitosan in which the enzyme sarcosine oxidase (SOx) is immobilized via crosslinking agent glutaraldehyde (Publication 7). Whole preparation process of the biosensor took about 3 hours which is relatively short period in comparison with the preparation procedure of some biosensors which take tens of hours. The biosensor was placed into the lab-made wall-jet cell and it worked in amperometric mode under conditions of the flow injection analysis. For electrochemical detection chitosan as a matrix has two very important advantages. Firstly, the chitosan layer is not active at high negative potentials so it is possible to work at about ~ –2 V without effect on the chemical structure. Second advantage is the electrolytic permeability of the chitosan hydrogel which allows easy diffusion of some compounds of the enzymatic reaction to the electrode surface.

A substrate of SOx (and at the same time an analyte) is natural amino acid sarcosine. Sarcosine pays important role as a differential metabolite concentration of which is highly increased during prostate cancer progression to metastasis and it also was studied in the connection with the treatment of schizophrenia and depression.

The enzymatic reaction witch takes place in the following way [101, 102]:

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{sox} \text{formaldehyde} + \text{glycine} + H_2O_2$$

According to this reaction oxygen dissolved in the mobile phase and sample is consumed. This decrease of oxygen concentration, which is directly proportional to the concentration of sarcosine, was amperometrically measured directly at the electrode surface of the biosensor.

Under optimal conditions (mobile phase [0.1 mol dm^{-3} phosphate buffer; 0.001 mol dm^{-3} Na_2EDTA; pH 8.0], detection potential –1.400 V, flow rate 0.10 ml min^{-1} and the injected volume 100 µl) the concentration dependence of model sample were measured. The calibration curve was linear in interval $7.5 \cdot 10^{-6}$–$5.0 \cdot 10^{-4}$ mol dm^{-3} with correlation coefficient R^2 = 0.998 and limit of detection $2.0 \cdot 10^{-6}$ mol dm^{-3}.

The biosensor had also a good repeatability. The relative standard deviation of 10 consecutive injections of sarcosine at the concentration of $1 \cdot 10^{-4}$ mol dm^{-3} was 1.6 %. The life-time of the biosensor was investigated for 14 days. During this period it was used at least 3 hours per day and then stored in mobile phase at laboratory temperature. Sarcosine oxidase lost about 50 % its activity after 8 days and after two weeks about 22 % remained. However, the progressive loss of the signal is not critical because the concentration of sarcosine can be found out by a method of standard additions, and a bigger injection loop can be used to obtain higher signal.

5. Conclusions

Newly developed tubular detector based on silver solid amalgam (TD-AgSA) was prepared and tested in model solutions of Cd^{2+}, Zn^{2+} and 4-nitrophenol in amperometric mode under conditions of flow injection analysis. The newly developed tubular detector is a simple, robust and inexpensive device with good repeatability and sensitivity. One of its main advantages is applicability for cathodic measurements in aqueous solutions at potentials up to –2 V. The developed tubular detector has also a good long-term stability – it is actively used in our laboratory for about 2 years and it provides stable and repeatable signals. Then TD-AgSA was successfully used for the determination of an active ingredient lomustine in pharmaceutical preparation CeeNU® Lomustine. As a detection method the non-stop-flow differential pulse voltammetry was used. The mean value of lomustine measured by proposed method well corresponded to the value declared by manufacturer.

For measurements in the flow system it was also necessarily to design and fabricate a small flow glass cell with miniature auxiliary and reference electrode. The reference electrodes (saturated calomel electrode, mercury-mercurous sulfate, and mercury-mercuric oxide electrode) in which metallic mercury was replaced by paste silver solid amalgam were proposed, fabricated, and tested for 14 months. All newly developed reference electrodes have proved to be very stable in both long-term and short-term tests. Their potentials are almost identical with their metallic mercury analogues. The saturated calomel electrode based on paste silver solid amalgam was shown to be the most resistant to polarization and it was used as a reference electrode in all further experiments described in this thesis.

