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Reverse-Micelle Synthesis of Electrochemically Encoded Quantum Dot Barcodes: Application to Electronic Coding of a Cancer Marker

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Abstract

Reproducible electrochemically encoded quantum dot (QD) barcodes were prepared by using the reverse-micelle synthetic approach. The encoding elements, Zn^{2+}, Cd^{2+}, Pb^{2+} were confined within a single QD, which eliminates the cumbersome encapsulation process used by other common nanoparticle-based barcode preparation schemes. The distinct voltammetric stripping patterns of Zn^{2+}, Cd^{2+}, Pb^{2+} at distinguishable potentials with controllable current intensities offer excellent encoding capability for the prepared electrochemical (EC) QDs. Additionally, the simultaneous modification of the QD barcode surface with organic ligands during the preparation process make them potentially useful in biomedical research. For proof of concept of their application in bioassays, the EC QD barcodes were further employed as tags for an immunoassay of a cancer marker, carcinoembryonic antigen (CEA). The voltammetric stripping response of the dissolved bardcode tags was proportional to log[CEA] in the range from 0.01 ng mL^{-1} to 80 ng mL^{-1}, with a detection limit of 3.3 pg mL^{-1}. The synthesized EC QD barcodes hold considerable potentials in biodetection, encrypted information and product tracking.

Introduction

With the rapid development of nanotechnology over the past decade, various nanomaterials, including nanowires, nanoparticles and nanotubes, have been synthesized. Due to their unique mechanical, electrical and optical properties, these nanomaterials have found important applications in drug discovery and delivery, catalysis, biodetections, information storage, environmental analysis and many other fields. One of the important applications of these nanostructured materials, however, is the use of these materials as barcodes for product tracking,[1,2] document protection and multiplexing of biological molecules.[3,4] In order to meet the needs for these important applications, different types of barcodes based on embedded organic dyes,[5,6] encapsulated QDs,[2,7] multisegmental nanowires[8–11] or layer-by-layer assembly of QDs[12,13] have been prepared and decoded by optical,[3–8,10] mangetic[9] or electrochemical[2,9,11,14] approaches. QDs have gained particular attention in bioanalysis recently owing to their unique size-tunable fluorescent properties.[15] The reported synthetic strategies for QD-based barcodes commonly rely on a two-step scenario. The barcoding QDs were first synthesized through a trioctyl phosphine/trioctyl phosphine oxide (TOP/TOPO)

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method under high temperature (260~300°C).[16] The synthesized QDs were then mixed with polymeric microbeads in organic solvents to allow the diffusion of QDs inside the microbeads, which eventually led to the formation of the QDs-encapsulated barcodes. Such barcode synthetic approach requires high temperatures and multiple steps, which make the preparation process technically complicated.

Herein, we report for the first time a convenient and reproducible route to prepare electrochemically-encoded QD barcodes based on a reverse-micelle system, which avoids the harsh conditions and encapsulation process common in other synthetic strategies. The reverse-micelle system has been widely used for nanoparticle preparation,[17–19] due to its simplicity and mild operation conditions. The reverse-micelle system normally consists of nanosized water droplets separated from the bulk organic phase and stabilized by a surfactant layer. These nanosized droplets act as micro-reactors, in which the size is controlled by the ratio of water to surfactant. The water droplets collide with each other constantly and exchange the water content, which result in the formation of the nanoparticles. By employing this reverse-micelle approach, the encoding ions (Zn^{2+}, Cd^{2+}, Pb^{2+}) are directly integrated into one single nanoparticle, instead of being encapsulated or assembled onto a microsphere carrier, leading to a more facile and reproducible QD barcodes preparation scheme. In addition, a layer of organic ligands is also introduced on the surfaces of the barcodes during the synthesis process for further functionalization with biomolecules. The acid dissolved QDs were decoded by the voltammetric stripping technique and the resulting patterns are dependent on the concentrations of the barcoding elements used for the QDs preparation. The peak positions and current intensities of the EC signatures of the QDs indicate the identities and pre-determined contents of the metal ions in the precursors used to synthesize the QD barcodes. Moreover, the synthesized EC QD barcodes were subsequently employed as tags in the immunodetection of the CEA cancer marker. The methodologies for reverse-micelle preparation of the EC QD barcodes and the barcode-tagged cancer marker biodetection are discussed in the following sections.

