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Electrochemical detection of quantum dots by stabilization of electrogenerated copper species

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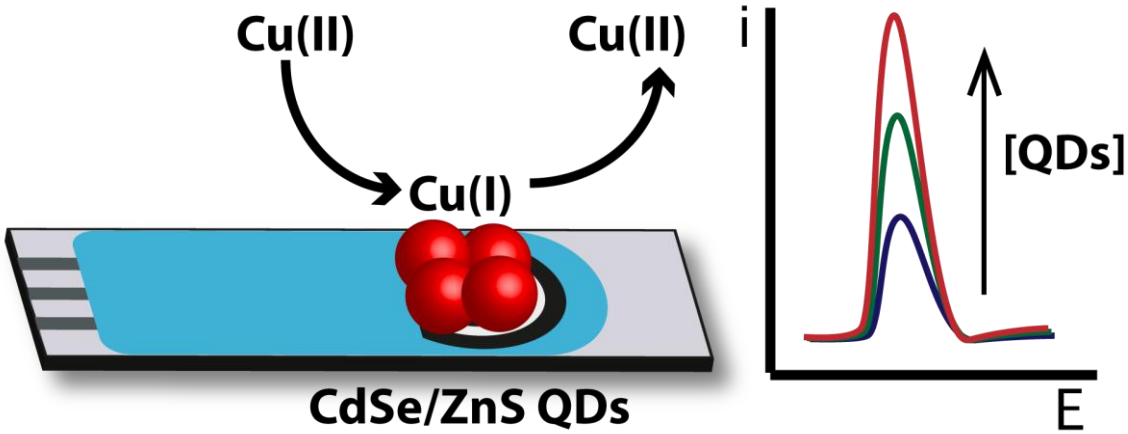
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- Copper species are stabilized at electrodes modified with quantum dots
- Amount of quantum dots on the electrode affected the copper voltammetric response
- A method for the determination of quantum dots was optimized
- Limit of detection was in the order of hundreds of amol of nanoparticles

ABSTRACT

Determination of quantum dots is particularly interesting as they are frequently used as detection label in electrochemical biosensors. In this paper, we describe a method for detection of very low concentrations of quantum dots using the voltammetric response of copper in ammonia medium. Copper species electrogenerated on the electrode surface are stabilized by the nanoparticles preventing their oxidation by dissolved oxygen, and therefore, a relationship between the nanoparticle concentration and the copper voltammetric response can be obtained. The developed method shows a linear range between 0.05 and 2 nM of quantum dots, with a limit of detection in the order of $9x10^7$ nanoparticles. This method could be interesting to improve the detection of electrochemical biosensors using quantum dots as label.

1. INTRODUCTION

Quantum dots (QDs) are small-sized semiconductor nanoparticles with interesting optical and electronic properties [1]. These properties and their easy functionalization allow them to be used as label in bioassays [2]. The electrochemical detection is usually performed after releasing the metals composing the nanoparticles to the solution by acid digestion [3,4]. This way, a high amount of metals is available for the detection. However, this increases the complexity of the detection process. Some methods have been proposed in order to simplify the detection such as the *in situ* digestion and detection on the surface where the bioassay is performed [5,6], or using flexible devices in ELISA well plates [7]. Some methods have been proposed to avoid the acid digestion of the nanoparticles, which would facilitate the electrochemical detection. For instance, the direct detection of cadmium from the nanoparticle allows their quantification but with a much lower sensitivity than performing the acid digestion [8]. Another method developed without acid digestion has been proposed by using the selective electrodeposition of silver on the QDs surface [9]. The stripping signal of the silver selectively deposited can be used for the determination of QDs concentration. In another interesting work, CdSe quantum dots were determined at low concentrations by the voltammetric reduction of borax, which is facilitated at the nanoparticle modified electrodes [10]. This apparent enhancement of the kinetics could be due to the organic capping agent of the nanoparticles, which could alter the mass transport or the local solubility of redox species [11]. Therefore, the development of new methods that improve the sensitivity, reduce analysis times using a simple procedure is a constant concern. In this work, we describe a novel method for the electrochemical determination of CdSe/ZnS quantum dots using the special copper voltammetric response in ammonia medium.

2. MATERIALS AND METHODS

2.1. Apparatus and electrodes

Electrochemical measurements were conducted with μStat 8000 (DropSens) potentiostat interfaced to an Apple Macbook Air laptop and controlled by the DropView 8400 2.2 software. All measurements were carried out at room temperature. 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 performed without removing oxygen from the solution, at room temperature and using an aliquot of 25 μL of the appropriate solution. All indicated potentials are related to the silver quasireference screen-printed electrode.