The formation of 11-mercaptoundecanoic acid monolayer on HMDE and on the electrodes based on solid amalgams (MF-AgSAE, m-AgSAE, m-BiAgSAE, m-CuSAE,m-CdSAE) was studied. The thiol formed the two-dimensional molecular films with lateral interactions between molecules. The created films completely blocked the surface of all electrodes. The lowest saturated surface coverage was observed at HMDE and the highest one at MF-AgSAE. Next, MF-AgSAE and m-AgSAE were used for preparation of several biosensors. First, monolayer of 11-mercaptoundecanoic acid was electrochemically formed at both electrodes. Then, avidin was bonded at MF-AgSAE and streptavidin at m-AgSAE via EDC/NHS chemistry. Thus prepared biosensors were successfully tested for the determination of biotin and biotin labeled albumin, respectively, by electrochemical impedance spectroscopy. From carried out experiments and from our previous experiences it could be concluded that the amalgam surface is suitable for modification by thiols and they can be used for the preparation of biosensors based on thiol monolayers.

The flow enzymatic biosensors based on the enzymatic reactor and the tubular detector were proposed. In the first case, the enzymatic reactor based on *porous* silver solid amalgam was prepared. The silver amalgam was modified by 11-mercaptoundecanoic acid. The immobilization of enzyme glucose oxidase at thiol layer was carried out using EDC/NHS chemistry. The biosensor (porous reactor + tubular detector) was then successfully used for the determination of glucose in commercial honey as a real sample.

In the second case, the enzymatic reactor contained *powdered* silver solid amalgam. The amalgam powder was modified by 4-aminothiophenol and enzyme was attached via crosslinking agent glutaraldehyde. Five different enzymes were used (ascorbate oxidase, glucose oxidase, catalase, tyrosinase, and laccase), so five different biosensors were prepared for the determination of ascorbic acid, glucose, hydrogen peroxide, catechol, pyrogallol, and dopamine. The biosensor with ascorbate oxidase was used for the determination of ascorbic acid in the vitamin tablets Celascon®. In general, it was found that sensitivity of biosensors with powder reactor is 2–3 times higher than that of the biosensors with porous reactor.

The last biosensor was constructed using polished silver amalgam electrode which was covered by layer of chitosan. Then, the enzyme sarcosine oxidase was immobilized at the surface of the chitosan via

crosslinking agent glutaraldehyde. The covalent bonding of sarcosine oxidase on chitosan layer was found to be an effective and relatively fast procedure (3 hours) of enzyme attachment. This technique is suitable especially for enzymes with lower activity. Thus prepared biosensor was used for determination of biologically important substance – sarcosine in model sample by amperometry in wall-jet arrangement.

6. References

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7. Appendixes

Curriculum Vitae

Personal Information

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Education

• 2005–2008

Charles University in Prague, Faculty of Science, Department of Analytical Chemistry, UNESCO Laboratory of Environmental Electrochemistry.
Bachelor's Degree
Bachelor Thesis: Voltammetric Determination of 1-Nitropyrene at Boron-doped Diamond Film Electrode.
Supervisor: Prof. RNDr. Jiří Barek, CSc.

• 2008–2010

Charles University in Prague, Faculty of Science, Department of Analytical Chemistry, UNESCO Laboratory of Environmental Electrochemistry.
Master's Degree
Diploma Thesis: Voltammetric and Amperometric Detection of Genotoxic Pyrene Derivatives at Boron-doped Diamond Film Electrode.
Supervisor: Prof. RNDr. Jiří Barek, CSc.

• From 2010

Charles University in Prague, Faculty of Science, Department of Analytical Chemistry, UNESCO Laboratory of Environmental Electrochemistry.
Ph.D. Studies
Ph.D. Thesis: Electrochemical Biosensors and Detectors Based on Silver Solid Amalgam for Analysis in Flow System.
Supervisor: Prof. RNDr. Jiří Barek, CSc.

• 2011 (8 days)

Summer School of Spectroelectrochemistry, Dresden, Germany.