EXPERIMENTAL SECTION

Chemicals and Materials

Tris-HCl, sodium bis(2-ethylhexyl)sulfosuccinate (AOT), cysteamine hydrochloride, 2-mercaptoethane sulfonate, 11-mercaptoundecanoic acid, bovine serum albumin (BSA), Tween-20, *N*-(3-dimethylamminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDAC), and *N*-hydroxysulfosuccinimid sodium salt (NHS) were purchased from Sigma (St. Louis, MO). Heptane, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Pb(NO_3)_2$, Na_2S, pyridine, hexane and methanol were from Kelong Chemical Inc. (Chengdu, China). Zn^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} standard solutions were received from the Chinese CRM/CM Information Center (Beijing, China). Anti-CEA pre-coated ELISA plate, anti-CEA and CEA were obtained from Biocell Co., Ltd (Zhengzhou, China). All chemicals were of analytical reagent grade.

Apparatus

All electrochemical measurements were performed at room temperature (25 °C) in an electrochemical cell containing a glassy carbon disk working electrode (3 mm in diameter, CH Instruments Inc.), a Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode, in connection to a μAutolab III analysis system with GPES 4.9 software (Eco Chemie).

Preparation of the EC QD barcodes

The EC QD barcode nanoparticles were prepared according to a slightly-modified reported procedure.[20] For this purpose, 14.0 g of AOT was first dissolved in a mixture of n-heptane

(196 mL) and water (4 mL). The resulting solution was separated into two volumes of 120 mL and 80 mL. A 0.48 mL aliquot of a mixture containing $Zn(NO_3)_2$ (0.402 M), $Cd(NO_3)_2$ (0.134 M), $Pb(NO_3)_2$ (0.134 M) was added to the 120 mL solution, while 0.32 mL of 2.0 M Na_2S was added to the 80 mL solution. The two solutions were stirred separately for one hour, and then mixed together under nitrogen with continuous stirring. The nanocrystals were capped with organic ligands by adding cysteamine hydrochloride (0.34 mL, 0.32 M) and sodium 2-mercaptoethane sulfonate (0.66 mL, 0.32 M) and mixing under nitrogen for 24 hours. The resulting QD barcodes (ZnS-3/CdS-1/PbS-1) were collected by evaporating the heptane *in vacuo* and washing with pyridine, hexane and methanol. The ZnS-1/CdS-3/PbS-1 and ZnS-1/CdS-1/PbS-3 QD barcodes can be prepared using a similar protocol by changing the concentrations of $Cd(NO_3)_2$ or $Pb(NO_3)_2$ to 0.402 M, while maintaining the concentrations of the other two metal ions as 0.134 M respectively in the mixture. The CdS-1/PbS-1 QDs were synthesized by using 0.48 mL of a mixture containing $Cd(NO_3)_2$ (0.355 M), $Pb(NO_3)_2$ (0.355 M) and 0.32 mL of 2.0 M Na_2S solution as the precursors.

Electrochemical decoding of the QD barcodes

Two mg of the QD barcodes were dissolved by adding 200 μL of 1.0 M HNO_3, followed by a 30-min sonication. Subsequently, 5.0 μL of this solution was added to an electrochemical glass cell containing 2 mL of acetate buffer (0.2 M, pH 5.2) and 10 mg L^{-1} Hg^{2+}. The electrochemical stripping detection involved a 1 min pretreatment at +0.6 V, and 2 min electrodeposition at −1.4 V, and stripping from −1.3 V to −0.3 V using a square wave voltammetric waveform, with a 5 mV potential step, a 25 Hz frequency and an amplitude of 25 mV. Baseline corrections were made using a 'moving average' technique by μAutolab III software for data processing.

