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Biosensor Array Based on the In-Situ Detection of Quantum Dots as Electrochemical Label

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ABSTRACT

A simple, fast, and inexpensive miniaturized electrochemical biosensor array with in-situ detection of CdSe/ZnS quantum dots (QDs) is presented.

This biosensor uses the affinity reaction between streptavidin and biotin, for biotin determination, but the methodology could be extended to other clinical biomarkers. Individual screen-printed carbon electrodes (SPCEs) and 8-channel screen-printed carbon electrochemical arrays (8xSPCEs) were modified with streptavidin and its analytical performance was evaluated using QDs labeled biotin (biotin-QD). After the biological reaction, Cd^{2+} ions released from the dissolution of the nanoparticles were determined in-situ by voltammetric stripping. A linear range of $5x10^{-10}$-$5x10^{-9}$ M and a limit of detection of $2x10^{-10}$ M (in terms of QD) were achieved for SPCEs (5.0% RSD, n=3). For 8xSPCEs, a linear range of $1x10^{-9}$-$1.2x10^{-8}$ M and a limit of detection of $3x10^{-10}$ M (in terms of QD) were obtained (8.5% RSD, n=3). A one-step competitive bioassay between biotin and biotin-QD was carried out using the biosensor array. A linear range of two orders of magnitude ($1x10^{-9}$-$1x10^{-7}$ M) and a limit of detection of $1.4x10^{-9}$ M were achieved for biotin (6.4% RSD, n=3).

KEYWORDS: Biosensor, Electrochemical array, Nanotechnology, Quantum dot, Screen-printed electrode.

INTRODUCTION

Quantum dots (QDs) are nanostructured semiconductor materials discovered in 1981 [1]. They have an approximately spherical shape with a size typically between 1-12 nm that provides unique optoelectronic properties due to the quantum confinement effect [2]. Their exceptional characteristics have been extensively studied by different techniques [3,4].

Typically, QDs have a core or core-shell structure. Former ones consist of only one semiconductor while the last ones, besides of the core, have an outer layer formed by another semiconductor. The outer layer has positive effects in the nanoparticle: protection of the core against oxidation and releasing of ions, increase of photostability and improvement of QDs surface defects [5]. QDs can be covalently bound to biomolecules, such as peptides, oligonucleotides, and proteins for different applications. Several methods of synthesis and modification of QDs have been developed in the recent years [6,7]. The small dimensions of QDs and the easy modification of its surface with biomolecules make possible their use as labels for bioassays [8]. QDs have generated great interest for optical biosensing because the size-controlled luminescence [9,10]. Compared to other labels, QDs are more stable and cheap and have an important versatility because the different materials and sizes available. The use of QDs instead of enzymatic labels may save a significant amount of analysis time because the enzymatic reaction can be avoided and may save costs because the enzymatic substrates are not necessary.

Wang et al. published the first studies employing core QDs as electrochemical label [11–13]. This procedure consisted on dissolving the semiconductor nanoparticles by acid attack for releasing metal ions, after performing a bioassay in microplates. Those metallic ions are easily determined by anodic stripping voltammetry after a final step of solution transfer to an electrochemical cell with a mercury film glassy carbon electrode. The signal measured is proportional to the amount of analyte in the bioassay. Synthesis of core QDs with several semiconductor materials (PbS, CdS, CuS, ZnS) opened the ability to perform multiplexed electrochemical bioassays [14,15]. Although in most of the works core QDs as electrochemical label are employed, the use core-shell QDs has advantages,

as they have a higher stability than core QDs and less metallic ions are lost from the core [5]. Pinwattana et al. used CdSe/ZnS quantum dots for electrochemical determination of phosphorylated bovine serum albumin [16]. Moreover, the core-shell QDs may be synthesized with different sizes. Bigger QDs contain a higher amount of metal atoms and for applications that require a high sensitivity, bigger QDs could be employed.

However, those works have some drawbacks such as the use of mercury film electrodes and conventional instrumentation with high volume electrochemical cells. Nowadays, the use of mercury is highly discouraged due the toxicity of this metal. Bismuth film electrodes are replacing mercury in heavy metal analysis [17]. Du et al. employed a glassy carbon electrode with a bismuth film for determination of neutravidin, using CdTe nanoparticles as label, after performing a bioassay on a gold surface [18].

An advantage of electrochemical instrumentation is that it can be miniaturized for fabricating portable point-of-care tools. One example of this miniaturization are the screen-printed electrodes. These devices are easy to use, require low sample volumes, allow an easy surface modification, are low cost and can be used as disposable devices [19]. Due to these features, the use of screen-printed electrodes as transducers of electrochemical biosensors is drastically increasing in the last years. Screen-printed electrodes have also been used to detect electrochemically cadmium ions from quantum dots in bioassays, but only after a final step where the solution is transferred to the electrode from the bioassay container [20,21]. In these works, screen-printed electrodes are not used as biosensor transducers but only as the measurement platform. The use of screen-printed electrodes as transducers is interesting because it simplifies highly the methodology approaching to the ideal point-of-care device. To the best of our knowledge, the use of screen-printed electrodes as transducers of biosensors detecting the QDs in the same electrode surface where the biological reaction takes place has not been reported.

