SYNTHESIS AND APPLICATIONS OF NANOPARTICLES IN BIOSENSING SYSTEMS

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Chapter 6

Electrochemical interrogation of cellular uptake of quantum dots decorated with a proline-rich cell penetrating peptide

Chapter 6 Electrochemical interrogation of cellular uptake

6.1 Introduction

Quantum dots (QDs) decorated with different types of biomolecule, for example antibodies[1-3], peptides[4] and DNA[5,6] are able to interact with cells and provide biological compatibility have been used. The use of QDs and other nanoparticles as tags for electrochemical detection of DNA[7] (including codified technology)[8,9] and proteins[10,11] has been extensively studied. An ICPMS-linked DNA assay based on gold nanoparticles immunoconnected through a peptide sequence has also been developed by our group[12].

Cell-penetrating peptides (CPPs) have been conceived as potential vectors to carry drugs that have low bioavailability across cell membranes[13-16]. Different CPPs have been described to efficiently deliver various types of cargo to the inside cells: from low molecular weight drugs to liposomes and plasmids. Among CPPs, the so-called SAP (Sweet Arrow Peptide) combines the ability to translocate the plasma membrane with a virtually complete lack of cytotoxicity[17,18]. SAP shares with other CPPs the presence of positively charged guanidinium groups, a feature that is considered very important for the cellular uptake[19]. In addition, in aqueous medium, SAP adopts a strongly amphipathic helical conformation[20].

We are particularly interested in electrochemical interrogation of the QDs cellular uptake because (1) previously reported methods based either on imaging techniques or on flow cytometry are both time consuming and usually related to more sophisticated and larger setups; (2) the electrochemical methods are simpler, lower cost and offer additional opportunities in relation to parallel monitoring of several other parameters where the electron transfer can be measured[21].

Following electrochemical methods the effect of anti-tumor drugs on the growth of immobilized tumor cells was performed by an irreversible voltammetric response related to the oxidation of guanine[22]. Ding et al. immobilized living cells on glassy carbon electrode and the proliferation of cells was measured by an electrochemical impedimetric technique due to the increase in the electron-transfer resistance by the growth of cells[23].

In this chapter we report on the use of square wave voltammetry to monitor the cellular uptake, in HeLa cells, of Quantum Dots (QD) decorated with Sweet Arrow Peptide (SAP).

6.2 Reagents and materials

Fmoc-Nα-protected amino acids were obtained from IRIS Biotech GmbH (Marktredwitz, Germany). The 2-chlorotrityl chloride resin was purchased from CBL-PATRAS (Patras, Greece). Coupling reagents: 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP) was obtained from Applied Biosystems (Foster City, CA); benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) from Novabiochem (Laüfelfingen, Switzerland); 1-hydroxy-7-azabenzotriazole (HOAt) from GLBiochem (Shanghai, China) and 2-(1Hbenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) from Albatros ChemInc. (Montreal, Canada). Trifluoroacetic acid (TFA) was purchased from Scharlab S.L. (Barcelona, Spain). Piperidine, dimethylformamide (DMF), dichloromethane (DCM) and acetonitrile were from SDS (Peypin, France). N,N-diisopropylethylamine (DIEA) was obtained from Merck (Darmstadt, Germany). Triisopropylsilane (TIS) was from Fluka (Buchs, Switzerland).

HeLa cells were obtained from ATCC (Manasas, VA) and cultured in DMEM (1000 mg/L glucose, Biological Industries) containing 10% fetal calf serum (FCS), 2 mM glutamine, 50 U/mL penicillin, and 0.05 g/mL streptomycin. Exponentially growing HeLa cells were detached from the culture flasks using a trypsin-0.25% EDTA solution, and the cell suspension was seeded at a concentration of 21.4 * 10^3 cells/cm^2 onto glass cover slips, 4-well Lab-Teck chambered coverglass, or plastic dishes (Nalge Nunc International, Rochester, NY).

CdS QDs glutathione was synthesized by arrested precipitation method (see chapter 3, section 3.1.5).

SAP (Sweet Arrow Peptide) was synthesized by Prof. E.Giralt's group at Barcelona University (www.pcb.ub.es/giralt/html/home.html) on 2-chlorotrityl chloride resin using the 9-fluorenylmethoxycarbonyl/tert-butyl (Fmoc/tBu) strategy and TBTU or PyBOP as coupling reagent. The peptide was cleaved using a cleavage mixture of 95% TFA, 2.5% TIS

and 2.5% water. It was purified by semipreparative RP-HPLC, and then characterized by analytical RP-HPLC and MALDI-TOF. For further details, see reference[17].

6.3 Equipment

Confocal laser scanning microscopy (CLSM) images were obtained using a Leica SPII microscope with a 63X objective.

The Z-potential measurements were performed using a zetasizer nano zs (Malvern).

All voltammetric experiments were performed using a PalmSens (Palm Instrument BV, Houten, The Netherlands) that consists of a portable potentiostat interfaced with a palmtop PC (155 mm × 85 mm × 35 mm) (see figure 4.1 section left). Electrochemical experiments were carried out using a screen-printed electrode (SPE) (Palm Instrument BV, Houten, The Netherlands). The screen-printing electrochemical cell (figure 4.1 section right) consists of a graphite working electrode (diameter 3 mm), a graphite counter-electrode and a silver pseudo-reference electrode.

6.4 QDs modified with SAP

SAP was added at a final concentration of 200 μM to a 2.4 mM CdS-QD solution in deionised water, and allowed to react under rapid stirring for 24h at room temperature. Dialysis (M.W.C.O. 6-8000) over deionised water for 3 days was performed to remove peptide excess.

6.5 Z potential measurements

The instrument was operating at a light source wavelength of 532 nm and a fixed scattering angle of 173°. These measurements were carried out at pH 7.0, ZP distribution was analysed by intensity.

6.6 Confocal fluorescence microscopy

HeLa cells were incubated with a CdS-QD or CdS-QD-C-SAP solution at 288 μM for 2 h; they were then washed three times with DMEM-10%FCS without phenol red. Then 1 μL of TRITC-WGA was added and confocal laser scanning microscopy was performed using a LeicaSPII microscope with a 63X lens under 364-nm excitation, acquiring 0.25 μm-thick optical sections.

6.7 Electrochemical measurements of HeLa cells

HeLa cells were incubated with a cultured medium containing CdS-QD or CdS-QD-SAP solution at 288 μM for 2 h at 37ºC under 5% CO_2.(figure 6.1 A) The supernatant was collected apart for the electrochemical measurements and the cells washed afterwards with PBS (3 times with 1.5 ml PBS during 1 min). 200 μl of 0.25 % trypsin-EDTA solution was added to the cells for detachment from the Petri dish followed by 5 min incubation at 37 ºC. 1 ml of DMEM was added then and the cell suspension was centrifuged (1000 rpm, 4 ºC, 4 min) (figure 6.1 B). The supernatant was removed and cells were resuspended in 200 μl of fresh DMEM; 100 μL of this cell suspension were kept in ice for the direct electrochemical measurements (figure 6.1 C). 1 ml of fresh DMEM was added to the rest and it was again centrifuged (1000 rpm, 4 ºC, 4 min). Supernatant was removed and 100 μl of water was added to the pellet and then cells were incubated during 1h at 37 ºC for cell lysis (figure 6.1 D).

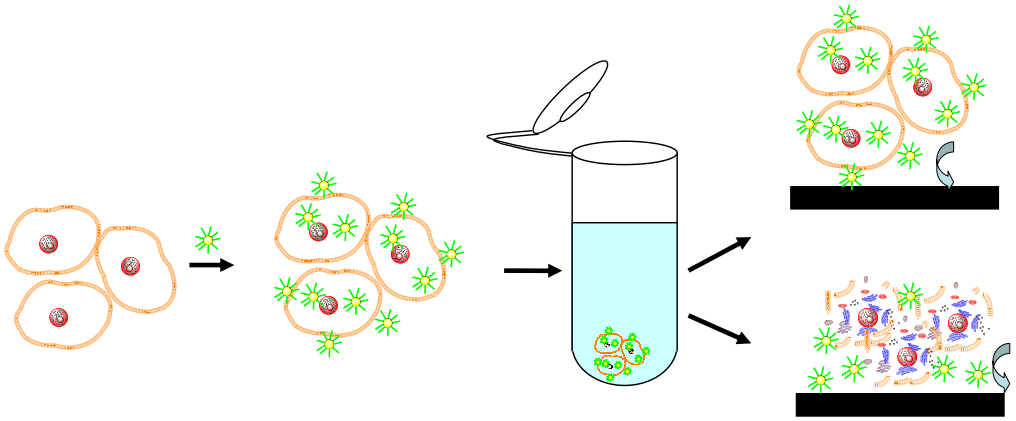

Figure 6.1 Schematic of the steps followed for the study of CdS QD-SAP interaction with HeLa cells. A) Positively charged CdS QD-SAP electrostatically interact with negatively charged membrane; B) The cells are separated; The electrochemical detection of CdS-QD-SAP adsorbed onto the cell surface (C) or after the cell lysis (D) have been performed.