QD barcodes (CdS-1/PbS-1) tagged EC detection of CEA

The CdS-1/PbS-1 QDs were functionalized with carboxyl groups by mixing 3.2 mg of the QDs with 300 μL of 0.1 M 11-mercaptoundecanoic acid (dissolved in ethanol:water at 4:1 v/v) for 60 min. The excess thiol was removed by a 1 min centrifugation at 3000 rpm, and the particles were suspended in 60 μL of 20 mM phosphate buffer solution (PB , pH 8.6). Twenty μL of the above suspension were diluted to 180 μL with PB, to which 1.0 mg EDAC and 1.0 mg NHS was added and mixed for 30 min. The mixture was centrifuged for 3 min at 5000 rpm to remove the supernatant, and was then dispersed in 220 μL PB. This was followed by adding 20 μL of 1000 ppm signal anti-CEA and mixing for 1 h. The CdS-1/PbS-1 QDs/signal anti-CEA conjugates were collected by centrifugation and re-suspended in 1600 μL of Tris-HCl buffer (50 mM Tris-HCl, 140 mM NaCl, 1% BSA pH=7.4) for further use.

Fifty μL of the CEA samples at different concentrations (diluted in 50 mM Tris-HCl, 140 mM NaCl, 1% BSA pH=7.4) were added to a capture anti-CEA pre-coated ELISA plate and incubated for 30 min. The plate was then washed five times with Tris-HCl washing buffer (50 mM Tris-HCl, 140 mM NaCl, 1% BSA, 0.05% tween-20 pH=7.4). After that, 50 μL of the signal anti-CEA conjugated CdS-1/PbS-1 QDs were added to the plate and incubated for another 30 min, followed by extra five times of washing. An aliquot of 100 μL 1.0 M HNO_3 was then added to the plate. After 2 h, the solutions were transferred to the glass EC cell for measurements.

RESULTS AND DISCUSSION

The successful reverse-micelle synthesis of the EC QD barcodes is illustrated in Scheme 1. The Pb^{2+}-Cd^{2+} or Zn^{2+}-Cd^{2+}-Pb^{2+} containing micellar solutions were added to the S^{2-} containing micellar solution under a nitrogen environment. The growth of the nanoparticles, due to the formation of the metal sulfide precipitation, resulted in the production of the hybrid

QD barcodes. During the particle formation process, an organic layer was capped on the surfaces of the QDs for stabilization and bio-functionalization purposes. The resulting QD barcodes were subsequently decoded by voltammetric stripping technique.

Square wave voltammetric measurements were carried out on an in-situ Hg-film plated glassy carbon electrode as described in the experimental section. Voltammetric stripping is a powerful tool for detection of trace metal ions down to the sub-ppb level. By using Hg as the electrode material, more than 20 metal ions (Zn, Cd, Pb, Ga, In, etc.) can be detected voltammetrically owing to the large cathodic window of Hg. The voltammetric signatures of the mixtures of the standard metal ions (Zn^{2+}, Cd^{2+}, Pb^{2+}) at different concentration ratios are displayed in Figure 1. We can see that the stripping voltammograms exhibit three well resolved characteristic peaks of the metal ions at distinct potentials (Zn^{2+}: −1.0 V, Cd^{2+}: −0.68 V, Pb^{2+}: −0.5 V). In Figure 1Aa, with an equal concentration ratio of Pb^{2+} to Cd^{2+} (denoted by Cd-1/Pb-1), similar stripping current intensities are observed (ratio of 1.0:1.2). Figure 1Ab shows the voltammetric stripping patterns of a mixture of the three standard metal ions at a Zn-1/Cd-3/Pb-1 level. A clear higher current response from Cd^{2+} (−0.68 V) is observed due to a higher concentration of Cd^{2+} over Zn^{2+} and Pb^{2+} (at a ratio of 3:1:1) in the mixture. The corresponding experimental current intensity ratio is determined to be 1.0:20.6:6.3. When the concentration ratios of the metal ions were switched to Zn-3/Cd-1/Pb-1 and Zn-1/Cd-1/Pb-3, the current intensity ratios changed to 1.0:2.1:2.5 (Figure 1Ac) and 1.0:5.3:21.0 (Figure 1Ad), respectively. It should be noted that Zn^{2+} displays a smaller current intensity in all cases even with a higher concentration level than Pb^{2+} and Cd^{2+}, which probably is attributed to the coupling of the lower plating efficiency of zinc (associated with the deposition potential used) with the shorter “effective” deposition of zinc (during the scan period compared to cadmium and lead) and the slower quasi-reversible kinetics of its stripping oxidation process (compared to the nearly reversible stripping of cadmium and lead). Nevertheless, despite these differences, the stripping voltammetric protocol yields distinct and reproducible electronic decoding patterns for the corresponding QD barcodes, reflecting its high reproducibility.