• 2013–2014 (4.5 months)

ERASMUS program in Universität Regensburg, Regensburg, Germany.

Job Experience

- 2012–2014

Research worker at Charles University in Prague, Faculty of Science, Department of Analytical Chemistry, UNESCO Laboratory of Environmental Electrochemistry.

- From 2012

Research worker at J. Heyrovský Institute of Physical Chemistry of AS CR, v.v.i., Department of Biomimetic Electrochemistry.

Language skills

- Ukrainian – mother tongue
- Czech – very good
- Russian – very good
- English – TOEFL level B1

List of Publications

1. Yosypchuk, O.; Pecková, K. a Barek,J.: *Voltametrické stanovení 1-nitropyrenu a 1-aminopyrenu na borem dopované diamantové filmové elektrodě.* **Chemické listy** 104, 186–190 (2010).

2. Yosypchuk, O.; Barek, J.: *Elektrochemická detekce karcinogenních derivátů pyrenu a jejich metabolitů.* **Chemické listy** 104, s61–s64 (2010).

3. Yosypchuk, O.; Barek, J.; Vyskočil, V.: *Voltammetric Determination of Carcinogenic Derivatives of Pyrene Using a Boron-Doped Diamond Film Electrode.* **Analytical Letters** 45, 449–459 (2012).

4. Yosypchuk, B.; Barek, J.; Yosypchuk, O.: *Preparation and Properties of Reference Electrodes based on Silver Paste Amalgam*. **Electroanalysis** 23, 2226–2231 (2011).

5. Yosypchuk, O.; Jiří Barek, Vyskočil, V.: *Determination of 1-Hydroxypyrene in Human Urine by HPLC with Electrochemical Detection at a Boron-doped Diamond Film Electrode.* **Analytical and Bioanalytical Chemistry** 404, 693-699 (2012).

6. Yosypchuk, O.; Karásek, J.; Vyskočil, V.; Barek, J.; Pecková, K.: *The Use of Silver Solid Amalgam Electrodes for Voltammetric and Amperometric Determination of Nitrated Polyaromatic Compounds Used as Markers of Incomplete Combustion*. **The Scientific World Journal** 2012, Article ID 231986, 12 pages, doi:10.1100/2012/231986

7. Yosypchuk, O.; Barek, J.; Yosypchuk, B.: *Tubular Detector of Silver Solid Amalgam for Electrochemical Measurements in Flow Systems*. **Electroanalysis** 24, 2230 – 2234 (2012).

8. Yosypchuk, B.; Fojta, M.; Yosypchuk, O.: *Thiolate Monolayers Formed on Different Amalgam Electrodes. Part II: Properties and Application*. **Journal of Electroanalytical Chemistry** 694, 84–93 (2013).

9. Josypčuk B., Barek J., Josypčuk O.: *Flow Electrochemical Biosensors Based on Enzymatic Porous Reactor and Tubular Detector of Silver Solid Amalgam.* **Analytica Chimica Acta** 778, 24–30 (2013).

10. Josypčuk O., Barek J., Josypčuk B.: *Application of Non-stop-flow Differential Pulse Voltammetry at a Tubular Detector of Silver Solid Amalgam for Electrochemical Determination of Lomustine (CCNU).* **Electroanalysis** 26, 306–311 (2014).

11. Josypčuk O., Barek J., Josypčuk B.: *Electrochemical Biosensors Based on Enzymatic Reactor of Silver Solid Amalgam Powder for Measurements in Flow Systems.* **Electroanalysis** 26, 1729–1738 (2014).

12. Josypčuk O., Barek J., Josypčuk B.: *Construction and application of flow enzymatic biosensor based of silver solid amalgam electrode for determination of sarcosine.* **Electroanalysis** (submitted).