The electrochemical measurements were performed suspending a volume of 20 µL over the sensor stripped in the horizontal position, to ensure electrical contact (complete circuit)[24]. Each SPE was pretreated, before using, by applying −1.1 V for 300 s[25], and then square wave voltammetric (SWV) scans were carried out until a low and stable background was obtained. SWV experiments were performed to evaluate the electrochemical behavior of the screen-printed electrode (SPE) for CdS QD detection. During this step, a drop of 20 µl containing an appropriate concentration of CdS QDs was suspended onto the SPE for 60 s and a potential of 0 V was applied (figure 6.2 A). The second step was the accumulation step. In this step a deposition potential of −1.1 V for 120 s was applied to promote the electrochemical reduction of Cd^{2+} ions contained in the CdS QD structure to Cd^0(figure 6.2 B). After the accumulation step, SWV was performed. During this step the potential was scanned from −1.1 to −0.5 V (step potential 10 mV, modulation amplitude 30 mV and frequency 15 Hz), resulting in an analytical signal due to the oxidation of Cd^0 (figure 6.2 C). After the SWV measurement the SPE was manually cleaned with a 0.1 mol l^{-1} phosphate buffer solution (pH 7.0). A blank subtraction method was performed[26]. The blank was measured using a separate blank solution (0.1 mol l^{-1}) phosphate buffer solution, pH 7.0). After this, using the same sensor, a determined volume of the QD suspension is added and measurements (SWV) performed by using the 'sample' option. In this way the subtracted curve was obtained.

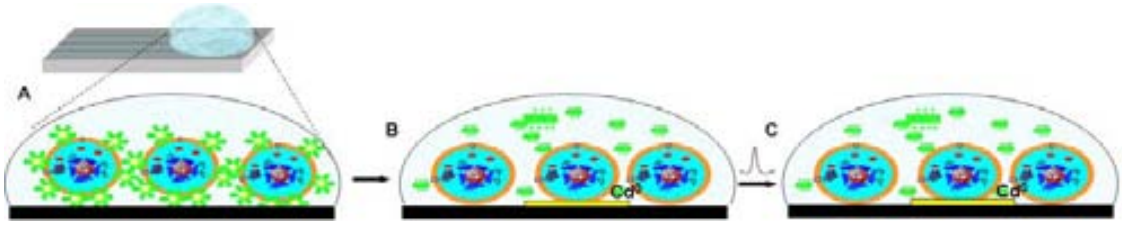

Figure 6.2 Schematic representation of electrochemical detection of HeLa cells labeled with CdS QDs-SAP. A). A drop of 20 µl CdS QD-SAP cell suspension is introduced onto the top of the SPE surface and a conditioning potential of 0 V was applied for 60 s. B). A deposition potential of -1.1 V for 120 s was applied during which the cadmium ions are reduced. C) The reduced cadmium is oxidized back to cadmium ions by SWV scanning from −1.1 to −0.5 V (step potential 10 mV, modulation amplitude 30 mV and frequency 15 Hz).

6.8 Results and discussion

The interaction of HeLa cells with QDs conjugated with sweet arrow peptide (SAP) have been followed by using square wave voltammetry technique.

Figure 6.3 shows that the charge of the QDs (-44 mV) after conjugation with SAP was shifted to less negative values (-2mV), a clear indication of the presence of the positively charged guanidinium groups from SAP molecules on the surface of the nanocrystal, a feature that should promote QD cellular uptake.

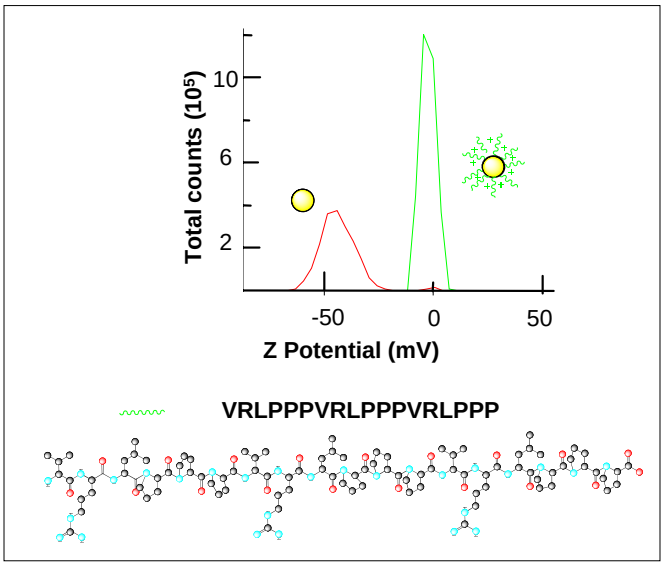

Figure 6.3 Upper part: Z potential diagram of CdS QD without (red) and with (green) SAP conjugated (Left). Lower part: Molecular structure of SAP.

The interaction of SAP decorated QDs with HeLa cells was first studied using confocal laser scanning microscopy (CLSM). The results obtained show that the QDs-SAP are partially internalized and partially remain on the cell membrane. On the contrary, the QDs without SAP did not show any kind of interaction with the cell (Figure 6.4 and CLMS images of figure 6.5)

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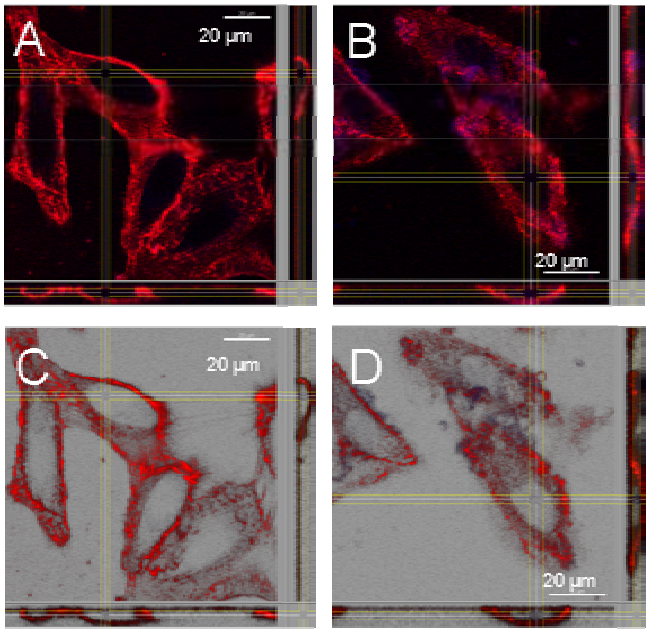

Figure 6.4 CLMS images of HeLa cells incubated with CdS QD (A, C) and HeLa cells incubated with CdS QD-SAP (B, D). Black contrast (A, B) and white contrast (C, D).

The confocal studies of both QDs and QDs-SAP interactions with the cells were followed by electrochemical detection using square wave voltammetry (SWV) as the measuring technique. SWV was previously shown to be a highly sensitive detection technique for CdS QD using SPEs.

Figure 6.5 shows the SWV curve obtained for the Hela Cells incubated with QDs (A, left) and QDs-SAP (B, left). The current magnitude is directly related to the quantity of CdS QDs through the SWV stripping of the corresponding cadmium ions. HeLa cells incubated with QDs without SAP do not interact with cell-membrane and consequently the SWV did not show a pronounced current peak. By the contrary, a current peak of around 2.4 µA is obtained for HeLa cells that interact with CdS QDs-SAP.