In order to conduct a clear comparison, we chose identical concentration ratios of the metal ions in Figure 1A as the precursors to prepare the QD barcodes. Figure 1B displays typical voltammograms of the dissolved QD barcodes [CdS-1/PbS-1(e), ZnS-1/CdS-3/PbS-1 (f), ZnS-3/CdS-1/PbS-1 (g) and ZnS-1/CdS-1/PbS-3 (h)] prepared by different concentration ratios of the encoding metal ions. As expected, each QD barcode yields a characteristic voltammetric decoding pattern, which corresponds to experimental current intensity ratios of 1.0:1.3, 1.0:20.0:6.9, 1.0:1.8:2.4 and 1.0:5.7:20.7, respectively. The resulting stripping signatures of the dissolved QD barcodes are in good agreement with those of the standard metal ion mixtures, which indicates that the compositions of the QD barcodes are uniquely controlled by the compositions of the metal ions in the precursors. Based on the fact that the encoding capability of the EC QD barcodes relies on the number of well resolved current peak positions and intensities, thousands of barcode patterns can thus be generated by using 3~4 metal ions and 5~6 current levels (the number of codes equals n^m-1, m is the distinguishable components and n is the number of intensity levels).

Practical applications require the production of highly reproducible barcodes. The reproducibility of the new QD barcodes synthetic route was examined by six constant preparations of ZnS-1/CdS-1/PbS-3 barcodes and the corresponding stripping voltammograms are displayed in Figure 2. The relative standard deviations of the current intensities of PbS, CdS and ZnS are calculated to be 8.3%, 5.7% and 3.6%, respectively. The mean current intensity ratio (1.0:5.5:20.9) is close to that of the Zn-1/Cd-1/ Pb-3 precursor used to synthesize the QDs, indicating a good reproducibility of the reverse-micelle approach.

For proof of concept of the QD barcodes application in bioanalysis, the CdS-1/ PbS-1 QDs were used as EC tags for sensitive immunoassay of the CEA cancer marker. In this assay, the CEA target molecules with various concentrations were added to a capture anti-CEA pre-coated ELISA plate. After a 30-min binding event, non-specifically adsorbed CEA was washed off and the CdS-1/PbS-1 QDs conjugated signal anti-CEA were introduced to the ELISA plate to form a capture anti-CEA/CEA/signal anti-CEA-QD barcodes "sandwich" complex. The QD barcodes were then dissolved by HNO_3 and the EC responses of the released metal ions thus generated the analytical signals for CEA quantitation. The QD barcodes- based protein assay protocol and the resulting calibration plot are illustrated in Figure 3A and Figure 3C, respectively. From Figure 3B, we can see that with the increasing concentrations of CEA in the sample, the EC current intensities of the released Pb^{2+} and Cd^{2+} increase accordingly. The corresponding calibration plot for logarithmic concentration of CEA (Figure 3C) shows a linear range from 0.01 ng mL^{-1} to 80 ng mL^{-1} with an estimated detection limit of 3.3 pg mL^{-1} (based on the mean current of blank plus three times the standard deviation) according to the EC responses of Pb^{2+}. This low detection limit for CEA is attributed to the amplified EC responses of the numerous Pb^{2+} released from the QD barcodes. With the QD barcode-based signal amplification our CEA assay protocol shows about a 13-fold improvement in terms of sensitivity compared to other universal enzyme-based approaches.[21,22] Based on these encouraging results, we believe that the EC QD barcodes could be a powerful tool in biodetection.