2.2. Reagents and solutions

Ammonia solution (25% w/v) and fuming hydrochloric acid were purchased from Merck. Copper nitrate trihydrate and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich. CdSe/ZnS Qdot® 655 Biotin Conjugate (QDs) was purchased from Life Technologies. The supplied material was 2 μM in particle concentration. Ultrapure water obtained with a Millipore Direct Q5™ purification system from Millipore was used throughout this work. All other reagents were of analytical grade. Working solutions of QDs were prepared in 0.1 M pH 7.4 Tris-HCl buffer. Cu(II) solutions were prepared in 1 M NH_3 aqueous solution. Copper ions in ammonia solutions are typically complexed: $[Cu(NH_3)_4]^{2+}$ for a +2 oxidation state, and $[Cu(NH_3)_2]^{+}$ or $[Cu(NH_3)_3]^{+}$ for a +1 oxidation state [12,13]. To simplify the notation, hereinafter these species will be written as Cu(II) and Cu(I). QDs concentration is always given as particle concentration. The commercial QDs used have a core-shell structure of CdSe/ZnS and are functionalized with an outer organic/polymeric coating for solubilization in aqueous solutions and for the conjugation with

biomolecules. The total size of the functionalized nanoparticles, with ellipsoid shape, is about 10 nm (larger diameter), as stated in the manufacturer specifications and as we observed experimentally by transmission electron microscopy (TEM) in a previous work [9]. However, the manufacturer does not provide the exact size of the coating and the nanocrystal and it cannot be differentiated by TEM.

2.3. Electrochemical detection of quantum dots

In order to carry out the electrochemical detection, QDs were physisorbed on the electrode surface by placing a drop (4 µL) on the working electrode. The adsorption time was maximal at 30 min and the analytical signal did not increase even when left overnight at 4 ºC. After washing with H_2O, a drop (25 µL) of 25 µM $Cu(NO_3)_2$ in 1 M NH_3 was added to the electrochemical cell and a potential of -0.4 V for 30 s was applied to carry out the reduction of Cu(II) to Cu(I). Then, square-wave voltammetry (SWV) was performed between -0.4 and +0.3 V with the following parameters: 20 Hz for frequency, 25 mV for amplitude, 20 mV for step potential and 3 s for equilibration time.

3. RESULTS AND DISCUSSION

We first describe briefly the voltammetric response of copper at bare and QDs-modified electrodes in aqueous ammonia medium, and then, we optimize a method in order to carry out the electrochemical detection of QDs using the copper voltammetric response.

3.1. Electrochemistry of copper at bare and QDs-modified screen-printed carbon electrodes

Recently, we have discovered the stabilization of electro-generated copper species in ammonia media at QDs-modified electrodes (unpublished results [14]). Briefly, the voltammograms obtained for 60 µM of Cu(II) in 1 M NH_3 at 50 mV/s show a clear difference for the electrochemical processes involving copper species at bare SPCEs and SPCEs modified with QDs (**Figure 1A**). The electrochemical processes found in the cathodic curve can be attributed to the reduction of Cu(II) to Cu(I), reduction of Cu(I) to Cu(0) and the oxygen reduction reaction (ORR), although the latter two

appear at a close potential and, therefore, their resolution is difficult. The electrochemical processes found in the anodic curve can be assigned to the oxidation of Cu(0) to Cu(I) and oxidation of Cu(I) to Cu(II) [15,16]. At initial Cu(II) concentrations below the concentration of dissolved O_2 (typically around 100 μM), a coupled chemical reaction to the electrochemical processes has a significant influence in the response obtained. This coupled reaction can be attributed to the oxidation of Cu(I) by O_2 to regenerate the initial Cu(II) at the electrode surface [17–19]. This is shown in the voltammograms as the appearance of a quasi-limiting current for the reduction of Cu(II) to Cu(I), and the disappearance of the anodic process, the oxidation of Cu(I) to Cu(II). This fact can be observed with higher resolution in the **Figure 1B**, where the voltammograms were recorded with a lower switching potential and lower Cu(II) concentration (40 μM). However, for electrodes modified with QDs, peak-shaped defined processes are observed. In this case, the influence of the coupled reaction is lower, the initial Cu(II) species is not regenerated as quickly at the electrode surface, and the Cu(I) species are available for the anodic reaction, which appears in the voltammogram as a peak-shaped response. Therefore, it seems clear that the QDs at the electrode surface are able to stabilize the Cu(I) species electro-generated in contrast to bare SPCEs, where the coupled reaction with O_2 occurs. The potentials of the electrochemical processes of the Cu(II)/Cu(I) redox couple are similar in presence and absence of QDs, so it seems that the stabilization is due to a weak adsorption of Cu(I) on the surface of the QDs, probably by interaction with the sulphide groups or with the functionalized layer. Something similar can be observed at more negative potentials for the Cu(I)/Cu(0) redox couple (see again **Figure 1A**). In presence of O_2 in NH_3 media, Cu(0) is typically oxidized to Cu(II) [17], so that the stripping process is quite diminished for bare SPCEs since Cu(0) is not stable. However, the response obtained for this same process at SPCEs modified with QDs is significantly higher. Therefore, the QDs are also able to stabilize the Cu(0) electro-generated at the electrode surface, preventing its oxidation by the O_2 present in the solution.