QDs have shown great potential for the electrochemical detection of biomolecules, but more research should be done in order to use this labeling system in point-of-care devices. Firstly, in the

published studies, the electrode is not used as sensing surface, the biological reaction is performed outside the electrode, and therefore, those bioassays are not strictly considered biosensors because the transducer is only used for measuring. On the other hand, Merkoçi et al.[22] measured CdS QDs directly on screen-printed electrodes, but the sensitivity obtained is rather low, probably due to the low availability of cadmium ions in solution because they do not destroy the QDs with an acid attack. A direct detection of QDs using screen-printed electrodes as sensing surface of a biosensor remains to be published.

In this work it is designed a sensitive electrochemical biosensor for biotin using CdSe/ZnS QDs as label. This biosensor is based in the affinity reaction between streptavidin and biotin, with in-situ detection of nanoparticles, on the surface of disposable 8-channel electrochemical screen-printed arrays (8xSPCEs) modified with a bismuth film.

MATERIALS AND METHODS

Apparatus and electrodes

Voltammetric measurements were performed with a µStat 8000 (DropSens, Spain) potentiostat interfaced to a Pentium 4 2.4 GHz computer system and controlled by DropView 8400 1.0 software. All measurements were carried out at room temperature. Screen-printed carbon electrodes (SPCEs) were purchased from DropSens (Spain). These electrodes incorporate a conventional three-electrode configuration, printed on ceramic substrates (3.4 x 1.0 cm). Both working (disk-shaped 4 mm diameter) and counter electrodes are made of carbon inks, whereas pseudoreference electrode and electric contacts are made of silver. These electrodes have an electrochemical cell of 50 µL. 8-channel screen-printed electrochemical arrays were purchased from DropSens (Spain). Each array is formed by eight 3-electrode electrochemical cells (30 µL volume) with carbon-based working and counter electrodes, whereas pseudoreference electrodes and electric contacts are made of silver. This device has dimensions of 4.0 x 7.9 x 0.06 cm (length x width x height) and the diameter of the working electrodes is 2.56 mm. **Figure 1A** shows an image

of the electrochemical array. The SPCEs and 8-channel arrays were connected to the potentiostat through specific connectors, DRP-CAST1X8 and DRP-CAST8X, respectively. An Elmasonic P ultrasonic bath (Elma GmbG, Germany) was also employed to disperse the nanomaterials in the solution. A JEOL 6610LV scanning electron microscope (30 kV, Japan) was used to characterize the working electrodes.

Reagents and solutions

Carboxyl modified multiwalled carbon nanotubes (MWCNTs) were purchased from Nanocyl (Belgium, ref. 3151). Carbon nanofibers (CNFs) and Graphene oxide (GO) were kindly provided by Grupo Antolín (Spain) and Nanoinnova Technologies (Spain), respectively. Bismuth(III) nitrate, cadmium(II) acetate, sodium hydroxide, sulfuric acid (97%), acetic acid (100%) nitric acid (65%), fuming hydrochloric acid (37%) and N,N-dimethylformamide (DMF) (99.8%) were purchased from Merck. Tris(hydroxymethyl)aminomethane (Tris), bovine serum albumin fraction V (BSA), streptavidin (molecular weight, 66 kDa), antimony(III) chloride and d-biotin were purchased from Sigma-Aldrich (Spain). Qdot® 655 Biotin Conjugate (biotin-QD) was purchased from Life Technologies (Spain). Ultrapure water obtained with a Millipore Direct Q5™ purification system from Millipore Ibérica S.A. (Madrid, Spain) was used throughout this work. All other reagents were of analytical grade. Working solutions of streptavidin, biotin, BSA, and biotin-QD were made in 0.1 M pH 7.2 Tris-HNO_3 buffer. MWCNTs solution was prepared by mixing 1 mg of MWCNT-COOH with 1 mL of a mixture DMF:water (1:1) by sonication using an ultrasonic bath for 2 h. A dilution of this solution was made for a final concentration of 0.1 g L^{-1} by sonication for 30 minutes. Graphene oxide and carbon nanofibers solutions were made in water using the same procedure. Unless indicated otherwise, a solution of $5x10^{-9}$ M (in terms of QD) of biotin-QD was employed for the optimization of the biosensor.

Modification of screen-printed electrodes with nanomaterials

The modification of SPCEs with carbon nanotubes was carried out following a method developed by Fanjul-Bolado et al [23]. Modification of SPCEs with graphene oxide or carbon nanofibers was carried out by depositing an aliquot of 10 µL of the graphene oxide or carbon nanofibers dispersion (0.1 g L^{-1} in water) on the working electrode surface. The solution was left at room temperature (20°C) until dryness. Modified electrodes were carefully washed with water and stored at room temperature.

Immunoassay procedure

An aliquot of 10 µL (4 µL for arrays) of streptavidin solution with an adequate concentration was dropped on the surface of the working electrode and was left overnight. After washing the SPCE with Tris-HNO_3 buffer, a blocking step was carried out placing 40 µL (25 µL for arrays) of BSA blocking buffer (2.0% BSA in 0.1 M Tris-HNO_3) solution for 30 minutes. After another washing step with Tris-HNO_3, 30 µL (25 µL for arrays) of biotin and biotin-QD solution (in Tris-HNO_3) was dropped on the modified electrode for 30 minutes. A last washing step was carried out with ultrapure water. Then, the electrode was connected to the potentiostat for the electrochemical analysis. In **Figure 2**, a scheme of the competitive assay on biosensor array is presented.

Electrochemical measurements

After the biological reaction, 1 µL of HCl 1.0 M was added on the working electrode to release Cd^{2+} from QDs. 40 µL (25 µL for arrays) of 0.1 M acetate buffer solution with 0.5 mg L^{-1} Bi (III) was added. A constant potential of +1.00 V was applied for 60 s to activate the working electrode. Cadmium was preconcentrated on the electrode surface by applying a potential of -1.10 V for 300 s, simultaneously a bismuth film was also formed during this step. The potential was swept from -1.10 V to -0.65 V using differential pulse voltammetry (DPV) with optimized.