Oral and Poster presentations

1. **XXIX. Modern Electroanalytical Methods, Jetřichovice, Czech Republic, 25.–29. 5. 2009**
   Oral presentation: *Voltametrické stanovení směsi 1-nitropyrenu a 1-aminopyrenu na borem dopované diamantové filmové elektrodě.*

2. **61. zjazd chemických společností, Vysoké Tatry, Tatranské Matliare, Slovakia, 7.–11. 9. 2009**
   Poster presentation: *Stanovení 1-nitropyrenu, 1-aminopyrenu a 1-hydroxypyrenu vysokoúčinnou kapalinovou chromatografií s elektrochemickou detekcí.*

3. **Modern Electroanalytical Methods 2009, Prague, Czech Republic, 9.–13. 12. 2009**
   Poster presentation: *Determination of 1-hydroxypyrene and 1-aminopyrene in human urine by HPLC with electrochemical detection based on boron doped diamond film electrode.*

4. **XXXI. Modern Electroanalytical Methods, Jetřichovice, Czech Republic, 23.–27. 5. 2011**
   Oral presentation: *Amperometric Determinations of Nitrocompounds with Flow Detector of Silver Solid Amalgam.*

5. **63. zjazd chemických spolecností, Vysoké Tatry, Slovakia, 5.–9. 9. 2011**
   Poster presentation: *HPLC stanovení nitrosloučenin pomocí průtokového detektoru ze stříbrného pevného amalgámu.*

6. **7th International Students Conference "Modern Analytical Chemistry", Prague, Czech Republic, 29.–30. 9. 2011**
   Oral presentation: *Electrochemical biosensor for determination of nucleic acid bases.*

7. **14th International Conference on Electroanalysis, Portorož, Slovenia, 3.–7. 6. 2012**
   Poster presentation: *Electrochemical biosensor based on the mercury covered solid silver amalgam electrode for determination of cytosine.*

8. **12th International Conference on Flow Analysis, Thessaloniki, Greece, 23.–28. 9. 2012**
   Poster presentation: *Tubular Detector of Silver Solid Amalgam for Electrochemical Measurements in Flow Systems.*

9. **XXXIII. Modern Electroanalytical Methods, Jetřichovice, Czech Republic, 20.–24. 5. 2013**
   Oral presentation: *Construction and Application of Tubular Detector and Porous Flow-through Detector/Reactor of Silver Solid Amalgam for Electrochemical Measurements in Flow Systems.*

10. **Euroanalysis XVII., Warsaw, Poland, 25. 8.–29. 8. 2013**
    Poster presentation: *Tubular Detector Based on Silver Solid Amalgam as a New Construction Arrangement of the Amalgams Electrodes Designated for Flow Measurements.*

11. **18th International Conference on Flow Injection Analysis, Porto, Portugal, 15.–20. 9. 2013**
    Poster presentation: *Electrochemical Flow Biosensor Based on the Silver Solid Amalgam Electrode for the Determination of Cancer Marker Sarcosine.*

12. **9th International Students Conference 'Modern Analytical Chemistry', Prague, Czech Republic, 23.–24. 9. 2013**
    Oral presentation: *Electrochemical flow biosensors based on the silver solid amalgam electrodes.*

13. **24th Anniversary World Congress on Biosensors, Melbourne, Australia, 27.–30. 5. 2014**
    Poster presentation: *Amperometric Flow Enzymatic Biosensor for Detection of Sarcosine Based on Polished Silver Solid Amalgam Electrode.*

Achievements

1. **2nd prize in the competition for the best scientific work in analytical chemistry**, Bratislava, Slovakia, 2008

2. **2nd prize in the 13th statewide competition for the prize of Merck company for the best student scientific work in analytical chemistry**,
   České Budějovice, Czech Republic, 2010

3. **Prize of the Metrohm company 2013 for the best publication** *Flow electrochemical biosensors based on enzymatic porous reactor and tubular detector of silver solid amalgam* (Anal. Chim. Acta 778, 24 (2013).

Grants

• *New Electrode Materials and Its Modification for Analysis of Biological Active Substances*,
Grant Agency of Charles University in Prague, 2011–2013.

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