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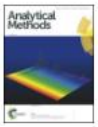

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Competitive electrochemical biosensing of biotin using cadmium-modified titanium phosphate nanoparticles and 8-channel screen-printed disposable electrodes

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Competitive electrochemical biosensing of biotin using cadmium-modified titanium phosphate nanoparticles and 8-channel screen-printed disposable electrodes

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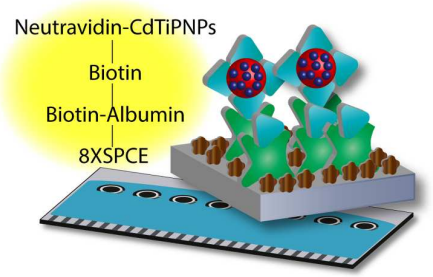

Detection of biotin was performed in multivitamin tablets with a competitive electrochemical biosensor using cadmium-modified titanium phosphate nanoparticles and 8-channel screen-printed electrodes

ABSTRACT

In this work, a method for the detection of biotin was developed using a competitive electrochemical biosensor and cadmium-modified titanium phosphate nanoparticles (CdTiPNPs) as detection label. The electrochemical behaviour of CdTiPNPs was studied and a method for the detection of very low concentrations of these nanoparticles by anodic stripping voltammetry was optimized. After their modification with neutravidin, the nanoparticles were used as labels for the biotin competitive biosensor, which was carried out on the surface of disposable 8-channel screen-printed cards modified with biotinylated albumin. These devices allowed the detection of eight samples simultaneously, and a limit of detection in the order of 1 nM of biotin was obtained. The method was evaluated in real samples by carrying out the determination of biotin in multivitamin tablets. These results show that the utilization of CdTiPNPs can compete in terms of analytical performance with quantum dots as detection label, but the methodology is simpler since the typical acid digestion step to release the metals to the solution is not necessary.

KEYWORDS: Nanoparticle; Titanium phosphate; Disposable biosensor; Screen-printed electrodes; Cadmium

1. INTRODUCTION

Biotin is a water-soluble vitamin also called Vitamin H, B7 or coenzyme R, which is found in small amounts in cells and has a critical function in some metabolic processes, cell growth or citric acid cycle. This vitamin is synthesized by bacteria and plants but cannot be generated by humans, so it must be obtained by dietary sources. Biotin deficiency could cause different health issues[1], especially complicated in children and pregnant women. Therefore, there are numerous products such as infant food or dietary supplements containing biotin[2] and its detection is important in the food industry. Biotin detection[3] is generally carried out using spectroscopic or chromatographic techniques, microbiological assays[4,5] or ELISA[6], among many others. These methods are complex and time-consuming, and some of them require expensive and sophisticated laboratory instrumentation. Therefore, the development of new sensitive and fast methods with cost-effective, portable devices is a constant concern.

Electrochemical biosensors[7,8] are devices with interesting properties such as high sensitivity, selectivity, reliability, cost-effective and portability and are widely employed to solve analytical issues of sectors as diverse as industrial, clinical, agri-food, environmental or forensic. Screen-printed electrodes (SPEs)[9], miniaturized devices fabricated using the thick-film technology, are widely used as transducers of electrochemical biosensors for Point-of-Care (POC) analysis and decentralized monitoring[10,11]. Their great success is due to the good electrochemical performance and the low manufacturing cost, ease of use, small size, disposability and portability. These electrodes have been applied in diverse applications[12–14]. SPEs are very helpful for biosensor development due to the possibility to use dozens of electrodes and perform bioassays almost simultaneously because their low cost compared to conventional electrodes, which leads to a higher number of analyses in the same time. The versatility of the fabrication technique allows to produce SPEs with different designs and multi-detection capabilities, either with several working electrodes sharing auxiliary and reference electrodes[15], or cards with multiple independent 3-electrode electrochemical cells such as the 8-channels cards[16] or the 96-cell electrochemical plates[17]. These

designs allow to perform simultaneous measurements with extraordinary simplicity in portable setups in contrast to conventional electrodes and electrochemical cells. Therefore, these multichannel devices are well placed to solve easily different analytical issues in a cost-effective way with a high frequency of measurements.

Numerous nanoparticles have properties such as biocompatibility or electroactivity, which make them very interesting for electrochemical biosensing. In addition, they generally exhibit greater stability than enzymes, offer high sensitivity, and the wide variety of available nanoparticles opens the door to multi-analyte assays. For these reasons, they have been widely used as label for electrochemical biosensors[18–20] in the last years. Porous nanomaterials, such as titanium phosphates, can be used in different applications exploiting their high surface area, pore size and modifiable structure. For instance, Liu et al.[21] described an easy method for the synthesis of core-shell titanium phosphate nanoparticles (TiPNPs) with high surface area and open nanopores. These nanoparticles can work as ion exchangers and can be employed as detection label in biosensors if indicator species are introduced into their structure. TiPNPs were used as label of electrochemical immunosensors after introducing Cd(II) in their structure by ion-exhange[22]. Conventional glassy carbon electrodes were used to conduct the bioassay and the detection of the metal was performed by square-wave voltammetry. Following the same strategy, TiPNPs modified with Cd(II) and Zn(II) were used for multiplexing detection of biomarkers of heart diseases[23], microRNA[24] or toxins[25]. Some works have described the electrocatalytic characteristics of these nanoparticles as an alternative detection method[26,27]. However, these works have a practical character and the authors did not study the effect of different experimental variables in the detection of TiPNPs. In addition, the use of conventional electrodes would not allow these methods to be applied in setups outside a laboratory: numerous electrodes and electrochemical cells are necessary to perform multiple simultaneous analysis, which is not a practical situation for biosensor development and it is against the current trends in Analytical Chemistry.

In this work, we describe the development of an analytical method for the detection of biotin in multivitamin tablets using a competitive electrochemical biosensor and cadmium-modified titanium phosphate nanoparticles (CdTiPNPs) functionalized with neutravidin as detection label. We carried out a fundamental study of the electrochemical properties of CdTiPNPs with the aim of optimizing an appropriate method for their sensitive detection. Factors such as the use of a bismuth film or the preconcentration of cadmium were evaluated in contrast to previous works using CdTiPNPs lacking these fundamental studies. Disposable 8-channel screen-printed cards were employed as biosensing platform, which serve both to carry out the biological reactions and the electrochemical detection. These cards allow to perform 8 simultaneous analysis and in combination with the possibility to use several cards concurrently led to a cost-effective high-throughput electrochemical method for biotin determination using a low volume of sample (25 μL). This system could allow to save considerable time and cost compared to other previously reported works, in which titanium phosphate nanoparticles were employed with conventional electrode systems.