CONCLUSIONS

In conclusion, we described the synthesis of a novel class of compositionally encoded electrochemical QD barcodes based on the reverse-micelle approach. Different encoding metal ions (Zn^{2+}, Cd^{2+}, Pb^{2+}) were confined in one single nanoparticle by using the reverse-micelle approach. The characteristic voltammetric stripping patterns of the dissolved QD barcodes correlate well with the predetermined metal ion concentration ratios used for the QD barcodes preparation. The distinct peak potential positions and current intensities of the metal ions reflect their high encoding capacities. The application of these barcodes as tags in sensitive immunoassays of CEA was also demonstrated. More powerful applications of the EC QD barcodes can be envisioned by eliminating the barcode dissolution step in connection to other decoding readout routes, such as X-Ray fluorescence (XRF), vibrating sample magnetometric (VSM)[9] and solid state EC monitoring. 23

Acknowledgments

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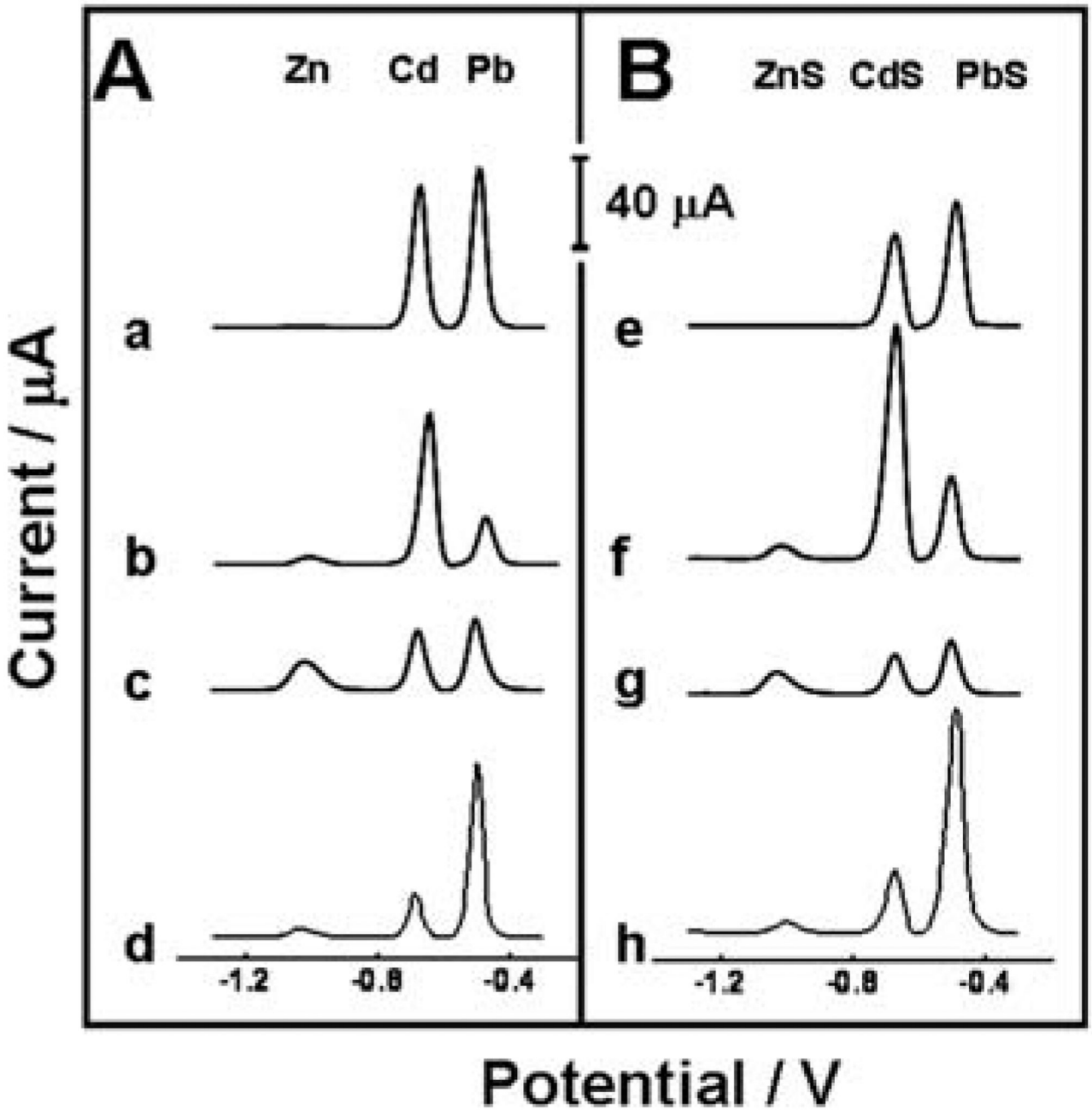