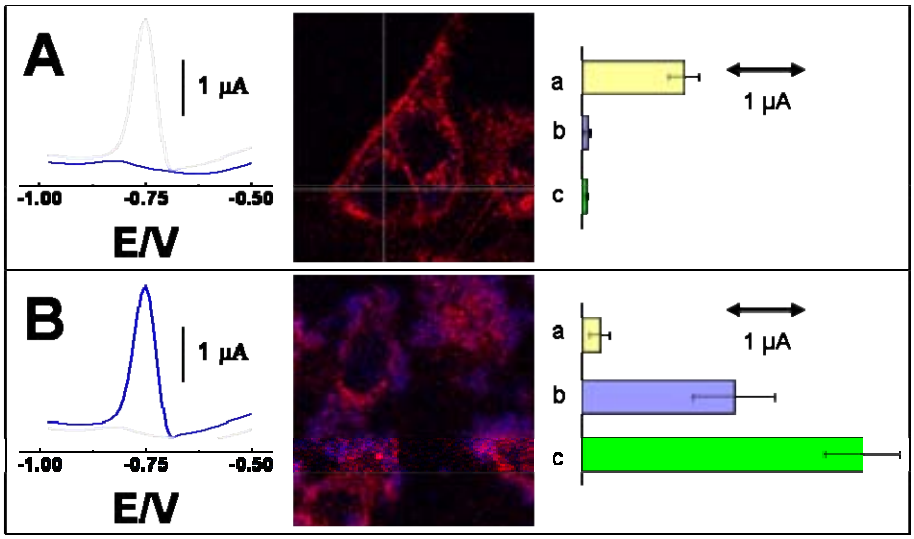

Figure 6.5 A) SWV of cells incubated with QDs (left); CLSM image of the same cells (the red color is related to the cell membrane); SWV peak of the supernatant (a), cells solutions (b) and lysis cells solution (c). B) SWV of cells incubated with QDs-SAP (left); CLSM image of the same cells (the red color is related to the cell membrane); SWV peak of the supernatant (a), cells solutions (b) and lysis cells solution (c).

Figure 6.5 A&B (right bars) shows the SWV peaks of supernatant (a), non lysed (b) and lysed (c) HeLa cells. It can be observed that in the case of HeLa cells incubated with QDs non-functionalised with SAP the supernatant contains almost all the CdS QDs used for incubation and consequently the SWV peak is higher (see Figure 6.5A-a). The SWV of the HeLa cell suspension (obtained after centrifugation and reconstitution with culture medium) did not give a significant peak (Figure 6.5A-b) which means that there were no QDs attached to the cell surface. The same SWV response was obtained after the HeLa cell lysis, which means no internalization occurred. The same experiments were performed for the case of HeLa cell incubation with SAP modified QDs. In that case, the QDs concentration in the supernatant was very low (Figure 6.5 B-a) due to the consumption of QDs, either due to cellular uptake (Figure 6.5 B-c) or due to adsorption on the plasma membrane (Figure 6.5 B-b).

The obtained results show that QDs-SAP either interact with the extracellular cell membrane matrix or translocate the bilayer. The first situation, membrane adsorption, is probably a transient state before cellular uptake. Both confocal microscopy and SWV results support the detection of this cellular internalization process.

6.9 Conclusions

The developed electrochemical detection strategy represents an interesting alternative due to the simplicity of the sensor (SPEs) and the measuring instrument (handheld potentiostatic unit).

The obtained results support the continuity of the application of this electrochemical interrogation technique. It can provide valuable insights into the study of peptide-mediated delivery as well as the design and development of nanoparticle probes for intracellular imaging, diagnostic and therapeutic applications. Another important field for future applications could be targeting specific intracellular organelles. Being the nucleus the desirable target because that is where the genetic information on the cell and transcription machinery reside, it could be an increasing research area for such applications. In addition, the described electrochemical interrogation is of low cost, is easy to use and offers future interest for diagnostics including cell analysis.

6.10 References

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Chapter 7

Global conclusions and future perspectives

Chapter 7 Global conclusions and future perspectives

7.1 Global conclusions

Several methods for the synthesis of nanoparticles (i.e. cadmium sulphide quantum dots, silver nanoparticles and gold nanoparticles) with interest in applications in electrochemical biosensing systems have been studied. Colloidal nanocrystals, dispersed in a solvent, allow stabilization in a way that prevents agglomeration. The most dispersed cadmium sulphide nanoparticles are those obtained by arrested precipitation method.

One of the most important attribute of colloidal nanocrystals for use in electrochemical sensing systems is the facility to be detected by any conventional electroanalytical method. Considering their applications as labels, their size distribution ensures enough reproducibility of the electrochemical bioassays.

A novel strategy for the electrochemical detection of quantum dots has been developed. The used stripping voltammetry method avoids the previous dissolution of the nanoparticles making possible their use as labels in affinity biosensors. The small label size, the established bioconjugation chemistry and the electrical properties make the CdS QDs an excellent tool for DNA detection.

The analytical signal used for the CdS QDs quantification comes from a mixed phenomenon detection which depends on the medium pH. The analytical protocols have been optimized. The results obtained show a wide, linear response range as well as a CdS QD detection limit that is of interest for various applications ranging from DNA analysis to immunoassays. The proposed technique represents a lower cost alternative to optical methods and will be of interest for fast screening as well as in field analysis.

Electrochemical method used for QDs label detection may be very promising taking into account their high sensitivity, low detection limit, selectivity, simplicity, low cost, and availability of portable instruments. Other QDs such as ZnS, PbS etc can be obtained and detected in a similar mode opening the way for multidetection based affinity biosensors.

Electrochemical genosensors based on labeling with QDs and the use of magnetic beads (MB) as platform for the immobilization of capture DNA probe have been also developed in this thesis. The developed magnetically assisted DNA sensor gave well defined signal whereas no signal was observed for non-complementary DNA. In addition to this, there is a possibility of applying the same detection methodology to an array composed of

several electrodes and using the same quantum dots but allowing the simultaneous and independent detection of each tag connected with the corresponding DNA target.

Beside the advantage of its use as capture platform, the MB bioconjugated can be separated from other species of the matrix simply by applying the magnetic force. This takes advantage of rapid magnetic separation (~30 s), which is in sharp contrast to conventional bioseparation processes (usually hours), such as chromatography and centrifugation. During the development of this thesis has been demonstrated that the use of MB offers unprecedented advantages in this respect, because are sufficiently robust to allow repetitive washing under moderately stringent conditions, thus exhibiting the ability to efficiently remove non-specific species. Moreover, using a handheld device and screen-printed electrodes the detection methodology is compatible with its possible use even in a doctor's office with the minimum waste of reagents and low cost.

CdS QDs are also conjugated to a peptide (SAP, Sweet Arrow Peptide) and their interaction with HeLa cells is studied. The electrochemical detection of cells with the adsorbed QDs-SAP or the lysed cells (uptake QDs-SAP released) was also carried out. The obtained results support the continuity of the application of this electrochemical interrogation technique for cell studies. It can provide valuable insights into the study of peptide-mediated delivery as well as the design and development of nanoparticle probes for intracellular imaging, diagnostic and therapeutic applications. In addition, the described electrochemical interrogation is of low cost, is easy to be used and it offers future interest for diagnostics including cell analysis

DNA and cell electrochemical sensing strategies based on CdS QDs are successfully demonstrated and their use for real samples is viable. Such DNA biosensors and cell sensor hold an enormous application potential principally for clinic diagnostic among other fields.