RESULTS AND DISCUSSION

For electrochemical biosensor development is critical to optimize the electrode surface. The electrochemical behavior of the analytical signal with different nanostructured surfaces was studied. SPCEs were modified with different carbon nanomaterials: carbon nanofibers, carbon nanotubes and graphene oxide and the measured signal was compared between these nanostructured transducers and the bare electrode. **Figure 3A** shows the typical differential pulse voltammograms for the different transducers using a solution containing 100 µg L^{-1} of Cd^{2+} and 1 mg L^{-1} of Bi(III). Previously the electrode surface was modified with streptavidin and BSA to obtain similar conditions to the real bioassay.

Peak currents obtained for the carbon nanostructured transducers were significantly lower than for the bare screen-printed electrode. This fact can be explained considering the increase of the capacitive current seen in the i-E curves. The modification of the electrode surface with nanomaterials produces a higher adsorption of proteins on the surface,[24] increasing the effect of the electrical double layer, and decreasing the signal/noise ratio. Although in the literature there are many examples where carbon nanomaterials improve electron transfer and sensitivity of different electrochemical sensors [25], in this case, the increment of the capacitive current plays a fundamental role in the analytical signal. Therefore, the best results were obtained with bare electrodes.

Preconcentration of cadmium on the electrode for the stripping step is performed on a metal film. The most common is the use of bismuth [17] and lately, antimony [26], since the analysis is more sensitive with these metals than in bare carbon surface. A study of different metal films (bismuth, antimony, and bismuth/antimony) to obtain the best surface for the preconcentration of cadmium was carried out. Thus, the concentration of ionic metal in solution for the in-situ film formation was studied. The peak current obtained using a solution containing 100 µg L^{-1} Cd^{2+} in acetate buffer (0.1 M, pH 4.5) was compared for different concentrations of Bi(III), Sb(III) and

mixture of Bi(III)/Sb(III). The best results were found to be: 0.5 mg L^{-1} of Bi(III), 5 mg L^{-1} of Sb(III) and for the Bi/Sb mix, 10 mg L^{-1} of Bi(III) and 0.2 mg L^{-1}.

Figure 3B shows that the optimal film for cadmium preconcentration was obtained with a solution containing 0.5 mg L^{-1} of Bi(III). Despite it, the use of Sb or Bi/Sb mixture could be interesting when a more acidic medium is necessary because the main advantage of using a Sb or Bi/Sb film for cadmium detection is that these metals can be used in a strong acidic medium (pH 2), since the reduction of the protons in solution is more difficult than using the Bi film [27]. Therefore, it might be useful for applications where it is needed to use a more acidic medium than it is possible with Bi.

Since the analytical signal is based on the anodic stripping voltammetry of Cd^{2+} ions released from QDs, the more degradation of the nanoparticles is achieved, the more Cd^{2+} ions existing in the solution and consequently, the methodology will be more sensitive. The fastest way to achieve this degradation of QDs is likely with a chemical acid attack. Several acids (HNO_3, H_2SO_4, HCl) and several volumes (1, 2, 5, 10 µL) for different concentrations (0.2, 0.5, 1.0, 2.0 M) of these acids, in combination with several concentrations (0.1 and 0.2 M) and pHs (4.5 and 5.0) of the acetate buffer were tested for breaking up the quantum dots. The highest analytical signals were obtained using 1 µL of 1.0 M HCl and 40 µL of 0.1 M acetate buffer (pH 4.5).

The effect of the time of dissolution of QDs was studied. After dropping 1 µL of HCl 1.0 M on the working electrode, a short time (0, 30, 60, 120 s) was waited before adding the acetate buffer and perform the electrochemical measurement. No significant differences were found between the several times studied. It appears that the dissolution of the nanoparticles occurs almost instantaneously in this acidic medium (HCl 1.0 M).

The activation potential step carried out before the electrochemical deposition of cadmium on the electrode surface was optimized. Several potentials were applied: +0.60 V, +1.00 V and +1.20 V for 60 and 120 s. It was found that after the application of this potential an improvement in the analytical signal was obtained. The highest peak current was obtained after applying +1.00 V

for 60s. In order to know the effect of this activation step, a drop of 10 µL of QDs without biotin (1 nM) was deposited on a bare (without streptavidin coating) electrode and the peak current obtained for the following experiments was measured: 1) neither adding HCl nor applying activation potential as in Merkoçi et al. work [22], 2) without adding HCl but applying the activation potential, 3) adding HCl and without applying the activation potential, 4) adding HCl and applying the activation potential. **Figure 3C** shows DPV signals for the four cases. It can be observed that the addition of HCl is fundamental to get an important release of Cd^{2+} ions to the solution and achieve a more sensitive assay. Moreover, there is a positive effect of the activation potential in the analytical signal because a higher peak is achieved by applying this potential. Therefore, even without protein material adsorbed on the electrode surface, the activation potential improves the analytical signal. This fact could be due to one (or both) of these reasons: for activation of the electrode surface increasing its area or for a higher degradation of the QDs with the consequent release of more Cd^{2+} ions. In a surface with proteins, the application of this potential may also help to desorb the proteins from the surface, improving the sensitivity.

