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# Electrodeposition of silver amalgam particles on ITO – Towards novel electrode material

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ABSTRACT

Silver solid amalgam represents up to now the most suitable alternative electrode material to metallic mercury in electroanalytical chemistry. Controlled electrodeposition of variable (sub)micrometer-sized silver amalgam particles (AgAP) on the surface of transparent indium-tin oxide (ITO) electrode from an electrolyte containing $Ag^+$ and $Hg^{2+}$ ions is reported here, as a novel perspective method suitable for preparation of nano-structured silver amalgam electrode material. Elemental analysis of the composition and morphology of the AgAP decorating the ITO was studied by scanning electron microscopy including energy-disperse X-ray spectroscopy and by image processing software. Particle composition, size, and surface coverage are controllable by selection of the $Ag^+/Hg^{2+}$ ratio in the electrodeposition solution and by setting of individual parameters of applied double pulsed/potential chronoamperometry. Applicable potential window of thus prepared ITO-AgAP electrode was found to be within +0.2 to −1.0 V in 0.2 acetate buffer pH 5.0. Utilized voltammetric and chronoamperometric methods revealed significant enhancement in electrochemical reducibility of selected model organic nitro-compound (shift of the peak potential about 300 mV to more positive potentials). Its further employment in UV/Vis spectroelectrochemical cell provided information about number of consumed electrons and kinetic characteristics. Furthermore preferential adsorption of calf thymus DNA at AgAP than ITO was observed by fluorescence microscopy indicating its potential applicability in (bio-)spectroelectrochemical methods. Further advantages and potential applications are also proposed and discussed.

## 1. Introduction

Metallic mercury represents unique electrode material with unrivalled electrochemical and mechanical properties: high hydrogen overpotential, and ideally smooth and easily renewable electrode surface. Great sensitivity of the mercury electrodes to biopolymers (nucleic acids, proteins, polysaccharides) and to their structural changes gave rise to broad spectrum of applications in biosensing [1–3]. However, low mechanical stability of the mercury drop hinder its application in development of (bio)sensors and miniaturized devices. On the other hand nanomaterials, e.g. gold nano-particles and/or carbon nanotubes, are commonly utilized in development of the (bio)sensors. They excel in selectivity and sensitivity, thanks to their simple functionalization and high charge transfer efficiency, which are frequently accompanied by catalytic effects, respectively [4].

Silver solid amalgam (AgSA) represents up to now the most suitable mechanically stable and non-toxic electrode material alternative to liquid mercury [5–9]. However, there is still a great potential to connect advantages of the metallic mercury and bimetallic colloids/nanoparticles in electrochemical and furthermore in spectroscopic applications [10,11]. All of the so far designed AgSA based electrodes (AgSAE), (bio)sensors [12–15] and (bio)reactors [16,17] have been prepared by direct dispersion of silver powder in liquid mercury in a suitable ratio or by electrodeposition of mercury film on silver or AgSA supports [18–20]. Unfortunately, lower homogeneity and charge transfer efficiency of AgSAE in comparison with mercury or nanomaterial- based electrodes, limits its common utilization [21,22]. Benefits of homogenous amalgam with nanostructured surface (1–4 nm) evincing more effective charge transfer have already been observed on crystals of silver amalgam (AgA) [23]. Even though these electrodes were

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successfully used in voltammetric and amperometric methods [24], prolonged preparation and fragility of the AgA crystals complicate their routine application.

Even though procedures for electrodeposition of silver nanoparticles [25–28], mercury film on ITO [29] and/or chemical preparation of Ag-Hg bimetallic colloids in solution [10] have already been presented, preparation of AgA using electrochemical co-reduction of $Ag^+$ and $Hg^{2+}$ ions on ITO for (spectro)electroanalytical purposes is reported here for the first time. The main aim of this work was to prove the concept of AgA electrodeposition on ITO, its following application in voltammetric and amperometric methods and to prove its spectro-electrochemical perspective and limitation. For this study a derivative of nitrobenzofurazan was selected as the model organic nitro-compound. Future perspective of AgAP in DNA analysis was also tested by adsorbed fluorescently labeled calf thymus deoxyribonucleic acid (C.T. DNA) on AgAP decorated ITO using a fluorescence microscopy. Other potential applications are also discussed.

## 2. Experimental

### 2.1. Chemicals and reagents

Solution of 0.01 mol $l^{-1}$ $AgNO_3$ (p.a. 99.8%, Safina) in 0.1 mol $l^{-1}$ $KNO_3$ (p.a. > 98%, Fluka) was used. Solution of 0.01 mol $l^{-1}$ $Hg(NO_3)_2$ in 0.1 mol $l^{-1}$ $KNO_3$ was prepared by dissolution of exact amount of metal mercury (99.999%, Polarografie Praha) in quantitative volume of concentrated $HNO_3$ (p.a. 65%, Sigma-Aldrich) in fume hood, then given amount of $KNO_3$ and deionized water was added to reach required concentrations. A 5 mmol $l^{-1}$ solution of *N*-methyl-4-hydrazino-7-nitrobenzofurazan (NBF, > 97.0%, Fluka) in dimethylsulfoxide (DMSO, p.a. > 99.9%, Sigma Aldrich) served as a model electrochemically reducible nitro-compound. All solutions were prepared from deionized water (18.2 MΩ cm, Purelab Option-Q, ELGA) and stored in dark borosilicate flasks in refrigerator at 4 °C. The C.T. DNA (for molecular biology, Sigma Aldrich) was dissolved in deionized water and labeled by a dsDNA Broad Range Fluorescence Assay, resulting DNA concentration 20 ng $\mu l^{-1}$, according to attached technical note 143 (both DeNovix).