7.2 Future perspectives

As reported in this thesis the lowest detection limit (LDL) obtained for the model genosensor was of 30 pmol for sandwich system assay by using 4 nm diameter CdS QDs. However, the obtained LDL could be further lowered by using diverse amplification techniques. Cadmium enhancement method, based on the catalytic effect of hydroquinone on the chemical reduction (electro catalytic deposition) of cadmium can be used to deposit

cadmium onto the surface of CdS QDs. This would increase the cadmium quantity available for stripping analysis increasing by this way the sensitivity. Another possible way to decrease the detection limit can be the previous 1:1 conjugation of quantum dots with thiolated DNA so as to control the number of thiolated DNA and avoid the net formation that decrease the sensitivity. (See figure 7.1)

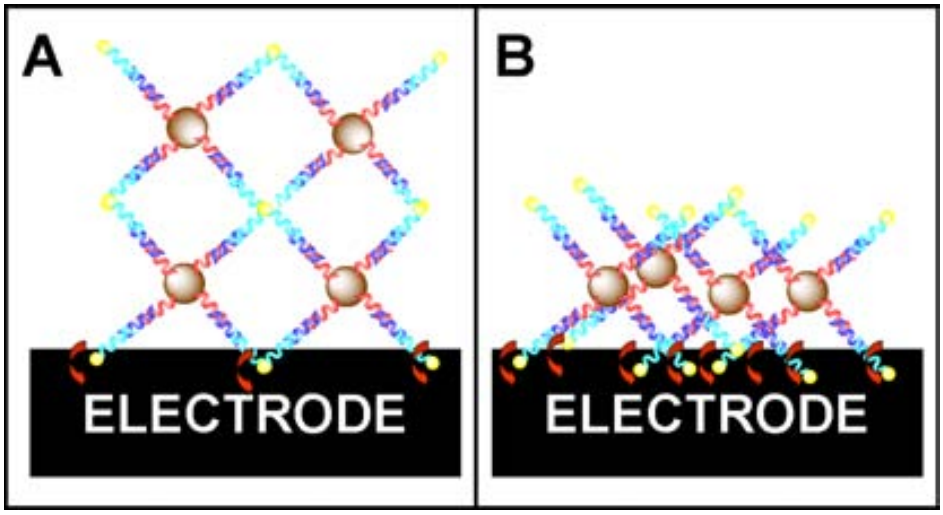

Figure 7.1 Schematic of MBs coated with DNA-QDs: A) Without previous conjugation of QDs with DNA-SH B) With previous 1:1 QDs / DNA-SH conjugation. The red arrow shows the electrical contact of QDs with the sensing surface. In the firsat case (A) there are less QDs in contact with electrode because of the the formed net.

The future applications of the developed strategies for DNA and cell studies require further developments in the following directions:

- Improvements of the transducing platforms. Control of the carbon ink composition so as to ensure enough reproducibility between different screen-printed electrode batches.

- Development of novel designs for the integration of sampling system. This would require development of microfluidic based sampling so as to reduce the volume of the sample as well as to facilitate the sample entrance.

- A strong collaboration with a clinical laboratory (among other fields) is necessary so as to strictly control and evaluate the interferences as well as compare the results obtained with standard methods (i.e. real time PCR for DNA, flow cytometry for cells etc.). This collaboration will also ensure finding of applications ‘niches’ for the developed sensing strategies.

- The application of the developed methods for protein detection would also open a broad range of novel immunosensing applications with interest for diagnostics between others.

Chapter 8

Publications

New materials for electrochemical sensing V: Nanoparticles for DNA labelling

Merkoçi A., Aldavert M., **Marín S**., Alegret S.

Trends in Analytical Chemistry

24, 341-349 (2005)

New materials for electrochemical sensing V: Nanoparticles for DNA labeling

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We present recent trends and challenges in the use of nanoparticles as DNA labels in electrochemical detection systems. We discuss nanoparticle synthesis procedures and methods for attaching biological molecules that enable their application in genosensors. We also discuss typical electrochemical procedures based on gold nanoparticles and quantum dots (QDs), along with QD carriers, such as carbon nanotube or polystyrene beads. High sensitivity and selectivity, simultaneous detection of various DNA sequences, and cost-efficient detection are advantages that seem to come using these novel labels in DNA electrochemical detection schemes.

Keywords: DNA; Electrochemical sensing; Genosensors; Nanoparticles; QD; Quantum dots; Semiconductors; Stripping potentiometry; Stripping voltammetry

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1. Introduction

Electrochemical sensors are playing a growing role in various fields where an accurate, low-cost, fast, on-line measuring system is required. Improvement of existing systems and the design of new concepts requires continual upgrading of all steps of the electronalytical process. The use of particulate materials (e.g., carbon powder) has been of great importance in electroanalysis. Conducting composites and biocomposites based on carbon powder mixed with polymers and biological materials are interesting alternatives for the construction of electrochemical (bio)sensors (Parts I and II) [1,2]. The particles, with diameters in the range 10–400 μm, are also known as beads or microspheres, which have been shown to be of interest for their application in flow systems with electrochemical detection (Part III) [3]. Even molecular imprinted polymers (MIPs), usually obtained as bead-particulate materials, have been applied in various electroanalytical systems (Part IV) [4]. As can be seen from the previous articles [1–4], various μm-size particles have been shown to bring a series of advantages in the design of novel electrochemical sensing systems. In the present article, we will show that the decrease of the material size from μm to nm scale has great impact, especially in the design electrochemical genosensors.

The development of sensitive non-isotopic detection systems has significantly improved many research areas, such as DNA sequencing, clinical diagnostics, and fundamental molecular biology. Affinity electrochemical biosensors, based on enzyme labeling, solve the problems of radioactive detection (e.g., health hazards and short lifetimes) and open new possibilities in ultrasensitive and automated biological assays. Nevertheless, biological research and other scientific and technological fields need a broader range of more reliable, more robust labels to enable high-throughput bioanalysis and simultaneous multi-analyte determination. The existing labeling techniques (e.g., enzymes or isotopes) have several drawbacks: the markers used have short life-times; and, they have a limited number of practical combinations (of more than two enzymes) for simultaneous analysis of various analytes.

To improve the sensitivity of electrochemical assays and to achieve better, more reliable analysis, there is a great demand for labels with higher specific activity. The most commonly used labels for electrochemical sensors to date have been enzymes and small molecules, such as electroactive indicators (e.g., dyes). In principle, nanoparticles provide a novel platform for improving specific activity of a

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label as well as affinity to the tracer molecules (DNA probes or other biomolecules). Nanosized particles have a chemical behavior similar to small molecules and can be used as specific electrochemical labels. Nanoparticles in general and quantum dots (QDs) in particular may be expected to be superior in several ways. A QD is a semiconductor particle that has all three dimensions confined to the 1–10-nm length scale [5]. Compared to existing labels, nanoparticles in general and QDs in particular are more stable and cheaper. They allow more flexibility, faster binding kinetics (similar to those in a homogeneous solution), high sensitivity and high reaction rates for many types of multiplexed assays, ranging from immunoassays to DNA analysis.

Despite the enormous opportunities clearly offered by electrochemical DNA sensing, some important hurdles remain. The first depend on the electrode probes themselves and their fabrication into useful arrays. Array sizes of the order of 10 have thus far been demonstrated, but arrays of 50–100 sequences will more typically be needed for clinical applications (e.g., genetic screening for cystic fibrosis carriers requires testing for 25 different mutations plus positive and negative controls [6]). Although it is not difficult to fashion electrode pads with reproducible dimensions of 1 μm or less, the electrochemical readout requires mechanical connections to each individual electrode. The construction of very large, multiplexed arrays (of the order of 10^3) therefore presents a major engineering challenge. Labeling technology using nanoparticles may provide a possible solution for this problem.

Nanoparticles, such as QDs or polystyrene particles loaded with QDs with distinguishable electrochemical properties, can be used to ''barcode'' DNA and proteins. The basic concept relies on the development of a large number of smart nanostructures with different electrochemical properties that have molecular-recognition abilities and built-in codes for rapid target identification. For example, the surface of a polymer bead can be bonded to biomolecular probes, such as oligonucleotides (short nucleotide chains) and antibodies, and identification codes are embedded in the interior of the bead. By integrating molecular recognition and electrochemical coding, each bead could be considered a tiny ''chemical lab'' (lab-on-a-bead) that detects and analyses a unique sequence or compound in a complex mixture.

This review will focus on current progress in applying nanoparticles to DNA-sequence detection based on electrochemical schemes. It covers both the synthesis of nanoparticles and some representative procedures to attach them to DNA targets and probes. We will show the application of electroactive labels ranging from gold nanoparticles and QDs to carbon nanotubes or polystyrene beads loaded with QDs in a variety of DNA electrochemical detection schemes.

2. Nanoparticles for bioanalytical applications

2.1. Synthesis

The most important attribute for colloidal nanocrystals to be useful in electrochemical sensing systems is the obvious facility to be detected by any conventional electroanalytical method. Their size distribution should be as narrow as possible to ensure enough reproducibility during the electrochemical assays when used as a label for biological molecules. Colloidal nanocrystals, dispersed in a solvent, should allow stabilization in a way that prevents agglomeration.