Other basic parameters affecting the affinity reaction as the streptavidin concentration and the time of reaction were optimized. The analytical signal measured for different streptavidin concentrations and reaction times reached a plateau at a concentration of $1x10^{-7}$ M and after 30 minutes of reaction time.

The parameters optimized for the stripping voltammetry were: deposition potential, deposition time, amplitude, step, modulation time and interval time. Between these parameters, the most relevant are deposition time, differential pulse amplitude and modulation time. Even a small change in the values of these parameters has an important effect on the magnitude of the analytical signal and can improve the sensitivity of the method. The optimized values were: -1.10 V for deposition potential, 300 s for deposition time, 0.05 V for amplitude, 0.01 V for step, 0.01 s for modulation time and 0.1 s for interval time.

The characterization of the surface of the working electrode was performed by SEM. There were no visible changes to the surface after performing each step of the bioassay. The presence of bismuth particles after the accumulation step was not obtained by SEM (**Figure 1B**), probably because the amount and size of those particles are very small and are not visible in the images. Coulometric measurements to obtain the deposited amount of bismuth were performed and the result was 10.2 ± 0.5 ng, and considering the surface area it was approximately 2 fg μm^{-2}.

After the optimization of the methodology the designed biosensors were used to perform a calibration with different concentration of biotin-QD. Analytical signal increased with increasing concentrations of biotin-QD. A linear range of $5x10^{-10}$-$5x10^{-9}$ M (in terms of QD) with a correlation (R^2) of 0.998 was obtained (i_p = 0.14 + 1.22 [biotin-QD] (10^{-9} M)). Detection limit of this method was $2x10^{-10}$ M. The reproducibility of the analytical signal obtained is about 5.0 % (n=3) in terms of RSD using different biosensor devices.

Recent developments in potentiostat technology facilitate the electrochemical measurement of several electrodes simultaneously. Within these advances are included the multichannel screen-printed electrode arrays shown in **Figure 1A**. The developed method for individual screen-printed electrodes was applied using an 8-channel screen-printed carbon electrode array. **Figure 4A** shows a calibration plot of increasing concentrations of biotin-QD using 8-channel SPCE arrays. A linear range of $1x10^{-9}$-$1.2x10^{-8}$ M (in terms of QD) with a correlation (R^2) of 0.990 was obtained. A detection limit of $4x10^{-10}$ M was calculated and the reproducibility between arrays was of 8.5 % (n=3) in terms of RSD. The sensibility (slope of the calibration plot) obtained is lower using arrays than individual electrodes and the relation between both slopes is in good agreement with the ratio of the electrode area (5.2 mm^2 vs 12.6 mm^2). Though the analytical characteristics are better using individual electrodes, the benefits using the arrays are evident. The simplicity of the methodology is clear because each step is performed with multichannel pipettes and washing steps are carried out simultaneously. Furthermore, it is noteworthy the advantages of these arrays when the procedure is time dependent, as in most developed electrochemical biosensors. Using arrays it is possible to

perform an exceptional amount of analysis in a short time, thus in situations where it is required to perform several tests, arrays are an excellent option to save costs.

The proper operation of the electrochemical biosensor array was evaluated for biotin determination. A one step competitive assay was performed between biotin and labeled biotin against the binding sites of the streptavidin. The voltammetric signal decreased with the increment of biotin in the sample. A constant concentration of $1.2x10^{-8}$ M of biotin-QD (in terms of QD) was chosen. A linear dependence between i/i_0 (%) and the logarithm of biotin concentration was found ($1x10^{-9}$-$1x10^{-7}$ M) (**Figure 4B**). i_0 was the current obtained in the absence of biotin and i was the corresponding current response when biotin was present. A detection limit of $1.4x10^{-9}$ M for biotin was achieved (5.9% RSD, n=3). The detection limit was calculated as the concentration that gives an analytical signal obtained after subtracting to the maximum signal three times the standard deviation of this signal.

CONCLUSIONS

There is a recent interest in developing miniaturized devices for solving clinical and environmental issues. Within the tools and detection labels being developed, the use of screen-printed carbon electrodes with in-situ detection of quantum dots provides an excellent platform for the development of electrochemical biosensors. The approach presented in this work has clear advantages: a simple and fast procedure (1 hour) using an array that allows to do eight simultaneous analysis and to get a high amount of data in a short time. The array system may be applied for different clinical biomarkers, with the possibility to use several quantum dots for multiplexed biosensing.

Future clinical analysis should be easier and cheaper and with the biosensor array developed we are closer to fulfill those goals.

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REFERENCES

[1] A. Ekimov, A. Onushchenko, Quantum size effect in three-dimensional microscopic semiconductor crystals, ZhETF Pis Ma Redaktsiiu. 34 (1981) 363–366.

[2] H. Weller, Colloidal Semiconductor Q-Particles: Chemistry in the Transition Region Between Solid State and Molecules, Angew. Chem., Int. Ed. 32 (1993) 41–53.

[3] S.K. Haram, B.M. Quinn, A.J. Bard, Electrochemistry of CdS nanoparticles: a correlation between optical and electrochemical band gaps., J. Am. Chem. Soc. 123 (2001) 8860–1.

[4] A.R. Montoro Bustos, J.R. Encinar, M.T. Fernández-Argüelles, J.M. Costa-Fernández, A. Sanz-Medel, Elemental mass spectrometry: a powerful tool for an accurate characterisation at elemental level of quantum dots., Chem. Commun. (Cambridge, U. K.). (2009) 3107–9.

