

# Selection of a Bismuth Film Electrode for Simultaneous Voltammetric Detection of Heavy Metals as the Final Step of an Immunoassay with the Quantum Dots

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## Abstract

Solid glassy carbon (GCE), metal disk, and commercial screen-printed electrodes (SPEs) were tested as suitable substrate(s) for deposition of bismuth films. The respective bismuth film electrodes were then used for the simultaneous voltammetric detection of selected heavy metals that had simulated the dissolved quantum dots of the immunoassays known as QLISA. According to the results obtained, it has been found that selected commercial SPEs are not suitable electrode substrates due to a parasitic anodic peak at DRP-150 and DRP-250AT, having significantly increased the background current under repetition of the same measurement at DRP-110, as well as decomposition of the supporting electrolyte at highly negative potentials at DRP-250BT. Unlike metal disk electrodes, GCE coated with thin bismuth film was applicable up to  $-1.5$  V.

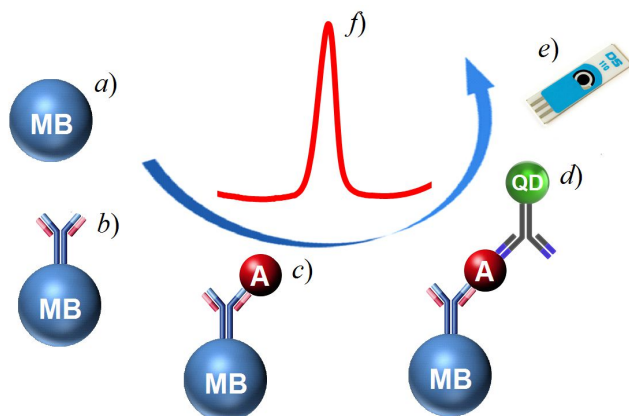
**Key words:** Stripping voltammetry, Bismuth film electrodes; Quantum dots; Heavy metals

## Introduction

Immunoassays can be defined as biochemical tests which are used for analytical purposes. These immunochemical techniques are based on specific reactions of antigenic (A) determinant with the binding site of the antibody (Ab) which can be differently labelled for the final instrumental detection<sup>1</sup>. Nowadays, the immunoassay utilizing an enzymatic labelling of antibody (*Enzyme-Linked ImmunoSorbent Assay*; ELISA) is already widespread in many areas of medicine<sup>2,3</sup>.

Analogically, novel labelling of antibody by homogeneous semiconductor nanocrystal<sup>4</sup>, known as the quantum dot (QD), was developed for luminescence measurements (*Quantum dot-Linked ImmunoSorbent Assay*; QLISA). From a chemical point of view, heavy metal-free colloidal QDs are binary compounds (CdSe, CdTe, PbS etc.) or their combination (InP/ZnS, CuInS/ZnS, CdSe/ZnS etc.); both being usually used in immunology for fluorescence detection<sup>5</sup>. It is evident that some sensitive voltammetric method for electrochemical determination of HMs (in the form of dissolved QDs) could potentially be used as the QLISA final step. The principles of the QLISA modified by magnetic beads (MB) with electrochemical detection are illustrated in Fig. 1, where the MB displayed serves for (pre-)concentrating and washing the immune complex formed<sup>6</sup>.

Several electrochemical methods based on anodic stripping voltammetry (ASV) at metallic film electrodes (consisting of Hg<sup>7</sup>, Bi<sup>8</sup> and Sb<sup>9</sup>) have already been described by our scientific group. These methods, in connection with differential pulse voltammetry (DPV) or square wave voltammetry (SWV) as the electrochemical techniques of choice, usually offer a very high sensitivity, which is, in general, necessary for all immunoassays. Herein, various ways of preparation of the bismuth film electrode (BiFE) have been examined in order to simultaneously detect (and determine) selected heavy metals (HMs) simulating the dissolved QDs.



**Fig. 1.** Principles of QLISA with electrochemical detection; *a)* MB (alone), *b)* MB with bounded Ab, *c)* binding of antigen, *d)* binding of Ab labelled by QD, *e)* dissolution of QD, and *f)* electrochemical detection.

### Experimental

For atomic spectroscopy standards solutions of heavy metal ions ( $\text{Ag}^+$ ,  $\text{Bi}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{In}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Tl}^+$ , and  $\text{Zn}^{2+}$ ) with concentration  $1000 \pm 1$  ppm were purchased from Sigma Aldrich. Chemicals of analytical purity, such as 35% hydrochloric acid, 99.8% acetic acid and sodium acetate needed for preparation of the supporting electrolytes were from Lach-Ner (Neratovice, Czech Republic). Ultrapure water ( $\rho = 18.3 \text{ M}\Omega \text{ cm}$ ) obtained by passing of the already deionized water again through a purification unit (Milli-Q system, Millipore) was used throughout the experimental work.