Nanoparticles can be synthesized from various materials, the most important from electrochemical point of view being gold and semiconductor materials (or QDs).

2.1.1. Gold nanoparticles. Homogeneous preparations of gold nanoparticles in the range 3–20 nm can be easily prepared. Various procedures on the preparation of gold nanoparticles have been reported [7,8]. Colloidal gold can be synthesized with high quality in organic or aqueous solution by inexpensive procedures. They are generally based on the reduction of Au(III) (from hydrogen tetrachloroacurate trihydrate, $HAuCl_4 \cdot 3H_2O$) to Au(0) by using sodium borohydride ($NaBH_4$) [9]. Most proteins can be easily coupled to colloidal gold particles and additionally they do not appear to lose their biological activity.

2.1.2. Quantum dots. Quantum dots, also known as semiconductor particles, are crystalline clusters of a few hundred to a few thousands of atoms with sizes of a few nm [5]. Fig. 1 presents the sizes of various materials, ranging from an atom to an animal cell, including also existing labels, where the size range of QD nanocrystals

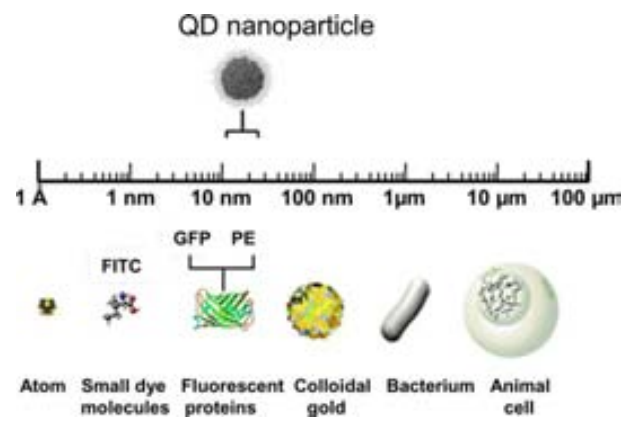

Figure 1. The size of a quantum dot (QD) compared to that of other materials. QD nanoparticle refers to quantum dots that have been solubilized and conjugated to affinity molecules. GFP, Green fluorescent protein; PE, Phycoerythrin; FITC, Fluoresceine-isothiocyanate.

that have been solubilized and conjugated to affinity molecules can be seen.

Several synthetic methods for the preparation of semiconductor nanoparticles have been reported [10]. They are based on pattern formation (colloidal self-assembled pattern formation by surfactant micellation) [11–14], organometallic thermolysis [15] or electrochemical deposition [16].

Pattern formation and organometallic thermolysis routes are the principal methods in use. Following these routes, QDs are produced as single-nanocrystaline materials, such as CdS, or else as core/shell hybrids, such as CdSe/ZnS. Although not frequently used in electrochemical sensing to date, cadmium selenide (CdSe) and indium arsenide (InAs) are most often reported due to their use as semiconductor particles. In the absence of stabilizing materials, the QDs are prone to aggregation, so they must be derivatized to preserve them as single, non-aggregated entities.

Organometallic thermolysis is generally performed in and organic solvent (trioctylphosphine oxide [TOPO] or trioctylphosphine [TOP]) at high temperature [17]. The precursors (Se; $Cd(CH)_3$; and, $InCl_3$ dissolved in tributylphosphine) are quickly injected into the rapidly stirred hot solvent. The QDs immediately start to nucleate. The desired size of the nanocrystals can be adjusted by changing the amount of injected precursors and the time that they are left to be grown in the hot surfactant solvent. The nanocrystals obtained are hydrophobic, since they are covered with a surfactant layer. The surfactant molecules also serve as stabilizers preventing agglomeration of the QDs.

The QDs can also be formed in the so-called reverse micelle mode. This technique is based on the natural structures created by water-in-oil mixtures upon adding an amphiphilic surfactant, such as sodium dioctyl sulfosuccinate (AOT) (see Fig. 2). By varying the water content of the mixture, the size of the water droplets suspended in the oil phase could be varied systematically. This led to the idea of using these self-enclosed water pools as micro-reactors for carrying out sustained nanoscale chemical reactions. A series of micelle-protected PbS nanoparticles were synthesized using lead acetate and alkanethiols [18,19].

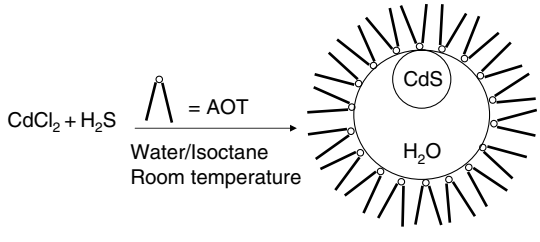

Figure 2. Growth of CdS QD nanoparticles in reverse micelles. This technique exploits natural geometrical structures created by water-in-oil mixtures upon adding an amphilic surfactant, such as sodium dioctyl sulfosuccinate (AOT). By varying the water content of the mixture, it was shown that the size of the water droplets suspended in the oil phase could be varied systematically.

A novel approach was developed to prepare thin films of nanosized ZnS-passivated CdS particles *via* metal–organic chemical vapor deposition (MOCVD) [20]. Another strategy, such as molecular beam epitaxy [21], has also been used for producing nanoparticles and can also be considered (along with other strategies not yet reported) in applications of electrochemical-sensor designs.

2.2. Water solubilization

Before application to bioanalytical assays, the hydrophobic nanoparticles produced (prepared as explained in Section 2.1) must first be modified again so as to be transformed to a water-soluble product. The resulting hydrophilic nanoparticles will then be attached to biological molecules through a bioconjugation step (see Section 2.4 below). Usually, these two steps are carried out independently. Alternatively, the water-solubilization and bioconjugation steps can be performed simultaneously. Using either strategy, it is important to maximize the stability of the linkages that connect the biomolecule to the nanocrystal.

As mentioned above, QDs are generally produced with an outer layer comprising one or more organic ligands, such as TOP or TOPO. These ligands are hydrophobic, so nanocrystals covered with these coatings are not compatible with aqueous assay conditions. For this reason, hydrophilic layer agents must be introduced after synthesis of the QDs. The easiest way to obtain a hydrophilic surface is by exchanging the hydrophobic TOPO or TOP surfactant molecules with bifunctional molecules, such as mercaptocarboxylic, mercaptoacetic, mercaptopropionic or mercaptodecanoic acids that are hydrophilic on one end and bind to QDs (for example ZnS) with the other end (–SH). Carboxyl groups are negatively charged at neutral pH. QDs capped with carboxyl groups repel each other electrostatically, thus avoiding aggregation [7].

Another strategy to convert QDs into water-soluble particles was used by Alivisatos and co-workers [22]. They coated CdS/CdS (core/shell) QDs with a layer of silica.

2.3. Encapsulation

Other strategies to maintain colloidal stability of QDs and decrease the non-specific adsorption are the encapsulation into micelles or other particles.

2.3.1. Phospholipid micelles. QDs can be encapsulated in the hydrophobic core of a micelle comprising *n*-poly (ethyleneglycol) phosphatidylethanolamine (PEG–PE) and phosphatidylcholine (PC) (see Fig. 3(a)) [23]. The

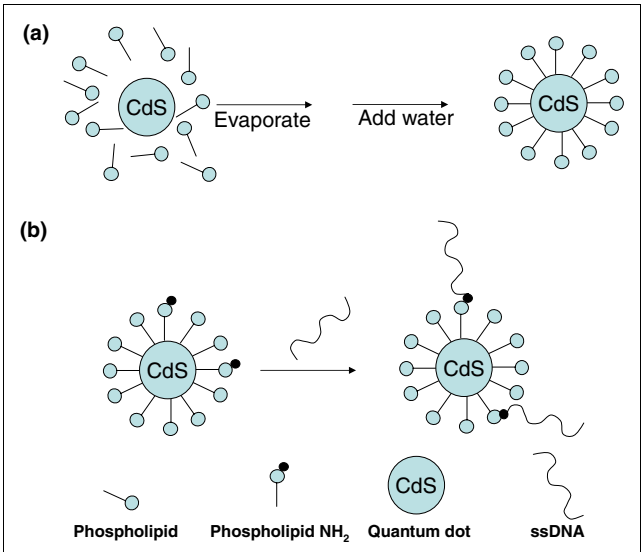

Figure 3. (a) Encapsulation of QDs in micelles; and, (b) QD–micelle conjugation with single-stranded DNA (ssDNA). CdS QDs can be encapsulated in the hydrophobic core of a micelle composed of a mixture of *n*-poly(ethyleneglycol) phosphatidylethanolamine (PEG–PE) and phosphatidylcholine (PC).

advantage of these micelles is that they are very regular in size, shape, and structure. In addition, their outer surface of PEG acts as an excellent repellent for biomolecules.