[5] M. Grabolle, J. Ziegler, A. Merkulov, T. Nann, U. Resch-Genger, Stability and fluorescence quantum yield of CdSe-ZnS quantum dots--influence of the thickness of the ZnS shell., Ann. NY. Acad. Sci. 1130 (2008) 235–41.

[6] M.T. Fernández-Argüelles, A. Yakovlev, R.A. Sperling, C. Luccardini, S. Gaillard, A. Sanz-Medel, et al., Synthesis and characterization of polymer-coated quantum dots with integrated acceptor dyes as FRET-based nanoprobes., Nano Lett. 7 (2007) 2613–7.

[7] Y. Xing, Q. Chaudry, C. Shen, K.Y. Kong, H.E. Zhau, L.W. Chung, et al., Bioconjugated quantum dots for multiplexed and quantitative immunohistochemistry., Nat. Protoc. 2 (2007) 1152–65.

[8] A.R. Montoro Bustos, L. Trapiella-Alfonso, J.R. Encinar, J.M. Costa-Fernández, R. Pereiro, A. Sanz-Medel, Elemental and molecular detection for Quantum Dots-based immunoassays: a critical appraisal., Biosens. Bioelectron. 33 (2012) 165–71.

[9] R. Gill, M. Zayats, I. Willner, Semiconductor quantum dots for bioanalysis., Angew. Chem., Int. Ed. 47 (2008) 7602–25.

[10] M.-C. Tu, Y.-T. Chang, Y.-T. Kang, H.-Y. Chang, P. Chang, T.-R. Yew, A quantum dot-based optical immunosensor for human serum albumin detection., Biosens. Bioelectron. 34 (2011) 286–290.

[11] J. Wang, G. Liu, R. Polsky, A. Merkoçi, Electrochemical stripping detection of DNA hybridization based on cadmium sulfide nanoparticle tags, Electrochem. Commun. 4 (2002) 722–726.

[12] J. Wang, G. Liu, G. Rivas, Encoded beads for electrochemical identification., Anal. Chem. 75 (2003) 4667–71.

[13] J. Wang, G. Liu, A. Merkoçi, Electrochemical coding technology for simultaneous detection of multiple DNA targets., J. Am. Chem. Soc. 125 (2003) 3214–5.

[14] G. Liu, J. Wang, J. Kim, M.R. Jan, G.E. Collins, Electrochemical coding for multiplexed immunoassays of proteins., Anal. Chem. 76 (2004) 7126–30.

[15] J.A. Hansen, J. Wang, A.-N. Kawde, Y. Xiang, K. V Gothelf, G. Collins, Quantum-dot/aptamer-based ultrasensitive multi-analyte electrochemical biosensor., J. Am. Chem. Soc. 128 (2006) 2228–9.

[16] K. Pinwattana, J. Wang, C.-T. Lin, H. Wu, D. Du, Y. Lin, et al., CdSe/ZnS quantum dots based electrochemical immunoassay for the detection of phosphorylated bovine serum albumin., Biosens. Bioelectron. 26 (2010) 1109–13.

[17] A. Economou, Bismuth-film electrodes: recent developments and potentialities for electroanalysis, TrAC, Trends Anal. Chem. 24 (2005) 334–340.

[18] D. Du, J. Ding, Y. Tao, H. Li, X. Chen, CdTe nanocrystal-based electrochemical biosensor for the recognition of neutravidin by anodic stripping voltammetry at electrodeposited bismuth film., Biosens. Bioelectron. 24 (2008) 869–74.

[19] P. Fanjul-Bolado, D. Hernández-Santos, P.J. Lamas-Ardisana, A. Martín-Pernía, A. Costa-García, Electrochemical characterization of screen-printed and conventional carbon paste electrodes, Electrochim. Acta. 53 (2007) 3635–3642.

[20] S. Marín, A. Merkoçi, Direct electrochemical stripping detection of cystic-fibrosis-related DNA linked through cadmium sulfide quantum dots., Nanotechnology. 20 (2009) 055101.

[21] D. Zhang, M.C. Huarng, E.C. Alocilja, A multiplex nanoparticle-based bio-barcoded DNA sensor for the simultaneous detection of multiple pathogens., Biosens. Bioelectron. 26 (2010) 1736–42.

[22] A. Merkoçi, L.H. Marcolino-Junior, S. Marín, O. Fatibello-Filho, S. Alegret, Detection of cadmium sulphide nanoparticles by using screen-printed electrodes and a handheld device., Nanotechnology. 18 (2007) 035502.

[23] P. Fanjul-Bolado, P. Queipo, P.J. Lamas-Ardisana, A. Costa-García, Manufacture and evaluation of carbon nanotube modified screen-printed electrodes as electrochemical tools, Talanta. 74 (2007) 427–33.

[24] M.M. Pereira da Silva Neves, M.B. González-García, C. Delerue-Matos, A. Costa-García, Nanohybrid Materials as Transducer Surfaces for Electrochemical Sensing Applications, Electroanalysis. 23 (2011) 63–71.

[25] M.T. Fernández-Abedul, A. Costa-García, Carbon nanotubes (CNTs)-based electroanalysis, Anal. Bioanal. Chem. 390 (2008) 293–298.

[26] E. Tesarova, L. Baldrianova, S. Hocevar, I. Svancara, K. Vytras, B. Ogorevc, Anodic stripping voltammetric measurement of trace heavy metals at antimony film carbon paste electrode, Electrochim. Acta. 54 (2009) 1506–1510.