Figure 1.

Square wave voltammograms of (A) mixtures of standard metal ion solutions at different concentration ratios. (a) Cd-1/Pb-1 (1.0:1.2), (b) Zn-1/Cd-3/Pb-1 (1.0:20.6:6.3), (c) Zn-3/Cd-1/Pb-1 (1.0:2.1:2.5), (d) Zn-1/Cd-1/Pb-3 (1.0:5.3:21.0). (B) Voltammograms of acid dissolved QD barcodes. (e) CdS-1/PbS-1 (1.0:1.3), (f) ZnS-1/CdS-3/PbS-1 (1.0:20.0:6.9), (g) ZnS-3/CdS-1/PbS-1 (1.0:1.8:2.4), (h) ZnS-1/CdS-1/PbS-3 (1.0:5.7:20.7). The numbers in the brackets indicate the corresponding current intensity ratios. Dissolution of the QD barcodes was carried out by the addition of HNO_3 (200 μL, 1.0 M) and sonication for 30 min. A 5.0 μL aliquot of the resulting solution was transferred to the electrochemical cell containing 2 mL of acetate buffer (0.2 M, pH 5.2) and 10 mg L^{-1} Hg^{2+}. Electrochemical stripping detection proceeded

with a 1 min pretreatment at +0.6 V, 2 min accumulation at −1.4 V, and scanning the potential from −1.3 V to −0.3 V using an amplitude of 25 mV, a potential step of 5 mV and a frequency of 25 Hz.