[FIGURE 1]

3.2. Electrochemical quantification of quantum dots by detection of stabilized copper species

Due to the enhanced voltammetric response of copper in presence of QDs, the anodic processes could be used with the aim to quantify these nanoparticles. **Figure 2A** shows the SWV response obtained for a solution of 40 µM Cu(II) in 1 M NH_3 after reduction at -1 V for 30s using bare SPCEs and SPCEs modified with 1 nM of QDs. A higher peak current was obtained for both anodic processes when QDs are present on the electrode. After the reduction of Cu(II) at -1 V, Cu(I) and Cu(0) species were generated and the QDs were able to stabilize these species, avoiding their chemical re-oxidation with dissolved O_2, and thus, showing higher voltammetric responses. Furthermore, this response increased with the concentration of QDs in the solution used for the electrode modification (data not shown). Both anodic processes could be used for the quantification of QDs, however, a study at different reduction potentials was conducted in order to find the largest signal/blank ratio. **Figure 2B** shows the SWV response obtained using a reduction potential of -0.8 and -0.4 V at bare and QDs-modified SPCEs. After reduction at -0.8 V, the largest signal/blank for the first anodic process (oxidation of Cu(0) to Cu(I)) was found, whereas -0.4 V was the optimal potential to discriminate the anodic signals of the Cu(I) to Cu(II) oxidation process. The latter anodic process and reduction potential was chosen for the following experiments as the ratio was larger in this case.

[FIGURE 2]

Other experimental parameters were optimized to find the optimal signal/blank ratio with the aim of being able to detect low QDs concentrations. A concentration of 25 µM Cu(II) was chosen from a wide range evaluated (10, 25, 40, 50, 100, 250, 500, 1000 and 2000 µM). With high Cu(II) concentrations, the behaviour on both electrodes (bare and modified with QDs) was similar. In such cases, Cu(II) is in excess of the O_2 concentration, so its effect is minimized. Interestingly, the scan

rate had a fairly significant influence for increasing the signal/blank ratio. In order to avoid increasing excessively the peak width, scan rate was optimized by varying the value of the potential step. The anodic signal obtained for the blank (bare electrode) remained practically unchanged after increasing the potential step, while that the signal obtained at QDs-modified electrodes increased with the potential step, which may indicate the different behaviour of the two processes. The optimum value of the potential step was 20 mV (scan rate of 400 mV/s), because at higher scan rates an interfering process appeared at a close potential, which is probably due to silver. Silver, composing the quasireference electrode, can also be oxidized in a NH_3 medium in presence of O_2 and Cu(II) [20], and its stripping signal (process governed by adsorption) increased significantly with the scan rate. The reduction time chosen was 30 s, enough to have the highest signal/blank ratio.

After the optimization of the experimental parameters that could affect the analytical signal, a calibration plot for increasing concentrations of QDs was registered. The voltammetric response is shown in **Figure 3A** and the peak current obtained was linearly proportional to the particle concentration of quantum dots in the initial solution (**Figure 3B**) following the equation: i_p (µA) = 1.0 ($\pm$0.2) + 16.2 ($\pm$0.2) [QDs] (nM), R^2=0.999. The linear range was from 0.05 to 2 nM, and the limit of detection, calculated as the concentration corresponding to three times the standard deviation of the estimate [21], was 37 pM. Considering the 4 µL employed as initial QDs solution, the detection limit corresponds to just 150 amol of QDs (9×10^7 nanoparticles), The reproducibility of the slopes was 4.8% (in terms of RSD, n=3). It is also interesting to estimate the surface coverage of the electrode with quantum dots and the fractional area covered at different QDs concentrations. Since these small nanoparticles cannot be clearly observed with techniques such as SEM or AFM due to the roughness and heterogeneous surface of screen-printed carbon, the total number of particles in the solution used for the modification was used. The area of the nanoparticles was estimated from TEM images obtained in a previous work [9], considering an ellipsoid shape.