[27] W.J. Yi, Y. Li, G. Ran, H.Q. Luo, N.B. Li, Determination of cadmium(II) by square wave anodic stripping voltammetry using bismuth–antimony film electrode, Sens. Actuators, B. 166-167 (2012) 544–548.

BIOGRAPHIES

Daniel Martín-Yerga obtained his B.Sc. degree in chemistry, focus on analytical chemistry in 2010 (University of Oviedo) and the M.Sc. degree in analytical and bioanalytical chemistry in 2011 (University of Oviedo). At present, he is a PhD student at the Nanobioanalysis Research Group of the University of Oviedo, supervised by Prof. A. Costa-García.

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Agustín Costa-García obtained his B.Sc. degree in chemistry, focus in analytical chemistry, in 1974 (University of Oviedo) and the Ph.D. in chemistry in 1977 (University of Oviedo). Since February 2000 he is professor in analytical chemistry (University of Oviedo). He leads the Nanobioanalysis Research Group of the University of Oviedo and has been supervisor of several research projects developed at the electrochemistry laboratories of the Department of Physical and Analytical Chemistry of the University of Oviedo. Nowadays his research is focused on the development of nanostructured electrodic surfaces and its use as transducers for electrochemical immunosensors and genosensors employing both enzymatic and non-enzymatic labels.

CAPTIONS OF FIGURES AND TABLES

Figure 1. **A)** Image of the 8-channel screen-printed electrochemical array and the corresponding connector. **B)** SEM image of the working electrode of the biosensor array after the deposition step.

Figure 2. Schematic diagram of the electrochemical biosensor array. The bioassay procedure is carried out using the working electrodes of the array as transducers and in-situ electrochemical detection of QDs.

Figure 3. A) Differential pulse voltammograms for 100 µg L^{-1} of Cd^{2+} and 1 mg L^{-1} of Bi(III) in acetate buffer (pH 4.5) using transducers modified with different nanomaterials: (-) bare electrodes, (-·-·-) carbon nanotubes, (- - -) graphene oxide, and (···) carbon nanofibers. **B)** Differential pulse voltammograms for 100 µg L^{-1} of Cd^{2+} in acetate buffer (pH 4.5) using different metallic films: (-)

0.5 mg L^{-1} of Bi(III), (- - -) 5 mg L^{-1} of Sb(III), and (···) 10 mg L^{-1} of Bi(III) and 0.2 mg L^{-1} of Sb(III). Inset: Mean peak currents (n=3) of the differential pulse voltammograms for the specified metallic films. **C)** Differential pulse voltammograms for 1 nM QDs in acetate buffer (pH 4.5): (-) after adding HCl 1 M and applying an activation potential of +1.00 V for 60 s, (- - -) after adding HCl 1 M but without applying the activation potential, (···) neither adding HCl nor applying an activation potential, and (-·-·-) without adding HCl but applying an activation potential of +1.00 V for 60s.

Figure 4. A) Differential pulse voltammograms and calibration plot for the biosensor array in the presence of different concentrations of biotin-QD (a-f): 1, 2, 4, 7, 10 and 12 $x10^{-9}$ M (in terms of QDs). Inset: calibration plot for the biosensor as a function of biotin-QD concentration. Activation potential: +1.00 V for 60 s. Deposition potential: -1.10 V for 300 s. **B)** Differential pulse voltammograms and calibration plot for the biosensor array recorded in the one step competitive bioassay in presence of different concentrations of biotin (a-f): 0, 1, 5, 10, 50 and $100x10^{-9}$ M. Inset: semilogarithmic plot of the corresponding calibration curve. The parameters of ASDPV were the same as in **Figure 3A**.