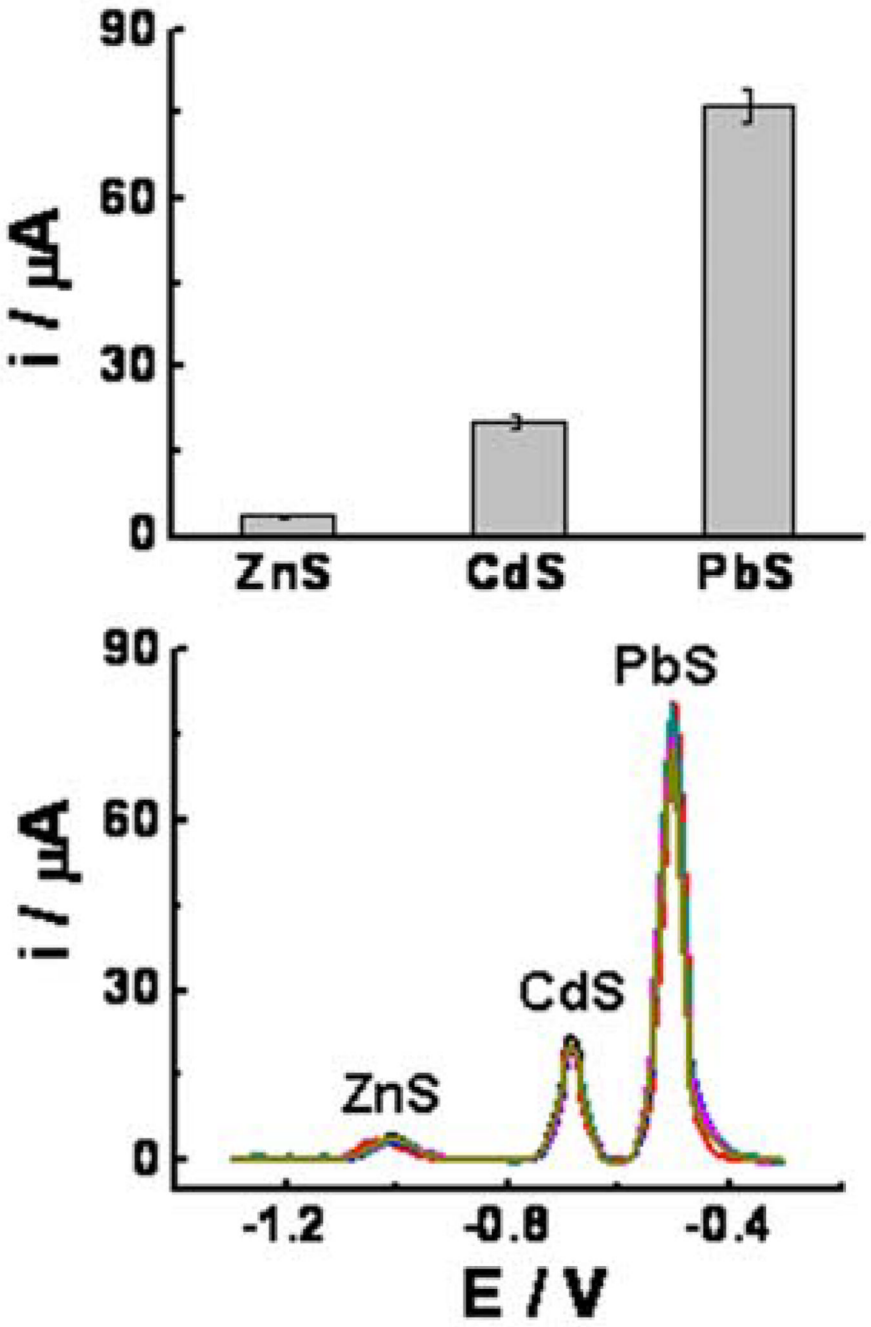


Figure 2.
Reproducibility investigation of the reverse-micelle approach for six repetitive preparations of the ZnS-1/CdS-1/PbS-3 QD barcodes at identical pre-determined metal ion concentration ratios. Other conditions, as in Figure 1.