The geometric area of the working electrode (5.15 mm^2) was also used for the calculations. The values obtained for the surface coverage were of 2.34x10^8, 4.68x10^9, 1.17x10^{10}, 2.34x10^{10}, 4.68x10^{10}, 9.35x10^{10} NPs/cm^2 and the fractional area covered was 0.005, 0.010, 0.024, 0.048, 0.096, 0.193 for the different quantum dots concentrations of 0.05, 0.1, 0.25, 0.5, 1 and 2 nM. After normalizing the net signal (blank subtracted) by the fractional covered area for each QDs concentration, very similar current values were obtained, in a range of 150-170 µA. This results show that the effect of the QDs is the same magnitude at different concentrations, and, probably, the nanoparticles behave independently of each other, at least in this range of surface coverages. The analytical figures of merit (sensitivity and detection limit) are improved over those presented by other electrochemical methods for the detection of QDs previously described in the literature [8,9,22–24], a fact that shows the high sensitivity of the method proposed in this work. In addition, in this case, it is not necessary to perform an acid digestion to release the metal ions from the nanoparticle in order to obtain a sensitive signal and, therefore, the method is simpler and the analysis take less time (just 30 s). Another work with a similar limit of detection for CdSe QDs (15 pM) was previously reported [10]. In this case, an external redox process such as the enhanced voltammetric response for borax reduction was also employed as the analytical signal. However, the sample volume used is larger than in our work, as we only used 4 µL. The good analytical characteristics of these studies show the usefulness of using external redox species for the detection of low concentrations of these nanoparticles. It is likely that these detection methods will ultimately displace the conventional electrochemical detection of QDs used in biosensing applications.

[FIGURE 3]

4. CONCLUSIONS

We report a novel electrochemical detection method for the determination of QDs concentration. This method uses the stabilization of electro-generated copper species in ammonia medium at QDs-modified electrodes. A great analytical performance is obtained using this method, being able to

detect QDs at the amol range, avoiding the tedious acid digestion typically performed for QDs electrochemical detection. This method could be useful to improve the detection in biosensing systems using QDs as electrochemical labels.

ACKNOWLEDGEMENTS

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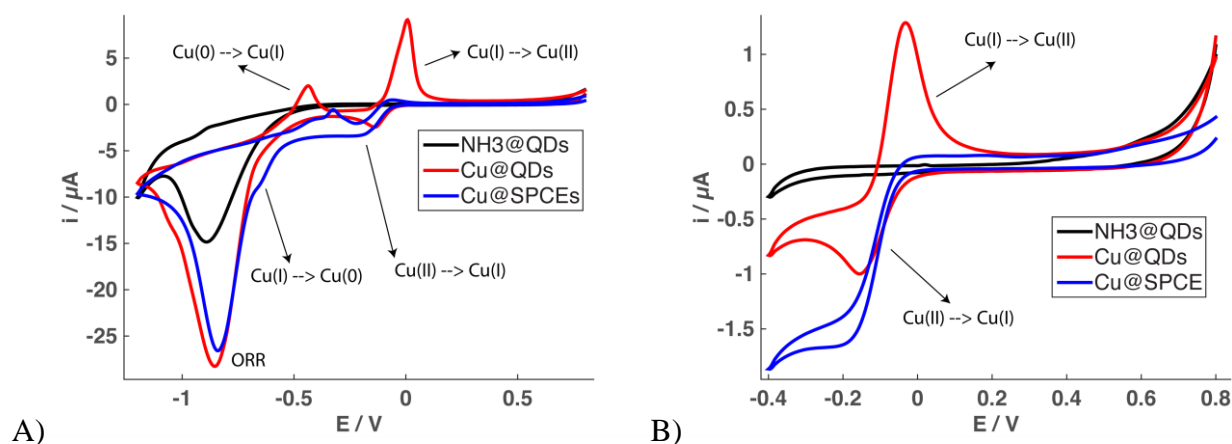
CAPTIONS

Figure 1. A) Cyclic voltammograms from +0.8 to -1.2 V of a solution of 60 μM Cu(II) in 1 M NH_3 at bare SPCEs and SPCEs-modified with 5 nM of QDs. **B)** Cyclic voltammograms from +0.8 to -0.4 V of a solution of 40 μM Cu(II) in 1 M NH_3 at bare SPCEs and SPCEs-modified with 5 nM of QDs. Voltammetric response from a blank (1 M NH_3) solution at QDs-modified electrodes is shown in both figures as comparison.

