



Tuning the incorporation of electroactive metals into titanium phosphate nanoparticles and the reverse metal extraction process: Application as electrochemical labels in multiplex biosensing



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ABSTRACT

In this work, titanium phosphate nanoparticles were modified with different electroactive metals (cadmium, bismuth, copper, silver and mercury), by a cation-exchange reaction. The amount of metal introduced into the nanoparticles depended strongly on the counter-ion used during the exchange reaction and the type of metal. Nanoparticles with a high metallic load could be generated. The electrolytic medium played an important role in the detection of these metal-modified nanoparticles, since the use of acid makes it possible to extract a large part of the introduced metal by reversing the cation-exchange reaction. The application of these nanoparticles in electrochemical detection was evaluated, the nanoparticles modified with copper and mercury proving superior in terms of sensitivity and selectivity in multiplex detection. As a proof of concept, these nanoparticles were used as labels in a multiplex electrochemical biosensor for the simultaneous detection of two analytes.

1. Introduction

Metal-based nanoparticles are widely used as labels in electrochemical biosensors [1–3] since they have interesting properties which include the possibility of biofunctionalization, multiple and easy synthesis methods, biocompatibility and, typically, electroactivity. The great variety of metallic nanoparticles available is very convenient for multiplex assays [4,5], where the simultaneous detection of several analytes is carried out. Nanoparticles that can be loaded with different electroactive species [6], such as liposomes [7], apoferritins [8,9] or other nanoparticles [10,11] are also an important resource in this field. Nevertheless, the development of novel or enhanced nanoparticles that can be loaded with a large amount of some electroactive species is still a relevant research problem which is aimed at improving the sensitivity of detection in electrochemical biosensing. For instance, titanium phosphate nanoparticles (TiPNPs) [12,13] are very interesting because they have a porous structure and an amorphous coating of acid phosphates that provides them with a strong ion-exchange functionality. However, these nanoparticles have so far only been modified with cadmium, lead or zinc for use as labels [14–17]. These metals are reduced at quite a negative potential, which can also reduce other species in the solution. Furthermore, this potential is usually quite close to that

of the hydrogen evolution reaction (in acidic solutions) and this could affect the efficiency of metal electrodeposition onto the electrode surface. Therefore, the evaluation and characterization of other electroactive metals introduced into these nanoparticles [18,19] may lead to better labels for electrochemical biosensors.

In this work, titanium phosphate nanoparticles were modified with several electroactive metals (cadmium, bismuth, silver, mercury and copper). The amount of metal introduced into the nanoparticles was tuned by changing the metal or the counter-ion used during the cation-exchange reaction. The choice of metals that are reduced at more positive potentials made it possible to use electrolytes capable of extracting the metals more efficiently than in previous studies reported in the literature. The multiplexing ability of these nanoparticles was demonstrated by the proof-of-concept development of a biosensor.

2. Materials and methods

2.1. Apparatus and electrodes

Electrochemical measurements were conducted with μ Stat 8000 (DropSens) potentiostat/galvanostat interfaced to an Apple Macbook Air laptop and controlled by the DropView 8400 2.2 software. 8-

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channel screen-printed carbon electrochemical arrays (SPCEs) were purchased from DropSens (ref. 8×110). These devices, with a circular working electrode of 2.56 mm diameter, have been described previously [20]. 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 related to the silver pseudoreference screen-printed electrode.

2.2. Reagents and solutions

Cadmium nitrate, cadmium acetate, cadmium acetylacetonate, mercury acetate, copper acetate, silver nitrate, bismuth nitrate, bovine serum albumin fraction V (BSA), phosphoric acid (crystalline), docusate sodium salt (AOT), poly-(allylamine hydrochloride), glutaraldehyde, titanium (IV) butoxide were purchased from Sigma. Sulfuric acid (98%), acetic acid (100%), phosphoric acid solution (85%), dried ethanol, sodium hydroxide and hydrochloric acid were purchased from Merck. Neutravidin (NTV) was purchased from Fisher Scientific. Human tissue transglutaminase was purchased from Zedira. Biotinylated goat anti-human IgA and IgG (anti-IgA-BT, anti-IgG-BT) were purchased from Life Technologies. Varelisa Celikey tissue transglutaminase IgA ELISA kit was purchased from Phadia. Ultrapure water obtained with a Millipore Direct Q5™ purification system from Millipore was used throughout this work. All other reagents were of analytical grade. Unless stated otherwise, 2 μL of a nanoparticle dispersion were used to modify the electrodes.

2.3. Synthesis of titanium phosphate nanoparticles modified with metals

The synthesis of titanium phosphate nanoparticles was carried out following a slightly modified version of a procedure found in the literature [12] and reported previously [18]. To synthesise the metal-modified titanium phosphate nanoparticles, an aqueous suspension (1 mL) of TiPNPs (40 mg/mL) was dispersed in 17 mL of a 10 mM aqueous solution of the appropriate metallic salt and the resulting mixture was stirred at 50 °C for 24 h. The final mixture was then centrifuged, the solid precipitate was washed three times with 10 mL of ultrapure water and the nanoparticles were dried overnight under vacuum. These nanoparticles were characterized in previous studies [18,19,21,22].