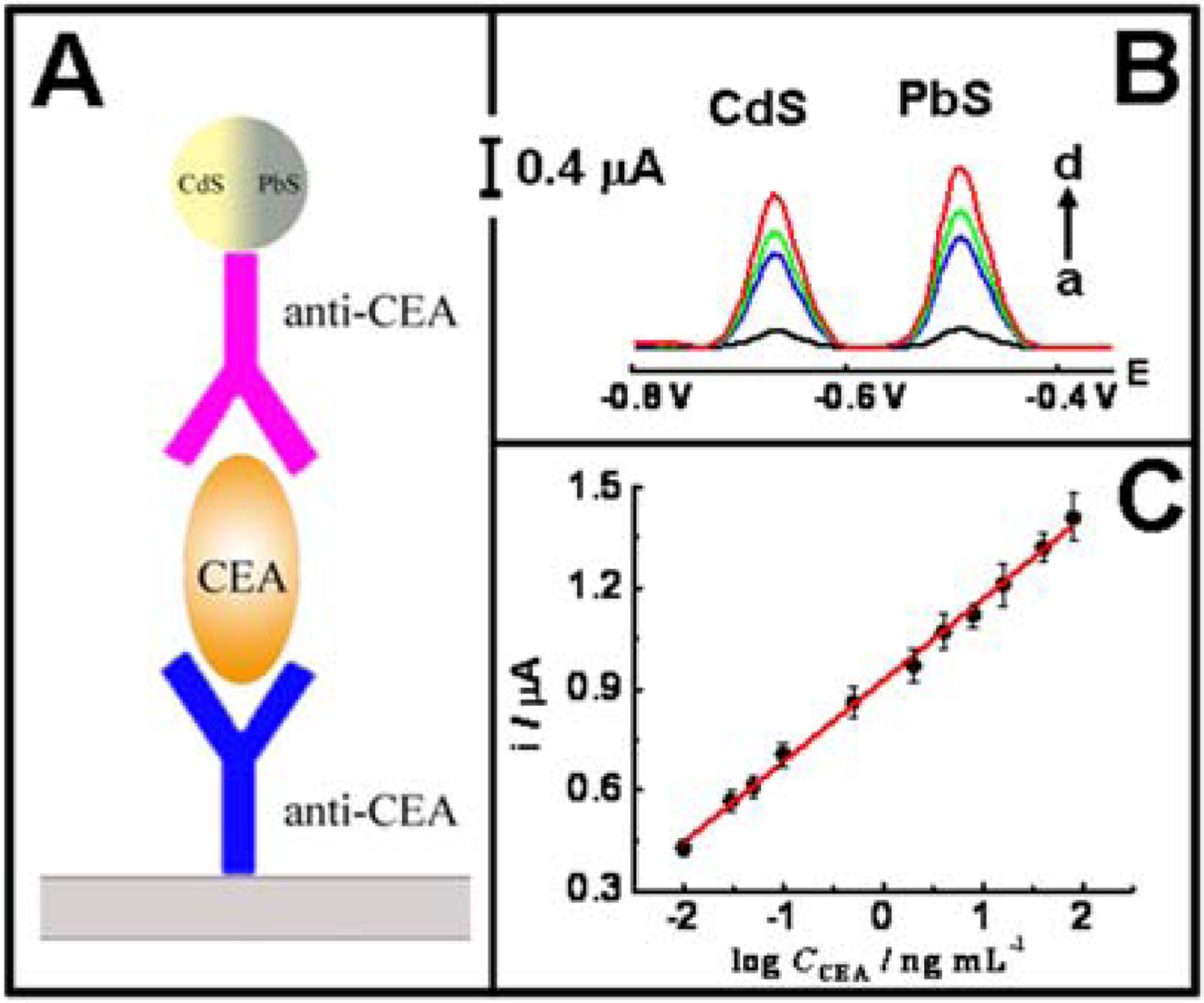
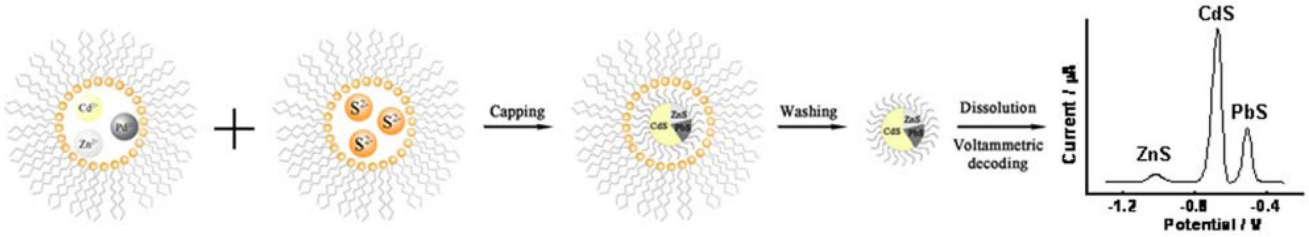


Figure 3.

(A) Schematic representation for the CdS-1/PbS-1 QD barcodes-tagged EC immunoassay of CEA. (B) EC Stripping responses of the dissolved CdS-1/PbS-1 barcode tags for increasing concentrations of CEA at (a) 0, (b) 0.5, (c) 4.0 and (d) 80.0 ng mL^{-1}. (C) The resulting calibration plot for log[CEA] over the 0.01 to 80 ng mL^{-1} range based on the EC responses of Pb^{2+} from the dissolved CdS-1/PbS-1 barcode tags. Error bars represent standard deviations of three parallel samples. Other conditions, as in Figure 1.



Scheme 1.
Illustration of the reverse-micelle-based QD barcodes preparation and of the EC stripping-voltammetric decoding of the acid dissolved QDs.