Figure 1

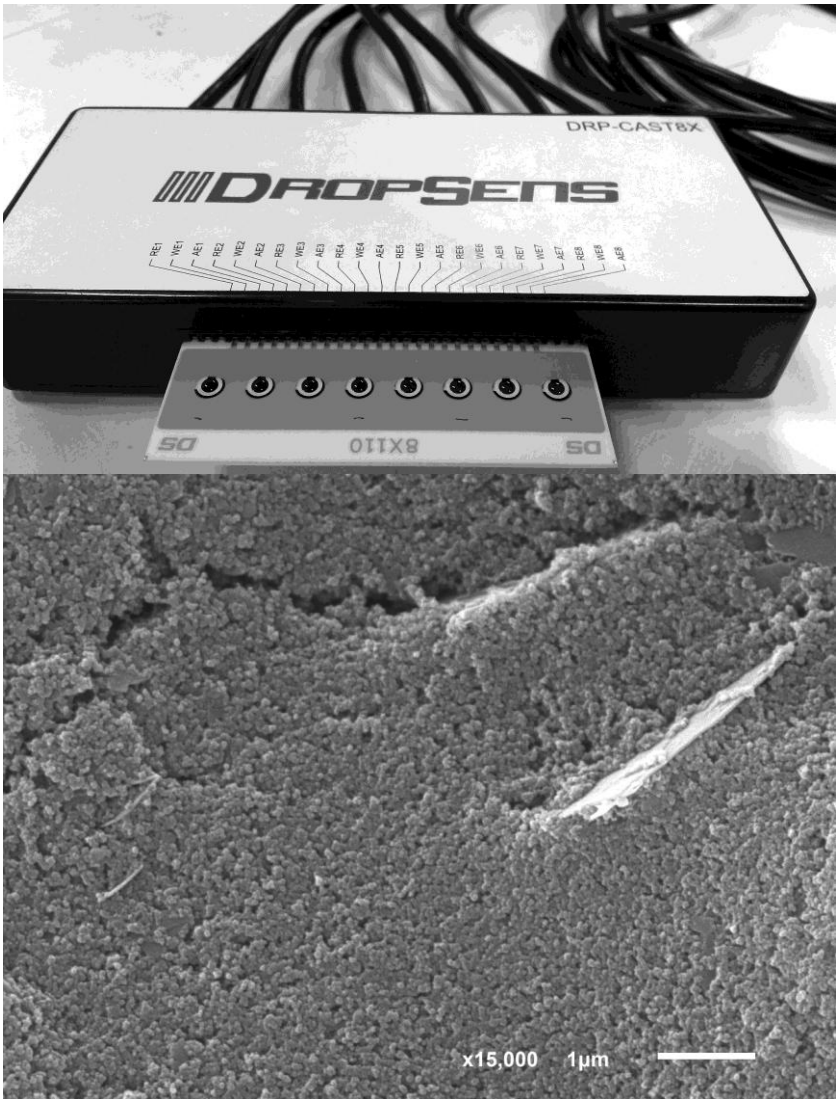

Figure 1. **A)** Image of the 8-channel screen-printed electrochemical array and the corresponding connector. **B)** SEM image of the working electrode of the biosensor array after the deposition step.

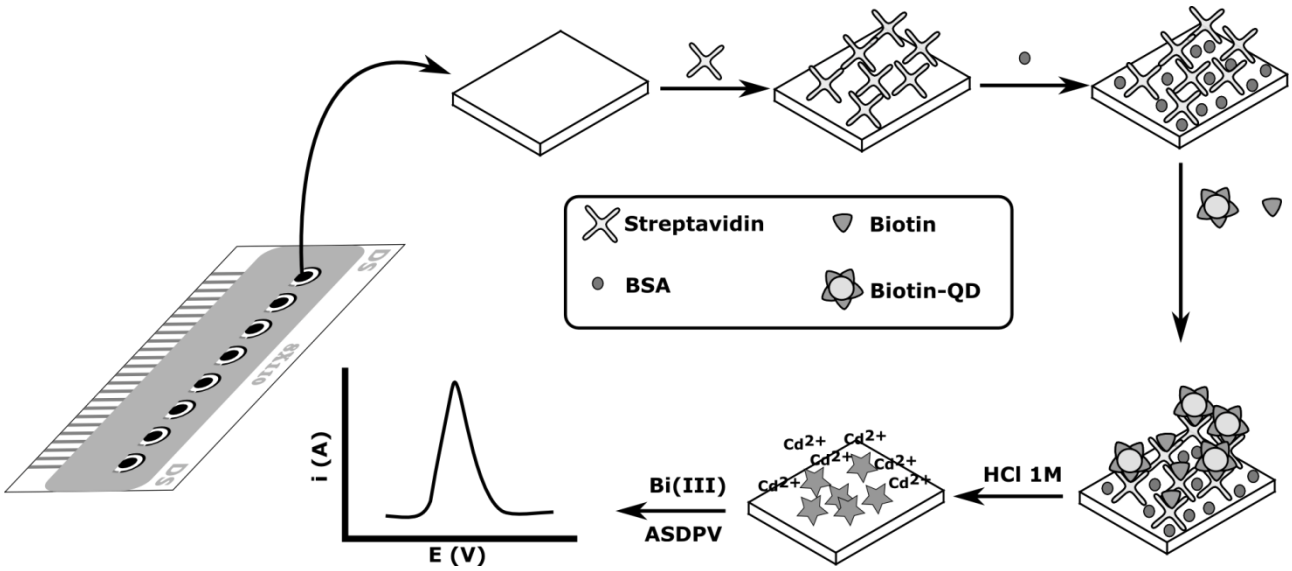

Figure 2. Schematic diagram of the electrochemical biosensor array. The bioassay procedure is carried out using the working electrodes of the array as transducers and in-situ electrochemical detection of QDs.