### 2.2. Instruments and procedures

Electrodeposition of the silver amalgam particles was preceded in homemade Teflon cell with delimited specific surface area by O-ring (i.d. 5 mm) at polished glass covered by ITO on one side (CG-51IN, Delta-Technologies). ITO was used as working electrode after 30 min bath in absolute ethanol (96%, GPR rectapur, BDH Prolabo - VWR) and desiccation on the air at ambient temperature. Silver wire (o.d. 0.5 mm) was used as pseudo-reference electrode (Ag) in two electrode system during AgAP electrodeposition. Contact to ITO was provided by copper wire attached by scotch tape. Electrochemical measurements were carried out by Autolab PGStat128N operated by Nova software Ver. 1.10 (both Metrohm-Autolab) in three electrode connection including: ITO/ITO-AgAP working electrode, Ag/AgCl/3M KCl reference electrode and glassy carbon rod counter electrode. Oxygen was removed from the solution by 5 min passing a stream of argon saturated with vapors of the water in all voltammetric and chronoamperometric experiments. All the measurements were carried out at room temperature 25 °C. Surface morphology of ITO-AgAP was analyzed by benchtop scanning electron microscope including energy-disperse X-ray spectrometer (SEM/EDX, NeoScop JMC-6000, Joel) using magnification (200 ×, 600 × 1300 × and 4000 ×), high vacuum mode and acceleration voltage 15 kV. Abundance of Ag, Hg, Sn, In, Si and O in the particles was observed by EDX detector and average particle size, distribution and surface density/coverage determined by freely available ImageJ software [30].

Spectroelectrochemical cell (Fig. 1) was composed of 1 cm quartz cuvette with attached heat shrink Teflon tube (I.D. 5 mm) enclosed by

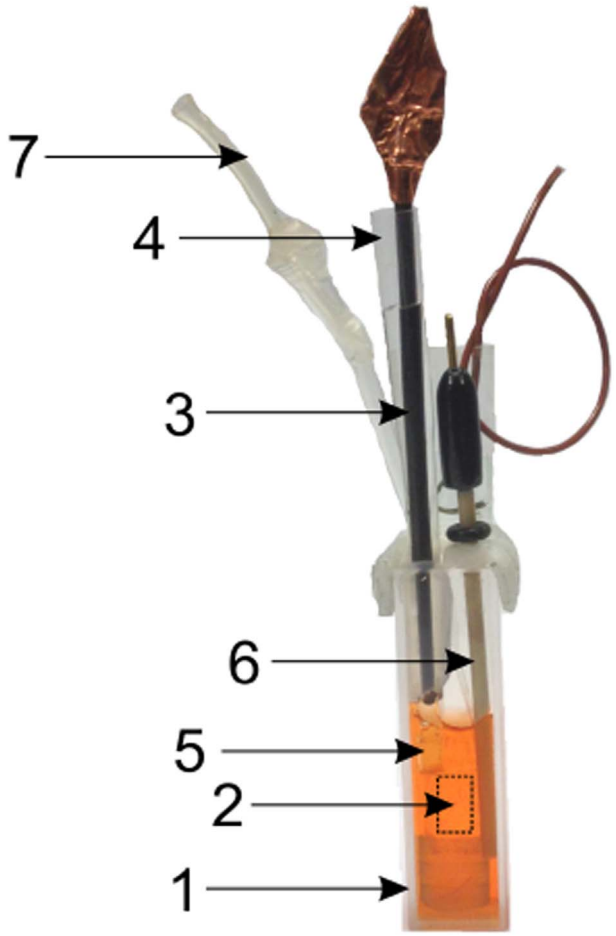


**Fig. 1.** Side view of the spectroelectrochemical cell assembled inside of the 1 cm quartz cuvette (1), using ITO or ITO-AgAP working electrode (2), glassy carbon rod auxiliary electrode (3) placed inside of the Teflon tube (4) and enclosed by CoralPor® (5), miniaturized Ag/AgCl reference electrode (6) and microloader pipette tip (7) for argon purging and mixing. Dashed rectangle shows free area for transmitted light beam.

CoralPor® filled by the electrolyte for separation of the auxiliary electrode (glassy carbon rod electrode, Metrohm-Autolab) from the studied solution, leakless miniature Ag/AgCl reference electrode (eDAQ), working ITO or ITO-AgAP electrode (geometric surface area 1.7 ± 0.1 $cm^2$) and microloader pipette tip for purging and mixing by Argon. Cyclic voltammetry together with chronoamperometry were controlled by potenciostat PalmSens 3 using PSTrace 5 software (both PalmSens). Spectra were registered within 300 and 600 nm in 10 s intervals by Specord 210 Plus (Jena) using software WinASPECT Plus Ver. 4.2.0.0 (both Jena).

Inverted fluorescence microscope (IX83, Olympus) using LED light source (pA-300$^{White}$, CoolLED), CMOS camera (ORCA-Flash 4.0 LT, Hamamatsu) and objective 60 × (UPLFLN, N.A. 0.90, W.D. 0.2, Olympus) was used for imaging of the ITO and ITO-AgAP surfaces with adsorbed fluorescently labeled C.T. DNA. Fluorescence was registered within 605–665 nm using excitation wavelengths within 540–580 nm.

## 3. Results and discussion

### 3.1. Electrochemical behaviour of $Ag^+$ and $Hg^{2+}$ at ITO

Before the electrodeposition of AgAP on ITO, electrochemical behaviour of the $Ag^+$, $Hg^{2+}$ ions and their mixture ($Ag^+$ + $Hg^{2+}$, 40% $w_{Ag}$) were individually studied using cyclic voltammetry (CV) within potential ranges of +0.6 to −1.6 V and +0.6 to −2.0 V (all starting at +0.6 V) at bare ITO (Fig. 2). Solution containing high concentrations