Quantum Dots-based Model System for Simultaneous Electrochemical Immunoanalysis of Clinical Biomarkers

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Abstract

Rapid and sensitive detection of significant clinical biomarkers are currently of great importance. The widely used analytical methods for their detection are based on enzyme-linked immunosorbent assays (ELISA). However, they have limitations in simultaneous detection of panel markers within one analysis. However quantum dots (QDs) represent recently new way in antibodies labeling and in connection to electrochemical SWASV detection are capable tags for immunoanalytical assays. Mixture of quantum dots made of three different materials for potential simultaneous detection of protein biomarkers and detected electrochemically is presented. The results obtained with mercury film and "mercury free" bismuth film electrodes are compared.

Key words

Quantum dots, electrochemical detection, screen-printed electrodes, mercury film, bismuth film.

Introduction

Reliable early stage diagnosis of cancer diseases is often based on detection of panel of biomarkers, often proteins. Currently, the detection of such biomarkers is based on immunoanalytical methods, where specific antibodies are involved. Most of these methods for biomarker (antigen) quantification require labeled antibodies (also known as conjugate). Enzymes, fluorophores and nanoparticles belong to the big group of possible options of labels [1]. The properties of selected label have an outstanding influence on final response affecting the limit of detection. Nevertheless, use of above mentioned labels run into limitations when utilized in simultaneous detection of multiple biomarkers.

Quantum dots are semiconductor nanocrystals, mostly synthesized from heavy metals (such as Cd, Pb, In, Ga, etc.) as compounds with Te, Se or P [2], which can be ranked into group of potential labels of antibodies. Apart from their specific optical properties, they have excellent potential for electrochemical detection. The electrochemical analysis of QDs is based on detection of above mentioned heavy metals compounds of, which can be easily detected by well-known electrochemical methods. The sensitivity is also boosted in connection with mercury based electrodes or with "mercury free" bismuth film electrodes[3,4] and hence enabling detection of low levels of target analytes. Moreover, regarding their varying composition resulting in peaks at different defined potential they are proper labels capable of simultaneous detection (see Fig. 1).

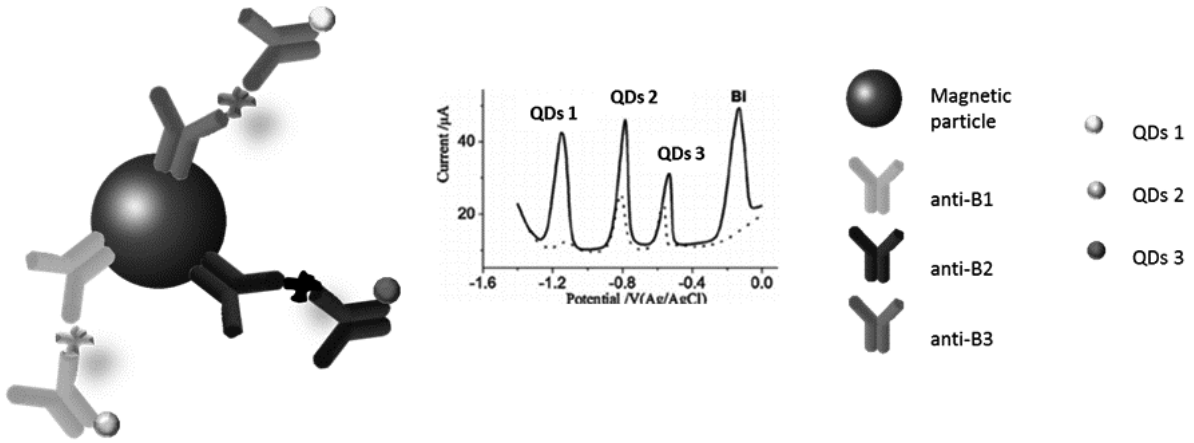

Fig. 1. Scheme of simultaneous electrochemical quantum dots-linked magneto-immunosorbent assay (QLISA) for 3 target analytes (B1, B2, B3).

The detection of one type of quantum dots were already described and can served for universal detection of any protein biomarker by the mere change in specificity of used antibodies. Unique options of quantum dots in simultaneous detection of multiple analytes could be utilized e.g. in analysis of panel of cancer biomarkers, moreover mutually complementing in their sensitivity and specificity. This type of analyses are currently fundamental for reliable cancer detection [5], especially important for early stage of disease [6,7]. In this contribution we present the combination of three types of quantum dots representing potential labels of antibodies for simultaneous target biomarkers detection.

Experimental

All electrochemical measurements were performed with portable electrochemical interface PalmSens (PalmSens, The Netherlands) controlled by software PSTrace 4.7 and connected to the three electrodes screen printed sensors with bismuth film (DropSens, Spain) or mercury film (ItalSens, Italy) modified carbon working, a platinum auxiliary and a silver pseudoreference electrode.

CdSe/ZnS carboxyl modified quantum dots were obtained from Invitrogen (USA), InP/ZnS and PbS carboxyl modified quantum dots were from Mesolight (USA). All other chemicals were form Penta (Czech Republic) and were of reagent grade.