2. MATERIALS AND METHODS

2.1. Apparatus and electrodes

Electrochemical measurements were performed with a μStat8000 (DropSens) potentiostat/galvanostat interfaced to an Apple Macbook Air laptop and controlled by the DropView 8400 2.2 software. 8-channel screen-printed electrochemical arrays (SPCEs) were purchased from DropSens (ref. 8X110). Each array is formed by eight 3-electrode electrochemical cells with carbon-based working and counter electrodes, whereas quasireference 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 circular working electrode is 2.56 mm. 8-channel arrays were connected to the potentiostat through a specific connector, DRP-CAST8X. All measurements were carried out at room temperature and using an aliquot of 25 μL of the appropriate solution. All reported potentials are versus the silver quasireference screen-printed electrode. The high-resolution

transmission electron micrographs (HRTEM) and Energy-dispersive X-ray spectroscopy (EDS) data were obtained on a JEOL JEM 2100 transmission electron microscope with an accelerating voltage of 200 kV.

2.2. Reagents and solutions

Bismuth(III) standard, Cadmium(II) standard, cadmium nitrate, bovine serum albumin fraction V (BSA), d-biotin (BT), biotinylated albumin (Alb-BT), phosphoric acid(crystalline), docusate sodium salt(AOT), poly-(allylamine hydrochloride) (PAH), glutaraldehyde (25%), titanium(IV) butoxide (TBOT), thiamine hydrochloride, riboflavin, ascorbic acid and pyridoxine hydrochloride were purchased from Sigma-Aldrich. Sodium hydroxide, sulfuric acid (97%), acetic acid (100%), phosphoric acid solution (85%), dried ethanol, Tween 20 and hydrochloric acid (37%) were purchased from Merck. Neutravidin (NTV) was purchased from Fisher Scientific. Ultrapure water obtained with a Millipore Direct Q5 purification system from Millipore was used throughout this work. All other reagents were of analytical grade. Phosphate buffer solution (PBS) (0.1 M, pH 7.0) was prepared by mixing the appropriate aqueous solution of H_3PO_4 and adjusting the pH with NaOH. Phosphate buffer solution with Tween (PBST) was prepared by adding 0.05% of Tween to this buffer. Acetate buffer was prepared by mixing the appropriate aqueous solution of acetic acid and adjusting the pH with NaOH. Working solutions of Alb-BT, NTV, BT, BSA, and CdTiPNPs-NTV were made in PBS buffer.

2.3. Preparation of titanium phosphate nanoparticles modified with cadmium

The synthesis of titanium phosphate nanoparticles was carried out following a procedure found in the literature[21]. Typically, 5.65 mmol of AOT was dissolved into 12.5 g of ethanol and H_3PO_4 (51 mmol) was added. AOT works as sodium source and structure-directing agent. This solution was filtered and the precipitate was removed. Then, a mixture of TBOT in ethanol (2.5mmol/15.5 mL) was fast dropped into the filtered solution, and stirred at 80 ºC for 6 h. The white solid product was washed with ethanol and ultrapure water for several times until neutral pH.

For the synthesis of cadmium-modified titanium phosphate nanoparticles, an aqueous suspension (1 mL) of TiPNPs (40 mg/mL) was dispersed in 17 mL of an aqueous solution of $Cd(NO_3)_2$ (10 mM) and the resulting mixture was stirred at 50 ˚C for 24 h. Then, the final mixture was centrifuged, the solid precipitate was washed three times with 10 mL of ultrapure water and the nanoparticles were dried under vacuum overnight.

2.4. Preparation of CdTiPNPs bio-functionalized with neutravidin

The bio-functionalization of CdTiPNPs with neutravidin was carried out by following a method previously reported in the literature[24] slightly modified. Briefly, 5 mg of CdTiPNPs were dispersed in 500 µL of ultrapure water and 500 µL of an aqueous solution of PAH (2 mg/mL) were added. The solution was placed in an ultrasonic bath for 20 min. The resulting solution was centrifuged, washed with ultrapure water (three times) and the solid was dispersed in 500 µL of a glutaraldehyde solution (0.25% in H_2O) and kept in an ultrasonic bath for 5 min. After centrifugation, washing with ultrapure water and PBS, the solid was dispersed in 500 µL of a neutravidin solution (0.05 mg/mL) in PBS. The mixture was stirred for 6 hours. The solution was centrifuged, washed with PBS and the product was re-dispersed in 5 mL of 0.1 M pH 7.0 PBS solution (for a final concentration of 1 mg/mL). All the procedure was carried out in low-binding centrifuge micro-tubes.

2.5. Bioassay and electrochemical detection procedures

The working electrodes of 8xSPCEs were modified with an aliquot of 4 µL of a 0.25 mg/mL solution of biotinylated albumin (in PBS) and were stored at 4 ºC overnight. After washing with PBS, surface blocking was conducted by adding 25 µL of a BSA solution (2.0% in PBS) and left in contact for 30 minutes. Then, a new washing step with PBS was performed, and the affinity reaction took place by adding 25 µL of a solution of CdTiPNPs-NTV (80 µg/mL in PBS) for the optimization experiments or a mixture CdTiPNPs-NTV and biotin for the competitive assay. **Figure 1** shows a schematic drawing of the biosensor used for the competitive assay (the same format was

used for the optimization but in absence of free biotin). After the biological reaction, the screen-printed card was washed with PBST (0.05% Tween) and ultrapure water and the electrochemical measurement was carried out. 25 µL of a 0.1 M pH 5.0 acetate buffer solution (with 10 mg/L of Bi(III)) were added to each electrochemical cell. The electrochemical detection was conducted using square-wave anodic stripping voltammetry under the following conditions: -1.3 V for deposition potential, 300 s for deposition time, 20 Hz as frequency, 30 mV as amplitude and 2 mV as step potential. Typically, all the measurements were performed by triplicate (n=3) using different 8-channel cards unless otherwise stated, and therefore, the standard deviations reported are between measurements in different cards.