Various commercial carbon (types DRP-150 and DRP-110) and gold (types DRP-250AT and DRP-250BT) screen-printed electrodes (SPEs) from DropSens (Asturias, Spain), home-made SPCE based on carbon ink type C10903P14 from Gwent Group (Pontypool, United Kingdom), the glassy carbon electrode (GCE) and metal disk electrodes (PtE and AuE) from Metrohm (Prague, Czech Republic) were tested as potentially suitable electrode substrate(s) for deposition of the bismuth film.

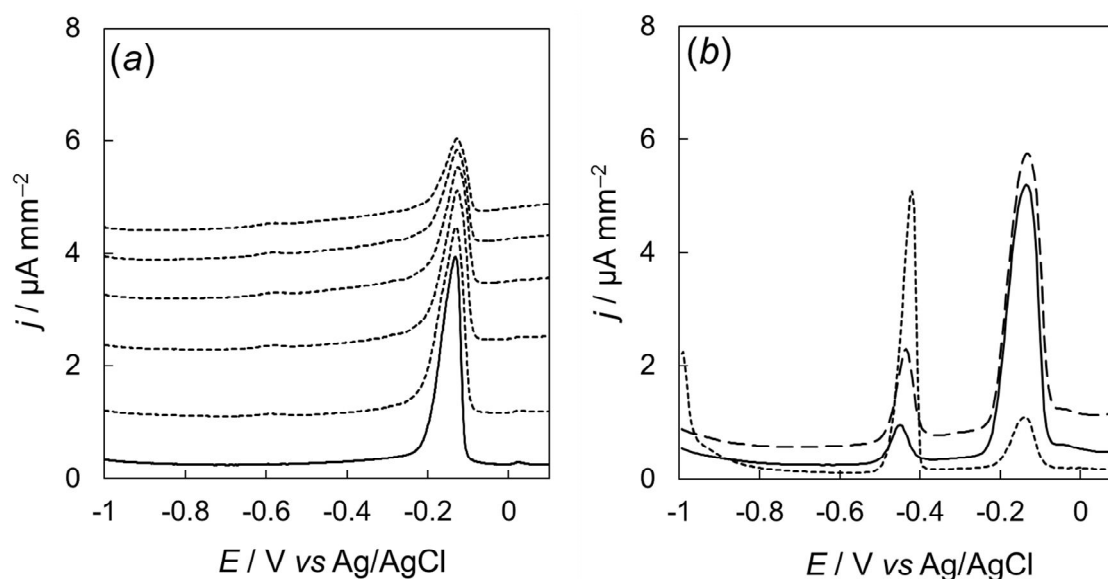
The ASV mode was carried out at conventional electrode setting consisting always one of the working electrodes tested, together with the reference ( $\text{Ag}/\text{AgCl}$  and 3.0 M KCl as salt bridge) and auxiliary electrode (Pt-wire). The resultant three-electrode system (or screen-printed electrode) was connected to a potentiostat (model "Autolab PGSTAT-101"; Metrohm Prague) being operated via the Nova software (Prague, Czech Republic).

All the BiFEs were prepared by plating of bismuth film(s) together with selected heavy metal standards when using an accumulation step at different potentials (varied within  $-0.8$  to  $-1.5 \text{ V}$  vs. ref.) for 120 s. Subsequently, an equilibrium period ( $t_{\text{eq}}$ ) was applied and kept for 10 s, whereas the following SWV scan was run from the deposition potential ( $E_{\text{dep}}$ ) chosen up to  $+1.0 \text{ V}$  vs. ref. at a pulse amplitude ( $E_{\text{ampl}}$ ) 25 mV, potential step / increment ( $E_{\text{step}}$ ) 5.0 mV and a frequency ( $f$ ) of 25 Hz. Otherwise, each change in the experimental conditions described above is specified below, in the legends of the corresponding figures.

### Results and Discussion

Generally, antibodies can be labelled by various kinds of quantum dots differing in the composition of HMs. Therefore, several ovarian tumor markers could be simultaneously

