## 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.