2.6. Real samples preparation

The determination of biotin in real samples was performed in multivitamin tablets (Deliplus and Centrum brands) obtained from a local supplier. Each Tablet #1 (Deliplus) and Tablet #2 (Centrum) contained 25 µg and 62.5 µg of biotin according to the product specifications. In order to obtain a solution with a biotin concentration within the dynamic range of the calibration plot, one tablet was dissolved in 100 mL of PBS buffer under an ultrasonic bath (10 min). The solution was filtered to remove large suspended insoluble materials and diluted 1:100 in PBS to obtain the final solution. Three different tablets were analysed for each brand.

2.7. Electrochemical determination of cadmium from the nanoparticles (in weight)

The amount of cadmium introduced into CdTiPNPs was estimated by a voltammetric technique. Briefly, 50 µL of 1 M H_2SO_4 were added to 20 µL of CdTiPNPs (in H_2O) and an ultrasound bath was applied to the solution for 1 min in order to facilitate the metal extraction by inverting the cation exchange reaction. Then, 930 µL of 0.1 M pH 5.0 acetate buffer (with 1 mg/L of Bi(III)) was added to generate a solution appropriate for the voltammetric measurement of cadmium. The concentration of Cd(II) in solution was obtained by the standard addition method. Square-wave

anodic stripping voltammetry was employed for the measurements with an initial preconcentration step by applying -1.3 V for 15 s. Considering the amount (in weight) of initial nanoparticles, 50±5 mg of Cd(II) per g of CdTiPNPs was obtained. Other authors have described metallic loads for CdTiPNPs of 58.6 or 70.4 mg of Cd(II) per g of nanoparticles[23,22], values close to the estimated in our work.

3. RESULTS AND DISCUSSION

3.1. Microscopic characterization of TiPNPs and CdTiPNPs

Titanium phosphate nanoparticles (TiPNPs) and modified with cadmium (CdTiPNPs) were characterized by TEM. **Figure 2** shows the TEM micrographs and the size distribution for both types of nanoparticles. No significant differences were observed in the size, geometry and appearance of the nanoparticles before and after the metal exchange, suggesting that the cation exchange reaction does not modify the morphological properties of these nanoparticles. The mean diameter of the TiPNPs was estimated as 30±5 nm versus 29±5 nm of the CdTiPNPs. **Table S1** shows the relative composition of TiPNPs and CdTiPNPs obtained by EDS. These data demonstrate the successful exchange of Cd cations inside the structure of the TiPNPs, reaching up to 1% of the total number of atoms. A detailed study of the incorporation of metals into the structure of TiPNPs has been recently published by our group[28].

3.2. Electrochemical detection of CdTiPNPs at screen-printed carbon electrodes

A specific method for the electrochemical detection of CdTiPNPs using screen-printed electrodes was firstly optimized to get the best conditions for the development of the biosensor for biotin detection. Cyclic voltammograms of CdTiPNPs at different concentrations were recorded in order to identify the electrochemical processes (**Figure 3**). Two cathodic processes can be observed in the forward curve, which are assigned to the oxygen reduction reaction (ORR), also observed in the blank solution, and the reduction of Cd(II) to Cd(0). Although the ORR may avoid to observe

perfectly the cathodic process, the anodic stripping process is the most interesting for analytical purposes, and therefore, the solutions were used without removing oxygen. Under the same conditions, the reduction of free Cd(II) in solution happened at a more positive potential than that of the cadmium from the nanoparticles, suggesting that the reduction of the metal takes place directly from the nanoparticles and not from free metal in solution, since, in that case, the reduction potentials would be similar. This is an interesting fact that has not been reported to date and explain the electrochemical behaviour of these nanoparticles. An anodic process assigned to the cadmium stripping (oxidation of Cd(0) to Cd(II)) is also observed in the voltammetric response. The similar peak potential of this process for the free Cd(II) and CdTiPNPs solutions indicates that the stripping of cadmium follows an oxidation process of the same nature, and that the presence of CdTiPNPs in solution does not affect the stripping step.

Anodic stripping voltammetry was chosen as the most appropriate technique for CdTiPNPs detection because it has been widely reported for the electrochemical detection of metals. Different parameters that could influence the stripping signal were evaluated such as the electrochemical technique (linear sweep voltammetry, differential-pulse voltammetry or square-wave voltammetry), the kind, concentration and pH of the buffer employed for the measurement or the effect of a bismuth film. These factors were not evaluated in previous works using CdTiPNPs and, therefore, it is unknown how they could influence the electrochemical detection of these nanoparticles. To perform these experiments, CdTiPNPs were previously adsorbed (2 μL in ultrapure water) on the electrode surface to mimic similar conditions to those used in biosensors. **Figure S1A** shows the effect of the electrochemical technique on the stripping current at different concentrations of CdTiPNPs (50, 200 μg/mL), obtaining higher signal/noise ratios using square-wave voltammetry. Acetate and citrate buffers at different concentrations and pHs were evaluated. The higher analytical signal was obtained for a 0.1 M pH 5.0 acetate buffer solution. In order to improve the preconcentration of cadmium on the electrode surface, the electrode was modified with a bismuth

film, a non-toxic metal with similar properties to mercury, commonly used for this purpose[29]. The enhancing in metal preconcentration is attributed to the bismuth property of forming fused alloys with heavy metals[30], analogous to the formation of mercury amalgams. Thus, the detection of CdTiPNPs with the aid of a bismuth film is a different approach from previous studies[23,22], where the carbon electrode surface was used directly. The bismuth film was generated following the typical *in situ* methodology, since it had given good results for the detection of cadmium using screen-printed electrodes previously[31]. The effect of the bismuth concentration (0, 0.5, 1, 10, 20 mg/L) was evaluated with a lower range of CdTiPNPs concentrations (10, 50 µg/mL). The highest stripping peak currents were obtained using a concentration of 10 µg/mL of Bi(III), as illustrated in the **Figure S1B**. The use of the bismuth film significantly enhances the analytical signal and the sensitivity in the detection of CdTiPNPs compared to bare carbon electrodes. Therefore, although a direct comparison with previous works using CdTiPNPs cannot be performed due to the lack of fundamental studies in those cases, it is expected that with the method proposed here, the sensitivity will be much higher according to the obtained results. Although a deposition potential is usually applied for the reduction and preconcentration of the metals in ASV measurements, in some of the reported studies using CdTiPNPs, the detection was carried out directly by sweeping the potential without a preconcentration step[23,22]. Therefore, the possibility of detection of these nanoparticles without performing the electrodeposition step was evaluated. The voltammograms obtained for 0, 10 and 50 µg/mL of CdTiPNPs with 30 s of deposition step (-1.3 V) or without it are shown in the **Figure S2**. These results indicate that even a short deposition time (30 s) is very important in order to obtain high stripping signals and, therefore, to detect lower concentrations of CdTiPNPs, since the signal for the blank solution is not significantly increased. 300 s was the optimized deposition time chosen for the following experiments.