2.3.2. Polymeric beads. Encapsulation of QDs within polystyrene beads has been developed for electrochemical identification [24]. Cadmium-sulfide, lead-sulfide and zinc-sulfide nanoparticles were deposited onto polystyrene beads to create a library of electrochemical codes.

2.3.3. Carbon nanotubes. Single-wall carbon nanotubes (SWCNTs) carrying a large number of CdS QDs are used as labels for DNA detection [25]. The SWCNTs were first acetone activated and then the CdS nanoparticles were anchored onto the activated surface followed by attachment of streptavidin. The SWCNT–CdS streptavidin were used as labels for the biotinylated DNA probes.

2.4. Biological modification

The chemistry by which oligonucleotides are attached to nanoparticles has a significant impact on their use in the analytical detection scheme. Nanoparticles functionalized in different ways have different oligonucleotide surface densities, different availability for hybridization to targets, and different tendencies to bind non-specifically to surfaces [26].

2.4.1. Gold nanoparticles. The attachment of oligonucleotides onto the surface of a gold nanoparticle can be performed by simple adsorption [27] or *via* biotin–avidin linkage, where the avidin is previously adsorbed onto the particle surface [28]. However, the most commonly used method to attach oligonucleotides onto gold nanoparticles is *via* thiol–gold bonds. Thiol-functionalized oligonucleotides stick strongly to gold surfaces. The attachment *via* thiol linkage to nanocrystals is much stronger and more efficient than non-specific adsorption. Unfortunately, the number of oligonucleotides attached per nanoparticle cannot be directly controlled. However, gold nanocrystals with a controlled number of attached oligonucleotides can be isolated using gel electrophoresis.

2.4.2. QDs. Several strategies (e.g., adsorption [29], linkage *via* thiol groups [30], electrostatic interaction [31] and covalent linkage [32,33]) have been reported for the conjugation of water-soluble QDs.

Fig. 4 represents schematics of QDs connected with DNA *via* streptavidin–biotin (Fig. 4(a)). The covalent linkage of proteins by mercaptoacetic acid [34] (Fig. 4(b)) can be also be extended for DNA.

DNA immobilization onto QDs is one of the most studied [26]. Mitchell et al. [35] immobilized the 5′- or 3′-thiolated oligonucleotides onto TOPO/mercaptoacetic acid-capped QDs. In this case, immobilization is formally non-covalent, since it arises as a result of displacement of some of the capping agents by the thiolated oligonu-

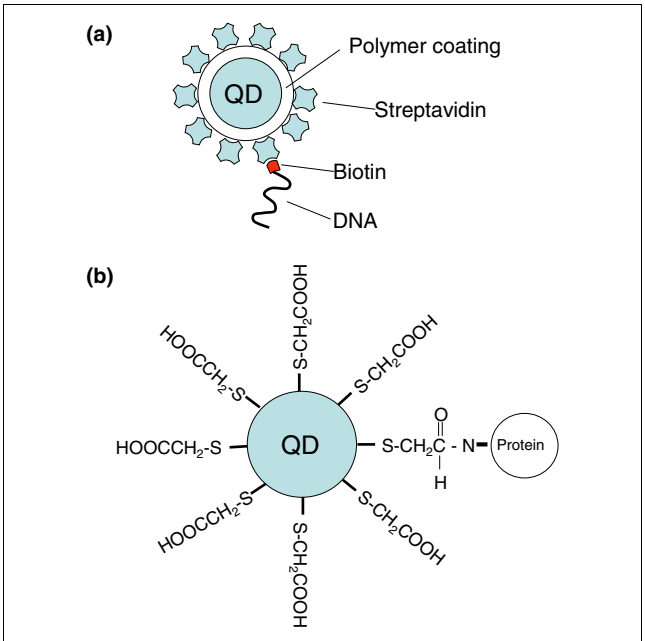

Figure 4. (a) A QD coated with a polymer modified with streptavidin. A biotin-functionalized DNA is attached onto the surface. (b) Schematic of a CdS QD that is covalently coupled to a protein through mercaptoacetic acid by using ethyl-3-(dimethyl-aminopropyl)carbodiimide) as a bifunctional reagent.

cleotides. by Willner et al. [36] also used cystamine-coated QDs to immobilize oligonucleotides.

2.4.3. QD micelles. QD micelles could be attached to DNA by replacing up to 50% of the PEG–PE phospholipids with an amino PEG–PE during micelle formation (Fig. 3(b)), thus introducing a primary amine to the outer surface of the micelle. Thiol-modified DNA was then covalently coupled to the amines using a hetero-bifunctional coupler, with non-coupled DNA removed by ultracentrifugation [22].

3. Electrochemical-detection formats

Nanoparticles can be used in a variety of bioanalytical formats with electrochemical detection. When nanoparticles are used as quantitation tags, an electrical/electrochemical signal emanating from the particles is quantified. Encoded nanoparticles used as labels rely on one or more identifiable characteristics to allow them to serve as encoded electrochemical hosts for multiplexed bioassays. This is analogous to the positional encoding of assays on microarrays, but in solution.

3.1. Nanoparticles as quantitation tags

By analogy to fluorescence-based methods, several electrochemical-detection methods have been pursued in which DNA sequences have been labeled with electroactive nanoparticles. The characteristic electrochemical response of the nanoparticle reporter signals the hybridization event.

3.1.1. Gold nanoparticles. Colloidal gold nanoparticles have been used to signal hybridization in various DNA-detection assays. Three strategies for the detection of gold tracers have been reported (see Fig. 5(A).

According to the first strategy, direct detection of the nanoparticle on the bare electrode without the need for dissolving is performed. A DNA biosensor based on a pencil-graphite electrode and modified with the target DNA was developed following this direct detection strategy [37]. To achieve their objective, the authors covalently bound PCR amplicons to a pencil-graphite electrode using carbodiimide/*N*-hydroxysuccinimide chemistry, and hybridized oligonucleotide–nanoparticle conjugates to these electrode-bound targets. Direct electrochemical oxidation of the particles was observed at a stripping potential of approximately +1.2 V.

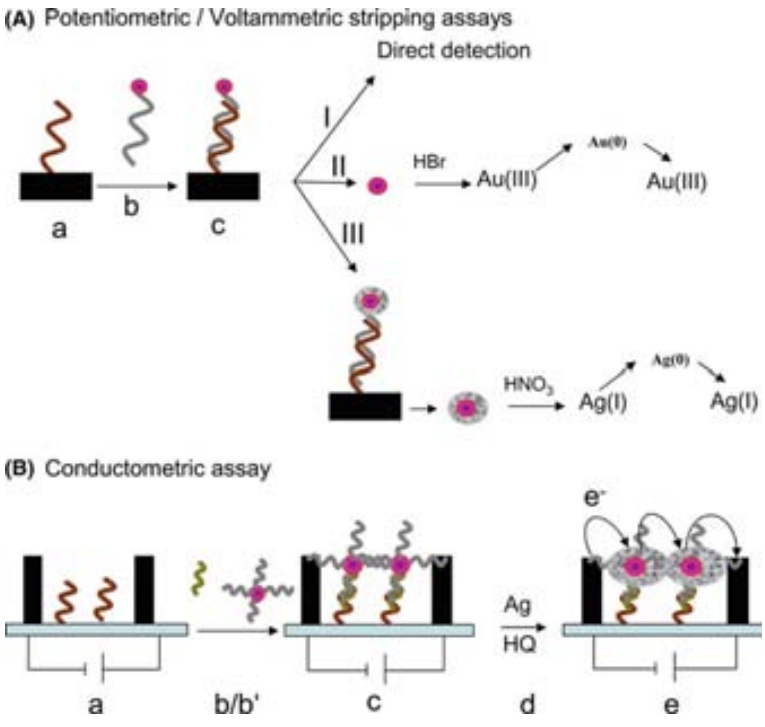

Figure 5. Detection strategies of gold nanoparticles. (A) Potentiometric/voltammetric stripping assay. The hybridization event occurs between DNA strand (a) and gold-tagged DNA (b). The gold-labeled duplex (c) formed is then detected according to each strategy: (i) direct detection of the nanoparticles onto the bare electrode without the need for tag dissolution; (ii) the gold nanoparticles are dissolved with HBr/Br_2 treatment and then detected by stripping techniques; and, (iii) The gold nanoparticles are first covered with Ag by a deposition treatment and then detected by stripping techniques *via* silver enhanced signal. (B). Conductivity assay. Probe DNA immobilized in a small gap between two electrodes (a) is hybridized with target DNA (b) and then with gold-modified DNA probes (b′). Gold is accumulated in the gap (c). Silver enhancement (d) is performed in the presence of hydroquinone (HQ). The silver precipitated onto the gold nanoparticles (e) improves the sensitivity of the assay by lowering the resistance across the electrode gap.