2.4. Bio-functionalization of MTiPNPs with neutravidin and antibodies

A previously reported method was employed to bio-functionalize the MTiPNPs [21]. The neutravidin–biotin reaction was used for the conjugation of MTiPNPs–NTV with biotinylated anti-IgA and anti-IgG antibodies. Briefly, in a low-binding micro-tube, a 1:1 mixture (in PBS) of biotinylated antibody solution (5 $\mu\text{g}/\text{mL}$) and MTiPNPs–NTV (100 $\mu\text{g}/\text{mL}$) were left to incubate for 50 min under constant stirring. After the reaction, a small amount of BSA was added to these solutions (final concentration of 0.25% BSA) in order to minimize possible non-specific adsorptions in the immunosensor.

2.5. Bioassay and electrochemical detection procedures

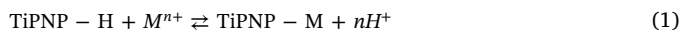
For the biosensor, the modification of the electrodes with a sensing element (transglutaminase) and various other steps were carried out using a method previously developed in our group [23] but using PBS as buffer solution. The reaction with the secondary antibodies was performed using bio-functionalized MTiPNPs. The electrochemical detection was carried out using square-wave anodic stripping voltammetry with a deposition step at -1 V for 60 s in 25 μL of 0.1 M H_2SO_4 .

3. Results and discussion

3.1. Tuning the incorporation of metals into titanium phosphate nanoparticles

Titanium phosphate nanoparticles modified with metals (MTiPNPs) can be detected by electrochemical methods after the introduction of electroactive metals into their structure. TiPNPs modified with different electroactive metals (cadmium, bismuth, copper, silver and mercury) were synthesized. These metals are easily measured by anodic stripping voltammetry, and the stripping processes usually occur at different potentials. In order to study the electroactivity of these nanoparticles, cyclic voltammetry was employed using 0.1 M pH 4.5 acetate buffer solution as an electrolyte suitable for the different metals. The surface of the screen-printed working electrode was modified with an aqueous suspension of nanoparticles (2 μL of 2 mg/mL). Fig. 1A and B show the cyclic voltammograms obtained for all the MTiPNPs. Although the cathodic processes are difficult to assign to the reduction of metals since the oxygen reduction reaction takes place at similar potentials and could affect the response, the most interesting process is the anodic stripping. Most of the nanoparticles showed one good resolved stripping peak due to the oxidation of the previously reduced metal. However, no stripping process was observed initially for the BiTiPNPs. To confirm the presence of bismuth in the nanoparticles, a preconcentration step was used (-1.4 V for 30 s), and then the scan was performed towards positive potentials, when the stripping process was observed. This suggests that a lower amount of bismuth is introduced into the nanoparticles or that the extraction is more difficult than for other metals (probably due to the lower solubility of bismuth cations). For the CdTiPNPs, the cyclic voltammogram was recorded up to -1.4 V in order to achieve reduction and observe the stripping process that appears at more negative potentials. These studies show that the kind of metal introduced into the MTiPNPs can be tuned and that the metal chosen has a strong influence on the detection of the nanoparticles.

It is thought that the cation-exchange reaction leading to the metallic nanoparticles follows the general mechanism given in Eq. (1). This mechanism suggests that increased extraction of protons from the initial nanoparticles would lead to an increased amount of metal introduced into the nanoparticle structure. Therefore, if a salt with a weaker base counter anion is used, it should be able to bind more protons, and to induce a shift in the reaction towards the products (Le Chatelier's principle).



The effect of two different anions on the electrochemical response of the CdTiPNPs was evaluated by using cadmium nitrate and acetate salts during the cation-exchange reaction. After obtaining the final product as described in the section 2.3 of the Materials and methods, 2 μL of a 2 mg/mL aqueous dispersion of the nanoparticles were adsorbed onto the working electrode, and square-wave anodic stripping voltammetry was used to detect the metal from the nanoparticles (0.1 M pH 4.5 acetate buffer was used). Fig. 1C shows that the highest signal was obtained for the CdTiPNPs synthesized with acetate, and the lowest signal was found for CdTiPNPs synthesized with nitrate, as expected theoretically. These results show that the amount of metal can be tuned by using different anions in the cation-exchange reaction, and that increasing the amount of metal would lead to more sensitive detection using electrochemical techniques. In previous work reported in the literature, only nitrate was employed for the cation-exchange reaction, which suggests that the detection of these nanoparticles would be less sensitive than for those synthesized in this work.

3.2. Enhancing the extraction of metals and detection of titanium phosphate nanoparticles

As the introduction of metals is carried out by a cation-exchange

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