Finally, several voltammograms were recorded using different concentrations of CdTiPNPs in order to obtain a calibration curve. The voltammetric responses and the calibration plot are shown in

Figure S3. A linear range between 0.5 µg/mL and 50 µg/mL was obtained following the equation: i_p (µA) = 1.98 (±0.02) [CdTiPNPs] (µg/mL) - 0.09 (± 0.1), R^2 = 0.996 (n=3, each point of the plot was measured in three different cards), with 7.1% RSD between slopes and a limit of detection (LOD) of 0.26 µg/mL, calculated as the concentration corresponding to three times the standard deviation of the estimate. Considering the amount of Cd(II) introduced in the nanoparticles (estimated as 50 mg Cd per g of CdTiPNPs), this LOD would be around 15 µg/L of Cd(II). Compared to the electrochemical detection of CdSe/ZnS quantum dots using a method previously developed[31], and using a rough value of 100 atoms per 3.26 nm quantum dot[32], the estimated LOD for quantum dots would be about 35 µg/L of Cd(II). These results indicate that a similar concentration of cadmium could be detected using CdTiPNPs compared to quantum dots but avoiding the acid digestion to release the metals to the solution, which is an essential step for the sensitive detection of quantum dots.

3.3. Optimization of the electrochemical biosensor for biotin detection

Some experimental conditions of the affinity biosensor for biotin detection were optimized. The analytical signal was the cadmium stripping response from the CdTiPNPs-NTV conjugate. A solution of 2% BSA in PBS was used for blocking the electrode surface for 30 min to prevent non-specific binding. The reaction time of CdTiPNPs-NTV with the sensor surface was also 30 minutes, similar to the time used for quantum dots in a previous assay[33]. Firstly, the operation of the biosensor using 0.1 mg/mL of Alb-BT, and a diluted solution of CdTiPNPs-NTV (20 µg/mL) was evaluated and the voltammogram obtained is shown in the **Figure 4A.** The blocking step was sufficient to avoid the non-specific adsorption of CdTiPNPs-NTV in this order of concentrations. Then, the concentration of Alb-BT was optimized by evaluating the response for different concentrations (0.01, 0.05, 0.1, 0.25 and 0.5 mg/mL). As shown in the **Figure 4B**, the analytical signal increased with increasing concentrations of Alb-BT up to 0.25 mg/mL, for which the higher signal was obtained. **Figures 4C** and **4D** shows the results obtained for the voltammetric response

and the calibration curve for different concentrations of CdTiPNPs-NTV with the signal increasing linearly from 5 up to 100 μg/mL of CdTiPNPs-NTV (1:10 dilution from the initial solution). The linear equation of the calibration plot was: i_p (μA) = 0.14 (±0.02) [CdTiPNPs-NTV] +0.2 (±0.1), R^2 = 0.991 (n=3, each point of the plot was measured in three different cards), with 9.1% RSD between slopes. A concentration of 80 μg/mL of CdTiPNPs-NTV was chosen for the subsequent competitive assay, since this concentration gives a high signal, it is in an area of maximum slope of the calibration plot, and was expected to respond sensitively to changes in the concentration of free biotin in solution.

3.4. Analytical characteristics of the competitive biosensor for the detection of biotin

Finally, the competitive biosensor for the detection of biotin under optimized conditions schematized in the **Figure 1** was developed. Free biotin can bind to CdTiPNPs-NTV and prevent their reaction with the sensor surface (Alb-BT). It is expected that for increasing concentrations of free biotin, less functionalized nanoparticles are bound to Alb-BT and, therefore, a lower signal would be obtained. A semi-logarithmic decreasing response was observed for increasing biotin concentrations as shown in the **Figure 5**. A linear dependence between i/i_0 (%) and the logarithm of the concentration of free biotin was obtained from $1x10^{-9}$ to $1x10^{-7}$ M, where i_0 is the current obtained in the absence of biotin, and i is the current obtained when free biotin is present in solution. The dynamic range was estimated between $2x10^{-9}$ and $4x10^{-8}$ M (response between 20 and 80% of the maximum signal), following a method described in the literature for a semi-logarithmic response[34,35]. The limit of detection estimated was $1x10^{-9}$ M (signal obtained for a decrement of 10% from the maximum response). The reproducibility between the slopes of the calibration curves (n=3, each point of the plot was performed in three different cards) was 7.1% (in terms of RSD), indicating a good reproducibility of the calibration plot. A precision study was performed by repeating the same measurement ($5x10^{-9}$ M of biotin) with five different screen-printed cards (inter-electrode reproducibility) and with the 8 electrochemical cells in the same card (intra-electrode

reproducibility). The inter-electrode reproducibility was 8.7% and the intra-electrode reproducibility was 5.1%, illustrating the good precision of the biosensor developed. The good precision and low cost of the screen-printed devices in combination with the possibility to perform 8 analysis at the same time leads to a high throughput method for the detection of biotin (the bioassays can be carried out simultaneously in different devices, and each 8-channel card can be measured every 5 min). In order to study the stability of the device, several biosensors were modified with Alb-BT as explained in the experimental section, washed the next day with PBS buffer and stored at 4 ºC for different time periods. The response of the sensor was evaluated over a 3-week period by measuring a solution of $5x10^{-9}$ M of biotin (**Figure 6A**). The responses during this period were not statistically different, indicating the good stability of the sensor element on the screen-printed electrodes. Comparable analytical characteristics were obtained for this method in comparison to a similar method using quantum dots as label[33] with the great advantage of avoiding the acid digestion of the nanoparticles. The use of CdTiPNPs as detection label of electrochemical biosensors using disposable screen-printed electrodes provides good results, leading to an interesting analytical method for detection of biotin at concentrations in the nM range. The method developed using CdTiPNPs as label shows a good analytical performance in comparison to other electrochemical methods for the determination of biotin previously reported (**Table 1**).