According to the second strategy (Fig. 5(B)), the intrinsic electrochemical signal of the nanoparticle can be observed after dissolving it with HBr/Br_2 [38]. The gold(III) ions obtained were preconcentrated by electrochemical reduction onto an electrode and subsequently determined by anodic-stripping voltammetry.

As 'tracer amplification', silver deposition on the gold nanoparticles after hybridization is also used and an enhanced electrochemical signal attributable to silver is obtained [39,40]. This represents the third strategy. Stripping detection is used for gold nanoparticles/silver enhancement related strategy.

Nevertheless, other interesting methods have been reported. Mirkin and colleagues [41] have exploited the silver-deposition technique to construct a sensor based on conductivity measurements. In their approach, a small array of microelectrodes with gaps (20 μm) between the electrodes leads is constructed, and probe sequences are immobilized on the substrate between the gaps. Using a three-component sandwich approach, hybridized target DNA is used to recruit gold nanoparticle-tagged reporter probes between the electrode leads. The nanoparticle labels are then developed in the silver-enhancer solution leading to a sharp drop in the resistance of the circuit (see Fig. 5(B), adapted from [41]).

3.1.2. QDs and other nanoparticles. A detection method of DNA hybridization based on labeling with CdS QD tracers followed by the electrochemical-stripping measurements of the cadmium have been developed [42]. Nanoparticle-promoted cadmium precipitation, using a fresh cadmium solution hydroquinone, is used to enlarge the nanoparticle tag and amplify the stripping DNA hybridization signal. In addition to measurements of the dissolved cadmium, it was demonstrated in direct ''solid-state'' measurements following a ''magnetic'' collection of a ''magnetic bead/DNA hybrid/CdS tracer'' assembly onto a thick-film electrode transducer. The low detection limit (100 fmol) is coupled to good reproducibility (RSD = 6%).

Besides QDs, gold-coated iron nanoparticles have also been used in DNA-detection assays [43]. After hybridization, the captured gold-iron nanoparticles are dissolved and the released iron is quantified by cathodic-stripping voltammetry in the presence of the 1-nitroso-2-naphthol ligand and a bromate catalyst. The DNA-labeling mode developed offers high sensitivity, well-defined dependence on concentration, and minimal contributions from non-complementary nucleic acids.

Electrochemical impedance spectroscopy (EIS) measurements based on CdS–oligonucleotides have been also possible, besides stripping techniques [44]. ESI was used

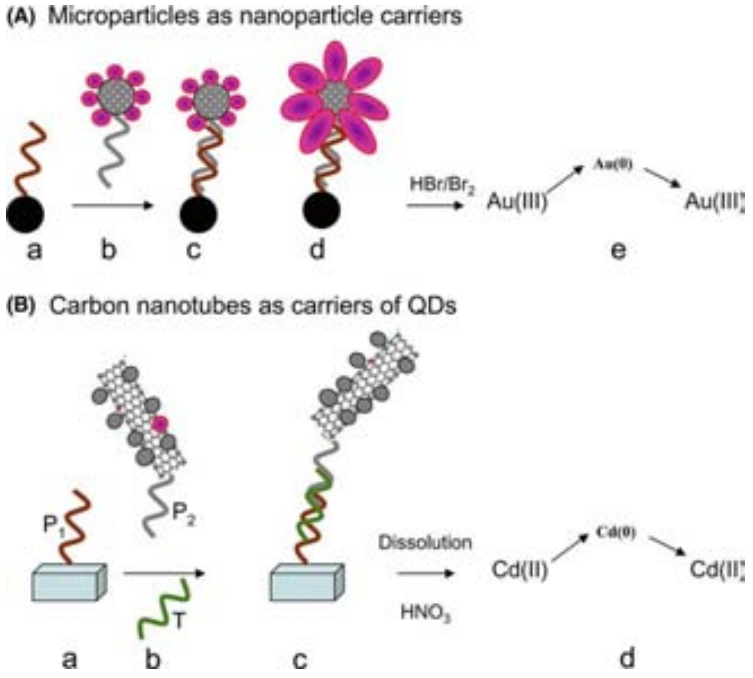

Figure 6. (A) Microparticles as nanoparticle carriers. The target DNA immobilized onto magnetic beads (a) hybridizes with the nucleic acid functionalized with Au-nanoparticle-carrier polystyrene beads (b) forming the Au-labeled hybrid (c) the tags of which are than enlarged (d) followed by magnetic separation and a dissolution process with HBr/Br_2 and then detection by stripping voltammetry (e). (B) Carbon nanotubes as carriers of QDs. The DNA probe P1 is first immobilized onto the well of a streptavidin-assay plate (a). The DNA target (T) and the single wall carbon nanotube (SWCNT)–CdS–labeled probe (P2) were then added, followed by a dual hybridization event (b) forming the final CdS-tagged sandwich (c). The QDs are dissolved with 1 M HNO_3 and then detected by stripping voltammetry using a mercury-coated glassy carbon electrode (d).

to detect the change of interfacial electron-transfer resistance (R_{et}) of a redox marker ($Fe(CN_6)^{4-/3-}$) from solution to transducer surface where the DNA hybridization occurs. It was observed that, when target ssDNA-CdS nanoconjugates hybridized with a DNA probe, the R_{et} value recorded increased markedly.

3.1.3. Nanoparticle carriers. Polymeric microbeads carrying numerous gold-nanoparticle tags have also been used as labels for DNA in electrochemical-detection procedures. The gold-tagged beads were prepared by binding biotinylated nanoparticles to streptavidin-coated polystyrene spheres. Such carrier-sphere amplification platforms are combined with catalytic enlargement of the multiple gold tags and with the sensitive electrochemical-stripping detection of the dissolved gold tags (Fig. 6(A)), allowing determination of DNA targets down to the 300-amol level [45].

SWCNTs carrying a large number of CdS QDs are used as labels for DNA detection [25]. A schematic view of the analytical protocol involving a dual hybridization event is shown in Fig. 6(B).

3.2. Nanoparticles as encoded electrochemical hosts

The potential of current DNA-microarray technology has some limitations. Both the fabrication and read-out of DNA arrays must be miniaturized to fit thousands of tests onto a single substrate. In addition, the arrays must be selective enough to eliminate false-sequence calls and sensitive enough to detect few copies of a target.

Nanoparticles hold particular promise as the next generation of barcodes for multiplexing experiments. Genomic and proteomic research demands greater information from single experiments. Conventional experiments utilize multiple organic fluorophores to barcode different analytes in a single experiment, but positive identification is difficult because of the cross-talking signal between fluorophores.

The labeling of probes bearing different DNA sequences with different nanoparticles enables the simultaneous detection of more than one target in a sample, as shown in Fig. 7. The number of targets that can be readily detected simultaneously (without using high-level multiplexing) is controlled by the number of voltammetrically distinguishable nanoparticle markers. A multi-target sandwich hybridization assay involving a dual hybridization event, with probes linked to three tagged inorganic crystals and to magnetic beads has been reported [46]. The DNA-connected QDs yielded well-defined and resolved stripping peaks at −1.12 V (Zn), −0.68 V (Cd) and −0.53 V (Pb) at the mercury-coated glassy carbon electrode (vs. the Ag|AgCl reference electrode).