Figure 2. A) Square-wave voltammograms for a solution of 40 μM Cu(II) in 1 M NH_3 at bare SPCEs (black line) and modified with 1 nM of QDs (red line) after reduction at -1.1 V. **B)** Square-wave voltammograms

for a solution of 40 μM Cu(II) in 1 M NH$_3$ at bare SPCEs and modified with 1 nM of QDs after reduction at -0.8 V (red and black lines) and -0.4 V (green and blue lines). Reduction time was 60 s for both figures.

Figure 3. A) Square-voltammograms for copper response in order to perform the electrochemical detection of QDs at different concentrations (0, 0.05, 0.1, 0.25, 0.5, 1 and 2 nM). **B)** Associated calibration plot for QDs concentration.

Figure 1 - Color

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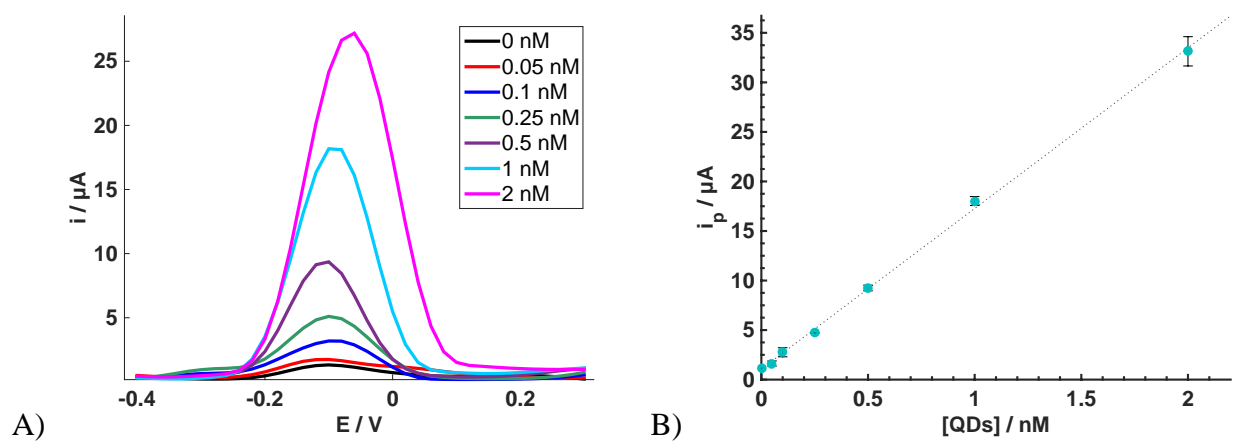
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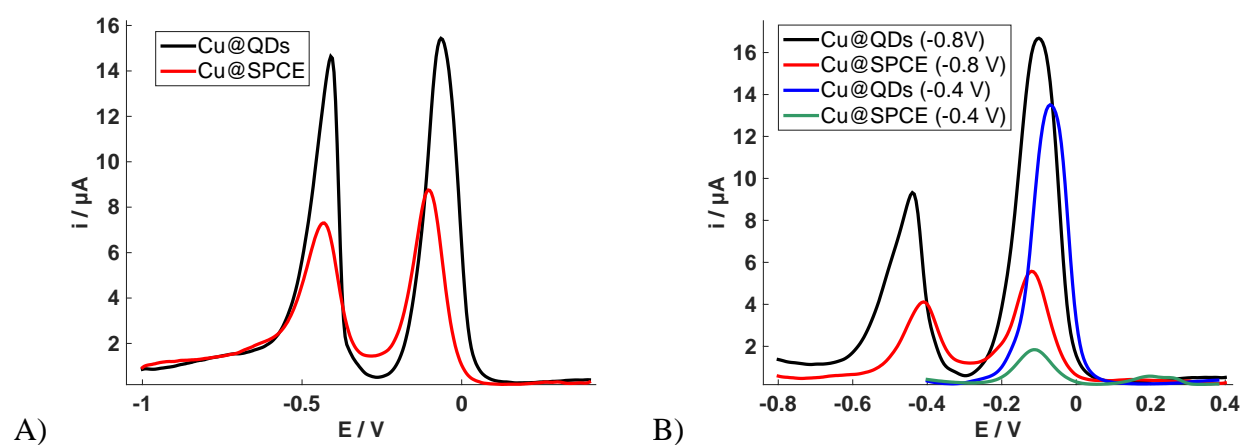
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