3.5. Selectivity study of the competitive biosensor for biotin detection

In order to evaluate the selectivity of the biosensor towards biotin, other vitamins such as riboflavin, ascorbic acid, thiamine and pyridoxine were tested. **Figure 6B** shows the analytical response obtained with the biosensor in presence of these compounds at concentrations typically found in real samples (multivitamin products) and in presence and absence of $1x10^{-8}$ M of biotin. These concentrations were $2x10^{-7}$, $2x10^{-5}$, $2x10^{-7}$ and $4x10^{-7}$ M for riboflavin, ascorbic acid, thiamine and pyridoxine, respectively. The responses of the biosensor were statistically comparable in presence

of the possible interfering species, which demonstrates the good selectivity of the biosensor to biotin due to the specific neutravidin-biotin interaction.

3.6. Determination of biotin in multivitamin tablets

To demonstrate the feasibility of the analytical method using the competitive biosensor, the detection of biotin in multivitamin tablets from two different brands was carried out. Tablets were dissolved in the measuring electrolyte according to the procedure described in the Experimental section. The competitive biosensor was carried out under optimized conditions, and the concentration of the samples was estimated by using the current obtained in the assay and the calibration plot of the competitive biosensor previously discussed. The estimated amount of biotin per tablet was 27 ± 2 μg for Tablet #1 and 59 ± 5 μg for Tablet #2, values in accordance with the data of the products specifications (25 μg and 62.5 μg per tablet, respectively). These results demonstrate the possibility of using the competitive biosensor for the detection of biotin in real samples.

4. CONCLUSIONS

This work describes the development of method for the detection of biotin using a competitive biosensor with cadmium-modified titanium phosphate nanoparticles as labels. These nanoparticles have shown a good analytical performance when used as detection label for disposable electrochemical biosensors using screen-printed electrodes. The analytical results of the biosensor are very competent being able to detect biotin at concentrations of the order of nM with a limit of detection of 1 nM. This fact demonstrates the good sensitivity of titanium phosphate nanoparticles, with similar results to those obtained for a previously described system using quantum dots as label. However, the methodology is simpler since there is no need for an additional acid digestion step to release the metals to the solution, as was the case for quantum dots. This work shows the possibility of using this type of nanoparticles as label of disposable biosensors, and open a promising door for

future developments of analytical methods with disposable multiplexing biosensors using screen-printed electrodes and titanium phosphate nanoparticles modified with different metals.

NOTES

The authors declare no competing financial interest.

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REFERENCES

1. D. M. Mock, A. A. DeLorimer, W. M. Liebman, L. Sweetman, and H. Baker, *N. Engl. J. Med.*, 1981, **304**, 820–823.
2. H. E. Indyk, B. D. Gill, and D. C. Woollard, *Int. Dairy J.*, 2014, **35**, 25–31.
3. E. Livaniou, D. Costopoulou, I. Vassiliadou, L. Leondiadis, J. O. Nyalala, D. S. Ithakissios, and G. P. Evangelatos, *J. Chromatogr. A*, 2000, **881**, 331–343.
4. T. Tsukatani, H. Suenaga, M. Ishiyama, T. Ezoe, and K. Matsumoto, *Food Chem.*, 2011, **127**, 711–715.
5. H. Tsuda, T. Matsumoto, and Y. Ishimi, *J. Nutr. Sci. Vitaminol. (Tokyo).*, 2011, **57**, 437–440.
6. G. J. Wellenberg and J. N. Banks, *J. Sci. Food Agric.*, 1993, **63**, 1–5.
7. J. Wang, *Biosens. Bioelectron.*, 2006, **21**, 1887–92.
8. P. T. Kissinger, *Biosens. Bioelectron.*, 2005, **20**, 2512–6.
9. J. P. Metters, R. O. Kadara, and C. E. Banks, *Analyst*, 2011, **136**, 1067–1076.
10. V. Gubala, L. F. Harris, A. J. Ricco, M. X. Tan, and D. E. Williams, *Anal. Chem.*, 2012, **84**, 487–515.
11. A. A. Kumar, J. W. Hennek, B. S. Smith, S. Kumar, P. Beattie, S. Jain, J. P. Rolland, T. P. Stossel, C. Chunda-Liyoka, and G. M. Whitesides, *Angew. Chemie Int. Ed.*, 2015, **54**, 5836–5853.
12. C. S. Lim, S. M. Tan, Z. Sofer, and M. Pumera, *ACS Nano*, 2015, **9**, 8474–8483.
13. M. M. P. S. Neves, P. Bobes-Limenes, A. Pérez-Junquera, M. B. González-García, D. Hernández-Santos, and P. Fanjul-Bolado, *Anal. Bioanal. Chem.*, 2016, **408**, 7121–7127.
14. B. Pérez-Fernández, D. Martín-Yerga, and A. Costa-García, *RSC Adv.*, 2016, **6**, 83748–83757.
15. M. M. P. S. Neves, M. B. González-García, C. Delerue-Matos, and A. Costa-García, *Sens. Actuators B*, 2013, **187**, 33–39.
16. D. Martín-Yerga, M. B. González-García, and A. Costa-García, *Talanta*, 2014, **130**, 598–602.