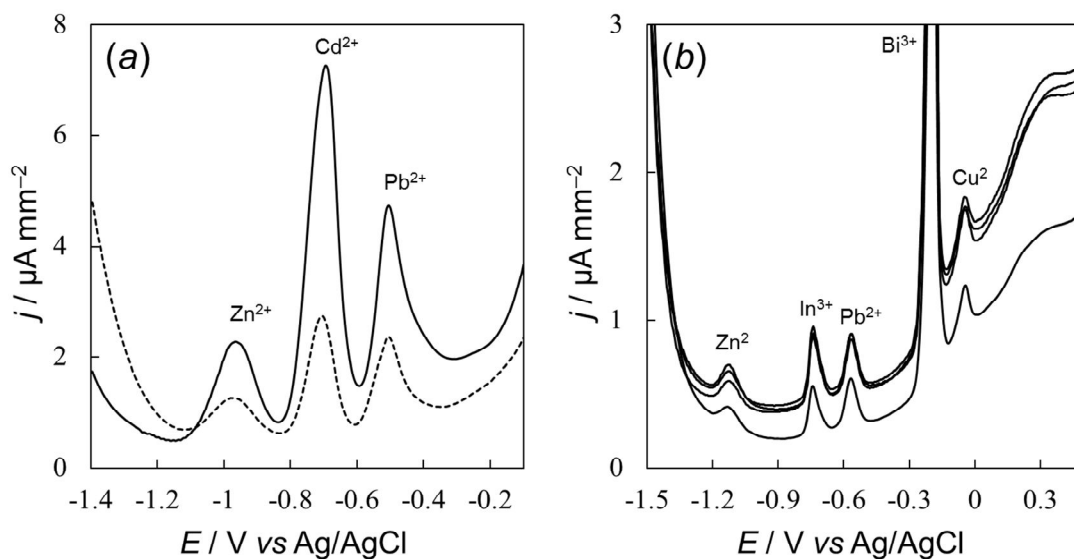
determined only during one analysis using QLISA with electrochemical detection. Firstly, it was necessary to know electrochemical behavior of all the bismuth film electrodes tested in 0.1 M acetate (pH 4.5) buffer. During these controlling measurements, it was observed that each used commercial SPE had shown significant deficiencies. For example, SPCE type DRP-110 was suitable only for disposable application due to the increase of negative background current after each replicate of the ASV scan (see Fig. 2a). Parasitic anodic peak at  $-0.45$  V was obtained at all the SPEs tested, such as DRP-150, DRP-250AT and DRP-250BT. From voltammograms in Fig. 2b, it is clear that the respective peak current response increased with the number of repeated measurements. Thus, it can be deemed that it has been caused by low stability of corresponding electrode materials during deposition step.



**Fig. 2.** ASV at SPCEs type DRP-110 (a) and DRP-150 (b) in 0.1 M acetate (pH 4.5) buffer, where solid lines present the first measurements, dashed line (the second repetition; b) and dotted lines (sequent repetitions). Conditions were set as follow: 5 ppm  $\text{Bi}^{3+}$ ,  $E_{\text{cond}} = +0.5$  V for 60 s,  $E_{\text{dep}} = -1.0$  V for 120 s,  $t_{\text{eq}} = 10$  s,  $E_{\text{step}} = 5$  mV,  $E_{\text{ampl}} = 25$  mV and  $f = 25$  Hz.

Also metal-disk electrodes, namely platinum (PtE) and gold (AuE), were also used for preparation of the corresponding BiFEs. Regarding the experiments, a decomposition of the electrolyte was observed with the Pt-BiFE for values of deposition potentials more negative than  $-0.6$  V vs. ref. Slightly better results were obtained at Au-BiFE, which was suitable for the detection of  $\text{Zn}^{2+}$  due to the stable behavior in the electrolyte at voltages lower than  $-1.4$  V vs. ref. However, the Au-BiFE exhibited rather low sensitivity and thus, it could be used only at relatively high concentrations of bismuth (see Fig. 3a).

Unlike other electrode substrates, a satisfactory sensitivity for QLISA was obtained only at the glassy carbon electrode plated with bismuth film (BiF-GCE) for the concentration of 200 ppb  $\text{Bi}^{3+}$  and deposition potential at  $-1.5$  V vs. ref. for 120 s. Unfortunately, a passivation of GCE surface was revealed and so poisoned surface could not be satisfactorily regenerated even by applying of conditional positive potential  $+1.0$  V vs. ref. at 400 rpm for 300 s. Thus, a mechanical polishing of electrode surface using alumina powder had to be done after each measurement to achieve the repeatability higher than 95%. This is confirmed by replicate ASV scans at GC-BiFE which is shown in Fig. 3b.



**Fig. 3.** ASV of 1.0 ppm  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  at Au-BiFE (dashed line) and GC-BiFE (solid line) in 0.1 M acetate (pH 4.5) buffer at following conditions: 5 ppm  $\text{Bi}^{3+}$ ,  $E_{\text{cond}} = +0.5$  V for 120 s,  $E_{\text{dep}} = -1.4$  V for 240 s,  $t_{\text{eq}} = 10$  s,  $E_{\text{step}} = 5$  mV,  $E_{\text{ampl}} = 25$  mV and  $f = 25$  Hz (a). Four ASV repetitions of 10 ppb  $\text{Zn}^{2+}$ ,  $\text{In}^{3+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  at GC-BiFE prepared from 200 ppb  $\text{Bi}^{3+}$  at  $E_{\text{dep}} = -1.5$  V for 120s,  $t_{\text{eq}} = 10$  s,  $E_{\text{step}} = 4$  mV,  $E_{\text{ampl}} = 25$  mV and  $f = 15$  Hz (b).

### Conclusions

From the results obtained, it can be concluded that only the standard GCE was suitable electrode material for preparation of the corresponding BiFE which could offer a satisfactory sensitivity for the desired purpose – simultaneous electrochemical detection of selected HMs as a simulation of soluble QDs. However, a combination of QDs containing Cd, In and Tl could not be used due to their close peak potentials. Last but not least, also a distinct interaction between the heavy metals examined, especially when being further combined with copper, was also ascertained and so far not satisfactorily solved. From these observations, it is therefore evident that, there is still a lot to improve and our following studies will be directed in this respect in order to develop indeed reliable QLISA with electrochemical detection.

### Acknowledgment

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