Quantum dots electrochemical detection

Quantum dots were detected as in our previous study [8]. Briefly, the incubation of quantum dots with 0.1M HCl was performed for 3 min at room temperature at final volume 50 µl and result solution of quantum dots was transferred to the surface of electrodes.

Square wave anodic stripping voltammetry (SWASV) was elected as detection method with previously optimized parameters. Each mercury film screen printed sensors required extra conditioning before use, which was done by running method in 20 mM HCl (50 µl) with condition potential -0.15 V for 45 s; deposition potential at -1 V for 300 s; potential range from -1 to -0.15 V; amplitude 28.5 mV and frequency 25 Hz, and followed by 10 cycles under conditions: condition potential -0.15 V for 45 s; without deposition step, potential range from -1 to -0.15 V;

amplitude 28.5 mV and frequency 15 Hz. The surface of electrode was than cleaned by distilled water and prepared for sample measurement.

Samples were detected in 0.1M HCl (50µl) for mercury film electrodes and samples for bismuth film electrode detection were prepared as mixture of 0.1M acetate buffer pH 4.5 containing 500 ppb Bi^{3+} and 0.1 M HCl. Measurements were performed under conditions: condition potential -0.15 V for 45 s; deposition potential at -1 V for 180 s; potential range from -1 to -0.15 V (from -1 V to 0 V in case of bismuth film electrode); amplitude 28.5 mV and frequency 25 Hz.

Results and discussion

The main goal of our work was to find at least two quantum dots applicable as suitable candidates for labeling antibodies and undoubtedly optimize their simultaneous electrochemical detection.

Commercially available core-shell quantum dots CdSe/ZnS, InP/ZnS and core-type PbS quantum dots were elected as possible convenient labels of specific antibodies for simultaneous detection of target biomarkers of ovarian cancer. All quantum dots were functionalized for possible conjugation with antibodies and also to improve solubility in aqueous medium.

For electrochemical detection we used screen-printed three electrode sensors with SWASV as detection method. Since electrochemical analysis of number of heavy metals in one sample at the same time can have impact to the selectivity and sensitivity we used sensors with two modifications of working electrode. Mercury film modified screen printed sensors were elected as sensors of first choice considering well known electrochemical detection of traces of heavy metals. The results measured with mercury film sensors were successfully compared to the bismuth film electrodes.

The combination of CdSe/ZnS and PbS quantum dots can be detected using both types of modified sensors (see Fig. 2). The InP/ZnS quantum dots are stored in solution of surfactants, which influence the response, mainly in case of bismuth film electrodes. However, this effect was reduced at low concentration. Also the shift in peaks potential was observed. While peak resolution measured with mercury film electrode was appropriate, peaks of InP/ZnS quantum dots measured by bismuth film electrode were hidden in high peak of PbS quantum dots.

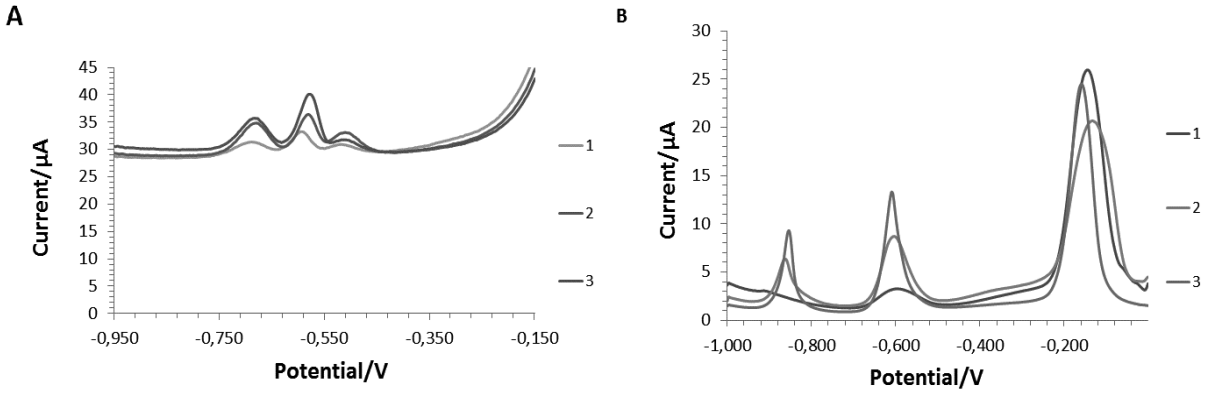

Fig. 2. SWASV voltammograms of CdSe/ZnS, InP/ZnS and PbS quantum dots measured on mercury film electrode (A) and bismuth film electrode (B).

Conclusion

All tested quantum dots can be detected by three electrode screen-printed sensors with mercury and bismuth film electrodes. However, simultaneous detection of all CdSe/ZnS, InP/ZnS and PbS quantum dots can be easily done by mercury film electrode, while under above mentioned conditions only CdSe/ZnS and PbS quantum dots can be detected by bismuth film modified electrodes. Nevertheless, all chosen quantum dots are suitable for coupling with specific antibodies, which meet requirements for conjugate creation for sensitive biomarker detection.

Acknowledgement

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