17. M. M. P. S. Neves, M. B. González-García, D. Hernández-Santos, and P. Fanjul-Bolado, *Electroanalysis*, 2014, **26**, 2764–2772.
18. A. Ambrosi, A. Merkoçi, and A. de la Escosura-Muñiz, *TrAC - Trends Anal. Chem.*, 2008, **27**, 568–584.
19. A. de la Escosura-Muñiz, C. Parolo, F. Maran, and A. Mekoçi, *Nanoscale*, 2011, **3**, 3350–3356.
20. M. M. Costa, A. de la Escosura-Muñiz, and A. Merkoçi, *Electrochem. commun.*, 2010, **12**, 1501–1504.
21. J. Liu, X. Wei, Y. Yu, J. Song, X. Wang, A. Li, X.-W. Liu, and W.-Q. Deng, *Chem. Commun.*, 2010, **46**, 1670–1672.
22. L.-N. Feng, J. Peng, Y.-D. Zhu, L.-P. Jiang, and J.-J. Zhu, *Chem. Commun.*, 2012, **48**, 4474–4476.
23. L.-N. Feng, Z.-P. Bian, J. Peng, F. Jiang, G.-H. Yang, Y.-D. Zhu, D. Yang, L.-P. Jiang, and J.-J. Zhu, *Anal. Chem.*, 2012, **84**, 7810–7815.
24. F. Cheng, T. He, H. Miao, J. Shi, L. Jiang, and J. Zhu, *ACS Appl. Mater. Interfaces*, 2015, **7**, 2979–2985.
25. M. K. Sharma, J. Narayanan, S. Upadhyay, and A. K. Goel, *Biosens. Bioelectron.*, 2015, **74**, 299–304.
26. D. Martín-Yerga, J. Carrasco-Rodríguez, M. B. González-García, F. J. García Alonso, and A. Costa-García, *Electroanalysis*, 2014, **26**, 2574–2579.
27. D. Martín-Yerga, J. Carrasco-Rodríguez, J. L. G. Fierro, F. J. García Alonso, and A. Costa-García, *Electrochim. Acta*, 2017, **229**, 102–111.
28. J. Carrasco-Rodríguez, D. Martín-Yerga, L. Garrido, A. Costa-García, and F. J. García Alonso, *Dalt. Trans.*, 2017.
29. A. Economou and P. R. Fielden, *Analyst*, 2003, **128**, 205–212.
30. A. Economou, *TrAC - Trends Anal. Chem.*, 2005, **24**, 334–340.
31. D. Martín-Yerga, D. Bouzas-Ramos, M. Menéndez-Miranda, A. R. M. Bustos, J. R. Encinar, J. M. Costa-Fernández, A. Sanz-Medel, and A. Costa-García, *Electrochim. Acta*, 2015, **166**, 100–106.
32. A. R. Montoro Bustos, J. R. Encinar, M. T. Fernández-Argüelles, J. M. Costa-Fernández, and A. Sanz-Medel, *Chem. Commun.*, 2009, 3107–3109.
33. D. Martín-Yerga, M. B. González-García, and A. Costa-García, *Sens. Actuators B*, 2013, **182**, 184–189.
34. Y. Sugawara, S. J. Gee, J. R. Sanborn, S. D. Gilman, and B. D. Hammock, *Anal. Chem.*, 1998, **70**, 1092–1099.
35. C. Wei, S. Ding, H. You, Y. Zhang, Y. Wang, X. Yang, and J. Yuan, *PLoS One*, 2011, **6**, e29196.
36. J. D. Wright, K. M. Rawson, W. O. Ho, D. Athey, and C. J. McNeil, *Biosens. Bioelectron.*, 1995, **10**, 495–500.
37. J. A. Ho, W.-L. Hsu, W.-C. Liao, J.-K. Chiu, M.-L. Chen, H.-C. Chang, and C.-C. Li, *Biosens. Bioelectron.*, 2010, **26**, 1021–1027.
38. S. V. Kergaravat, G. A. Gómez, S. N. Fabiano, T. I. Laube Chávez, M. I. Pividori, and S. R. Hernández, *Talanta*, 2012, **97**, 484–490.
39. J. Biscay, M. B. González García, and A. Costa García, *Talanta*, 2015, **131**, 706–711.
40. J. Biscay, M. B. G. García, and A. C. García, *Sens. Actuators B*, 2014, **205**, 426–432.

FIGURES AND TABLES

Table 1. Analytical characteristics of different electrochemical methods for the detection of biotin reported in the literature.

Reference	LOD (nM)	Linear range (nM)
Avidine-HRP[36]	-	285 - 8000
anti-BT and BT-liposome[37]	14	1 - 1000
MB-STV + HRP-BT[38]	84	94 - 240
MB-STV + HRP-BT (FIA)[39]	0.008	0.01 - 1
MB-STV + HRP-BT (8xSPCEs)[40]	0.2	0.2 - 250
QDs-BT-STV (8xSPCEs)[33]	1.4	1 - 100
This work	1	2 - 40

Figure 1. Schematic drawing of the competitive biosensor for the detection of biotin using CdTiPNPs as label. Biotinylated albumin (Alb-BT) was used as sensing element, bovine serum albumin (BSA) as blocking agent, cadmium-modified titanium phosphate nanoparticles conjugated with neutravidin (CdTiPNPs-NTV) as label, and biotin (BT) was the analyte. Square-wave anodic stripping voltammetry (SWASV) was employed as detection technique.

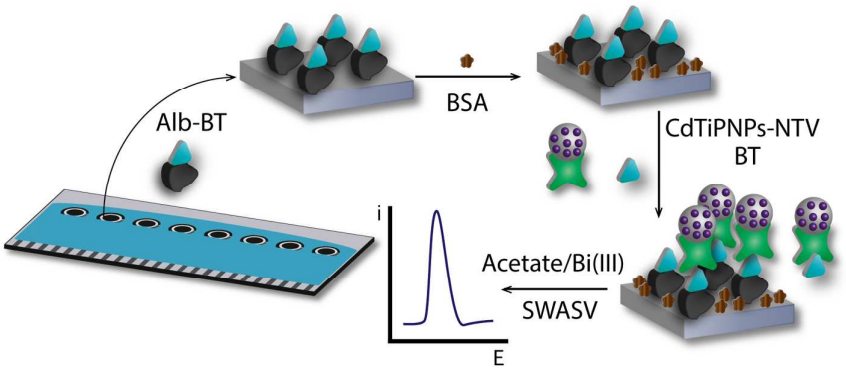


Figure 2. TEM micrographs and size distribution for TiPNPs and CdTiPNPs.