Figure 3

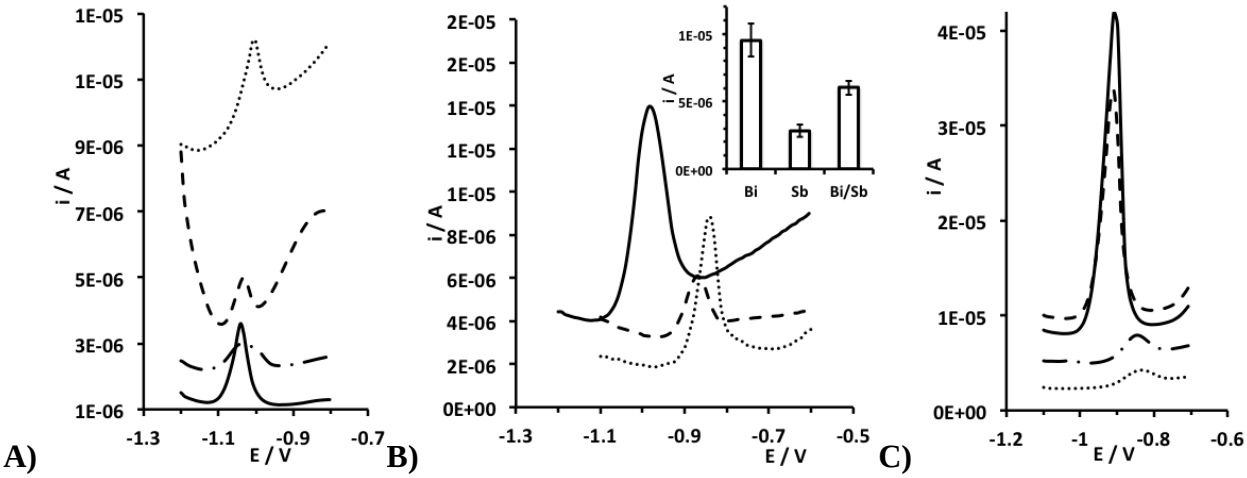

Figure 3. A) Differential pulse voltammograms for 100 µg L^{-1} of Cd^{2+} and 1 mg L^{-1} of Bi(III) in acetate buffer (pH 4.5) using transducers modified with different nanomaterials: (-) bare electrodes, (-·-·-) carbon nanotubes, (- - -) graphene oxide, and (···) carbon nanofibers. **B)** Differential pulse voltammograms for 100 µg L^{-1} of Cd^{2+} in acetate buffer (pH 4.5) using different metallic films: (-) 0.5 mg L^{-1} of Bi(III), (- - -) 5 mg L^{-1} of Sb(III), and (···) 10 mg L^{-1} of Bi(III) and 0.2 mg L^{-1} of Sb(III). Inset: Mean peak currents (n=3) of the differential pulse voltammograms for the specified metallic films. **C)** Differential pulse voltammograms for 1 nM QDs in acetate buffer (pH 4.5): (-) after adding HCl 1M and applying an activation potential of +1.00 V for 60 s, (- - -) after adding HCl 1 M but without applying the activation potential, (···) neither adding HCl nor applying an activation potential, and (-·-·-) without adding HCl but applying an activation potential of +1.00 V for 60s.

Figure 4

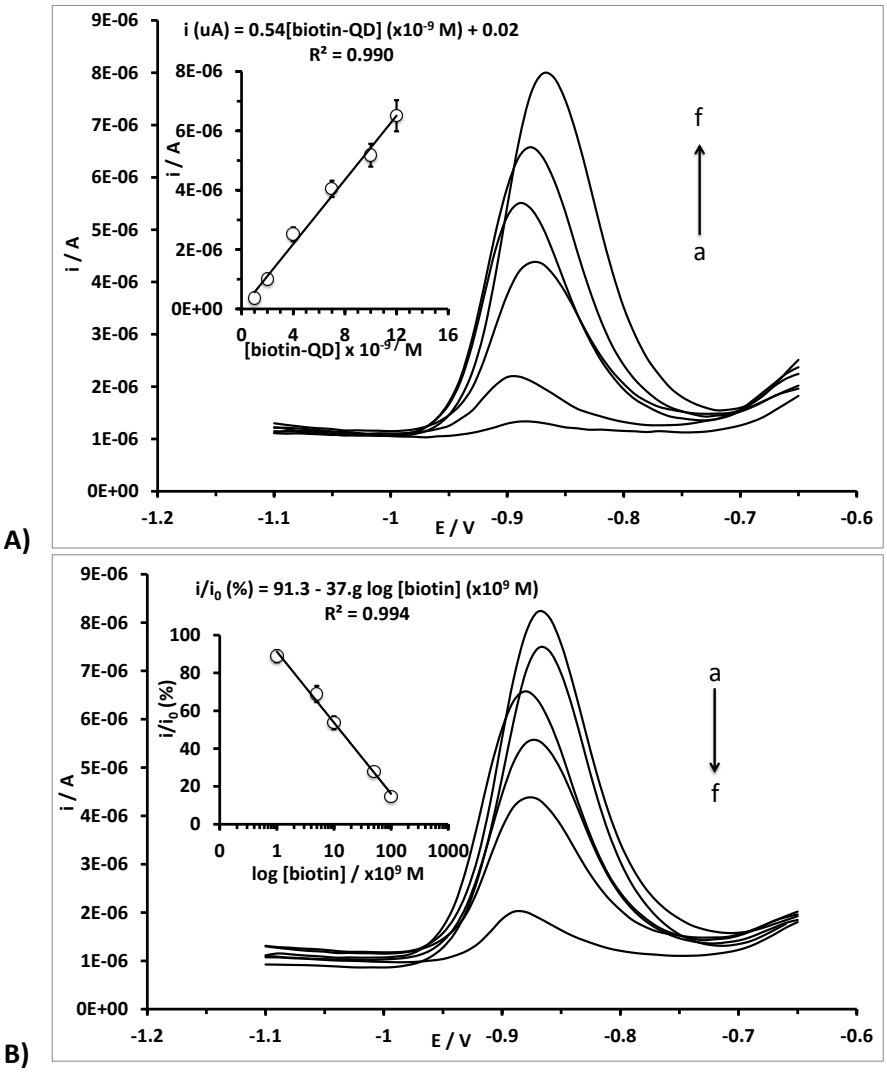

Figure 4. A) Differential pulse voltammograms and calibration plot for the biosensor array in the presence of different concentrations of biotin-QD (a-f): 1, 2, 4, 7, 10 and 12 x10^{-9} M (in terms of QDs). Inset: calibration plot for the biosensor as a function of biotin-QD concentration. Activation potential: +1.00 V for 60 s. Deposition potential: -1.10 V for 300 s. **B)** Differential pulse voltammograms and calibration plot for the biosensor array recorded in the one step competitive bioassay in presence of different concentrations of biotin (a-f): 0, 1, 5, 10, 50 and 100x10^{-9} M. Inset: semilogarithmic plot of the corresponding calibration curve. The parameters of ASDPV were the same as in **Figure 3A**.