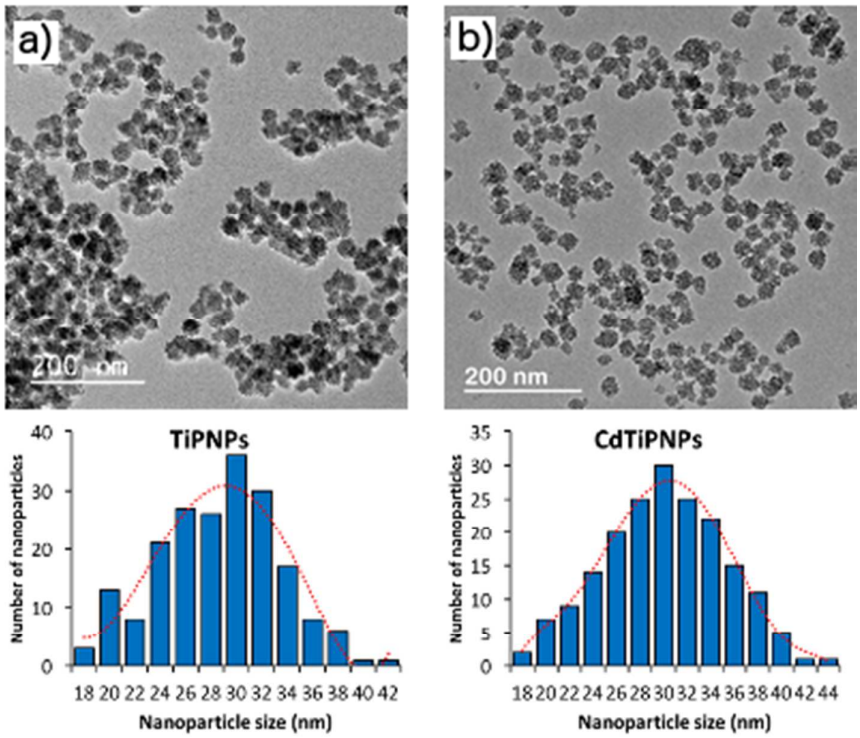


Figure 3. Cyclic voltammograms for solutions containing 100 mg/L of Cd(II), 1 mg/mL or 2 mg/mL of CdTiPNPs. The voltammogram for the blank solution (0.1 M pH 4.5 acetate buffer) is also shown for comparison.

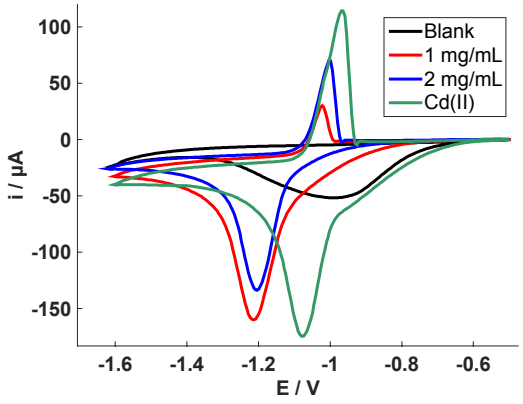
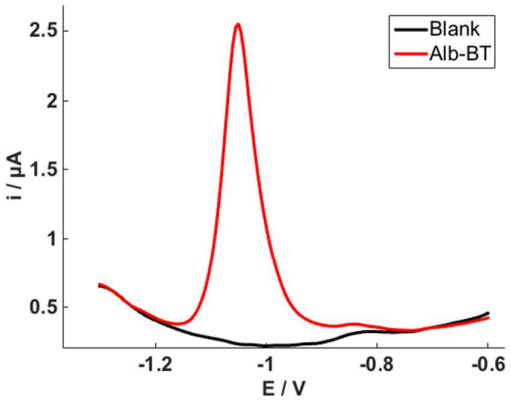
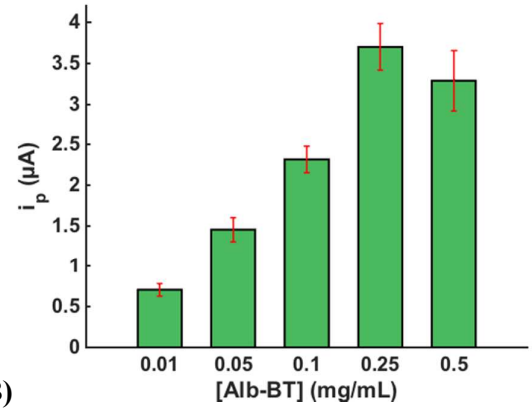


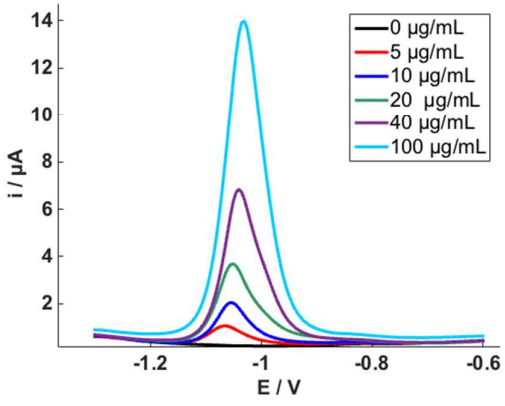
Figure 4. A) Voltammetric response for the biosensor using CdTiPNPs-NTV as label in absence (black line) and presence (red line) of Alb-BT on the electrode surface. **B)** Effect of the Alb-BT concentration on the cadmium stripping peak current after conducting the affinity reaction. **C)** Voltammetric responses of the affinity biosensor for increasing concentrations of CdTiPNPs-NTV and associated calibration plot **(D)**.



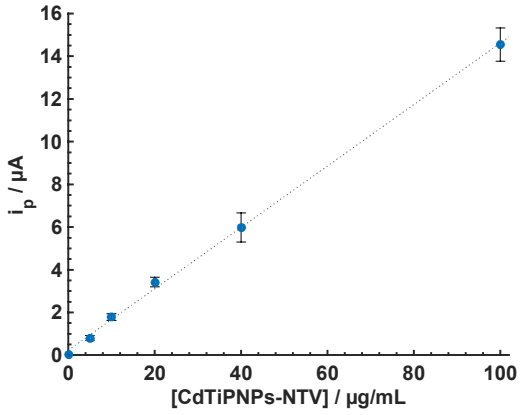
A)



B)

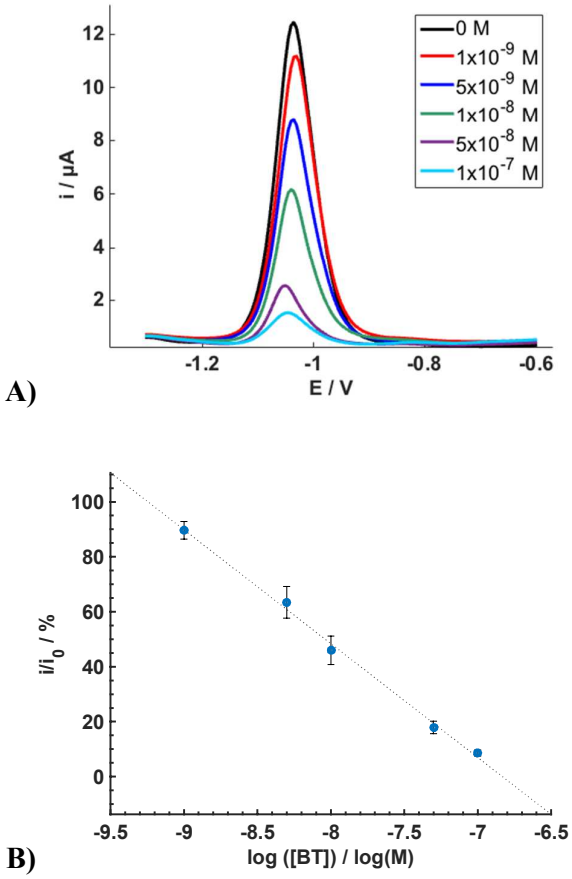


C)



D)

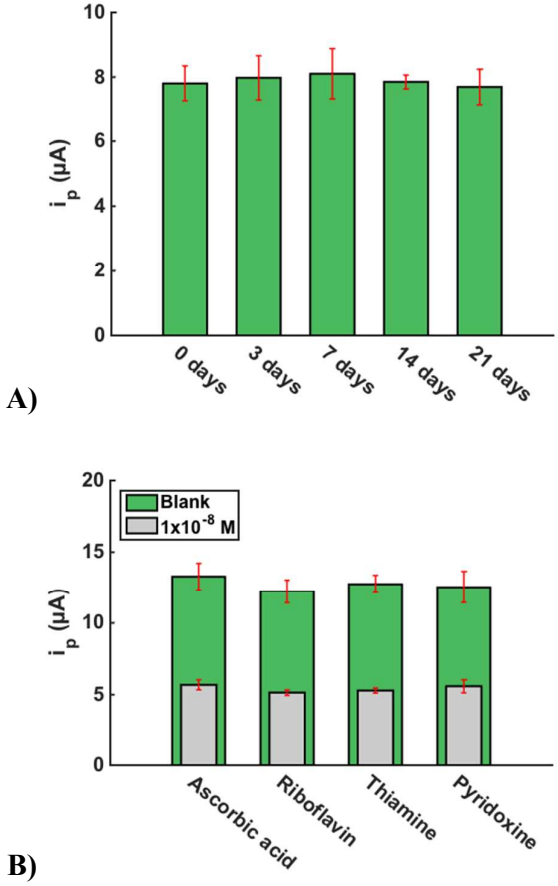
Figure 5. Voltammetric response for the competitive biosensor for different concentrations of biotin **(A)** and the associated semi-logarithmic calibration plot **(B)**.



A)

B)

Figure 6. A) Stability study of the electrochemical immunosensor for the detection of biotin. **B)** Effect of several possible interfering species on the analytical signal of the proposed competitive biosensor in absence and presence of $1x10^{-8}$ M of biotin.



SUPPLEMENTARY INFORMATION

Competitive electrochemical biosensing of biotin using cadmium-modified titanium phosphate nanoparticles and 8-channel screen-printed disposable electrodes

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Table S1. Relative concentration of O, Na, P, Ti and Cd for TiPNPs and CdTiPNPs obtained from the EDS data in the TEM measurements.

	O (%)	Na(%)	P(%)	Ti(%)	Cd(%)
TiPNPs	66±5	3.7±0.1	22±3	9±1	
CdTiPNPs	66±1	2.36±0.01	21±1	10.5±0.3	1.02±0.03

Figure S1. Effect of the electrochemical technique **(A)** and Bi(III) concentration **(B)** on the cadmium stripping peak of CdTiPNPs at different concentrations.

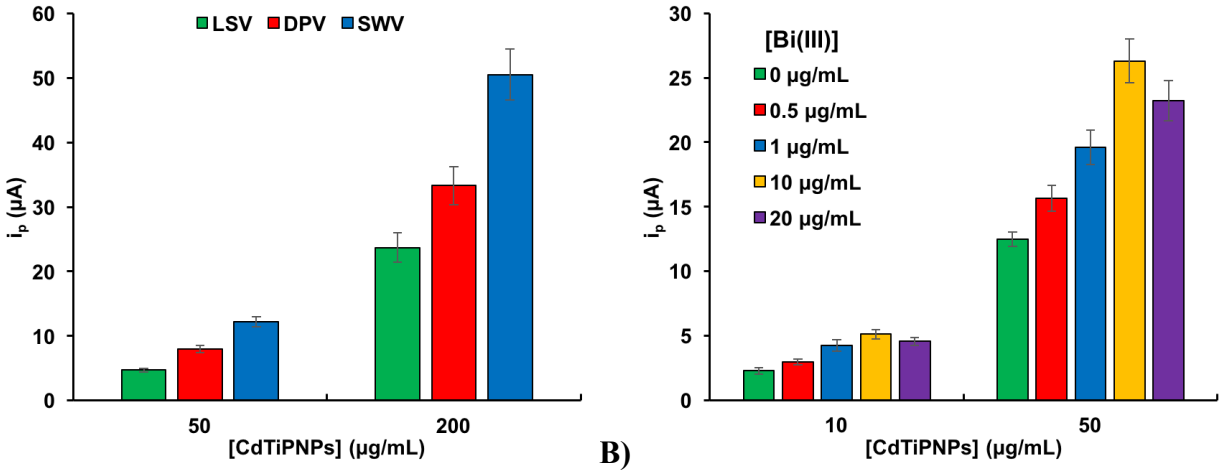

Figure S2. Voltammetric responses for several concentrations of CdTiPNPs after the electrodeposition step (by applying -1.3 V for 30 s) and the direct measurement without electrodeposition step.

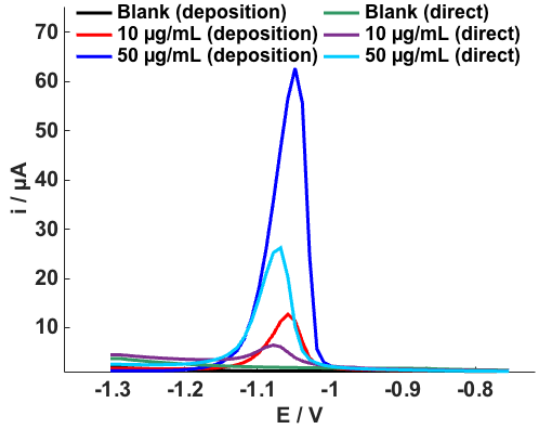

Figure S3. Voltammetric response for increasing concentrations of CdTiPNPs (A) and associated calibration plot representing relationship between the stripping peak current and the nanoparticle concentration (B). Inset of A) is the voltammograms at low concentrations.