Other attractive nanocrystal tracers for creating a pool of non-overlapping electrical tags for such bioassays are ZnS, PbS, CdS, InAs, and GaAs semiconductor particles, in view of the attractive stripping behavior of their metal ions.

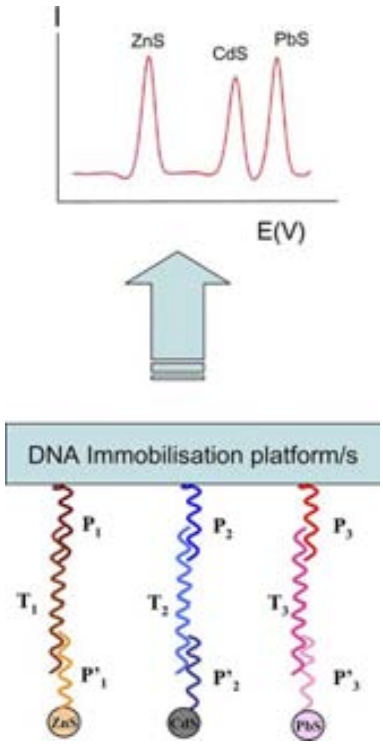

Figure 7. Schematic of multiple detection of DNA. DNA probes (P′1, P′2 and P′3) bearing different DNA sequences with different nanoparticles (ZnS, CdS and PbS, respectively) that enable the simultaneous detection of three DNA targets (T1, T2 and T3) hybridized with corresponding DNA-capturing probes (P1, P2 and P3) immobilized onto a direct or an indirect (magnetic particles) transducing platform/s.

4. Conclusions and future prospects

The integration of nanotechnology with biology and electrochemistry is expected to produce major advances in the field of electrochemical sensors. Recent progress has led to the development of functional nanoparticles that are covalently linked to biological molecules, such as peptides, proteins and nucleic acids. To be biologically relevant, nanoparticles need to have surface functionality amenable to biological modification, solubilization solubility and long-term stability in a range of buffered saline solutions and pH values, and limited non-specific binding.

Various electrochemical strategies to detect DNA hybridization by employing nanoparticles as labels have already emerged. Table 1 summarizes some of analytical parameters of ongoing strategies. Gold nanoparticles are the labels reported most. The low detection limits (a few fmols) obtained for gold-nanoparticle-based assays have

Table 1. Reported nanoparticle labels and analytical parameters of the assays developed

Nanoparticle label	Label connection with DNA	Detection technique	Hybridization separate from detection	DNA detection limits	RSD	Reference
Au	Au–SH–DNA	DPV at pencil-graphite electrode	No	0.78 fmol/mL	~8%	[37]
Au	Au–SH–DNA	PSA and silver catalytic enhancement at screen-printed electrodes	Yes	150 pg/mL	7%	[39,40]
Au	Au–SH–DNA	Conductivity at micro-electrodes	No	500 fM	–	[41]
Au carried into PVC beads	PVC (Au) strep-tavidin–biotin–DNA	PSA and silver catalytic enhancement at screen-printed electrodes	Yes	40 pg/mL	13%	[45]
CdS QDs	CdS NH–DNA	EIS with gold electrode	No	1.43×10^{-10} M	–	[44]
CNTs loaded with CdS QDs	CNT–CdS–strep-tavidin–biotin–DNA	DPV at Hg-film electrode	Yes	40 pg/mL	6.4%	[25]
Au–Fe (core/shell)	Fe–Au–SH–DNA	DPV at Hg-film electrode	Yes	50 ng/mL	6.3%	[43]
CdS QDs	CdS–SH–DNA	PSA and catalytic enhancement with Cd at screen-printed electrodes	Yes	20 ng/mL	6%	[42]
CdS QDs PbS QDs ZnS QDs	CdS–SH–DNA PbS–SH–DNA ZnS–SH–DNA	Simultaneous detection with SWV at Hg-film electrode	Yes	5 ng/mL	9.4%	[46]

PSA, Potentiometric stripping analysis; DPV, Differential pulse voltammetry; SWV, Square wave voltammetry; EIS, Electrochemical impedance spectroscopy.

been obtained by combining gold tracing with silver enhancement.

Enhancement has also been reported for QDs, leading to improved detection limits. Nevertheless, the use of QD carriers, such as polystyrene beads or carbon nanotubes, has significantly improved the limits of detection (by up to 500-fold). The potential for detecting single-molecule interactions by detecting individual gold-colloid labels opens the way to new detection limits. The electrochemical detection of these labels using stripping methods allows the detailed study of various biomolecule interactions.

Most of the electrochemical strategies reported to date suffer from the fact that the hybridization event is still separated from the detection. Only in a few cases (e.g., in the graphite-pencil electrode [37], impedance measurement [44] or conductivity [41]) are these two processes already integrated, the whole electrochemical assay being compacted in a classical sensor model.

The electrochemical properties of QD nanocrystals make them extremely easy to detect using simple instrumentation. QDs nanocrystals are made of a series of semiconductor nanoparticles that can easily be detected by highly sensitive techniques, such as stripping methods. In addition, these electrochemical properties may allow the design of simple, inexpensive electrochemical systems for detecting ultra-sensitive, multiplexed assays.

The electrochemical coding developed could be adapted to other multi-analyte biological assays, particularly immunoassays. The electrochemical-coding technology is therefore expected to open up new opportunities for DNA diagnostics and for bioanalysis, in general.

Clearly, nanoparticles have a promising future in designing electrochemical sensors. Their utilization will be driven by the need for smaller detection platforms with lower limits of detection. Further efforts should be directed at encapsulating different nanoparticle markers within the polystyrene beads as well as micelles, both in relation to multi-target DNA detection.

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Crystal and electrochemical properties of water dispersed CdS nanocrystals obtained via reverse micelles and arrested precipitation

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Abstract
Different methods of synthesis for the production of electroactive nanocrystals (NCs) for use as labels in DNA sensing systems are presented. They are based on two general ways of controlling the formation and growth of the nanoparticles: (a) physical restriction of the volume available for the growth of the individual nanoparticles by using templates such as reverse micelles; (b) arrested precipitation that depends on exhaustion of one of the reactants. The water dispersed nanocrystals thus obtained are then characterized by optical or electrochemical techniques so as to evaluate the quality of the prepared NCs. A novel direct electrochemical stripping detection protocol that involves the use of a bismuth modified graphite epoxy composite electrode is developed and applied so as to quantify the CdS NCs. The electrochemical study revealed a linear dependency of the stripping current upon the concentration of CdS NCs with a detection limit of around 10^{15} CdS NCs cm^{-3}. The obtained NCs are of great interest for future applications in electrochemical genosensors.

M Supplementary data files are available from stacks.iop.org/Nano/17/2553

1. Introduction

Fluorescent and enzyme labels can be considered as the preferred labelling schemes in the detection of biomolecules and array applications, but an increasing emphasis on higher sensitivity has led to the search for novel alternatives. Nanocrystals (NCs) represent a promising strategy in certain bioanalytical and imaging applications where there is a continuous need for new and improved types of labelling materials. An NC, also called a quantum dot (QD), is a semiconductor particle that has all three dimensions confined to the 1–10 nm-length scale [1].

There are practical constraints associated with current DNA labelling technologies: fluorescent molecules or enzymes are not stable, and moreover their application in multidetection is complicated [2]. However, they allow more flexibility, faster binding kinetics [3], high sensitivity and high reaction rates for many types of multiplexed assays of interest for DNA analysis. It is possible to 'bar-code' DNA using nanocrystals (NCs) [4]. Although the principal interest in NCs lies in their optical properties [5], which are dramatically affected by quantum confinement, the application

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of NCs as electrochemical labels has been the object of an increasing number of publications [6]. Different nanoparticles with distinctive electrochemical properties (electrochemical stripping potentials) have been used as labels for detection of DNA hybridization. The use of three different NCs for the multidetection of DNA [7] opened up the possibility for further research to improve the electrochemical properties of NCs as well as to find new alternatives for their direct electrochemical detection [6].

Several synthetic methods for the preparation of semiconductor nanoparticles have been reported [8]. They are based on pattern formation (colloidal formation of self-assembled patterns by surfactant micellation) [9–11], organometallic thermolysis [12] or electrochemical deposition [13]. The pattern formation and organometallic thermolysis routes are the principal methods in use. Following these routes, NCs are produced as either single nanocrystalline materials such as CdS or else as core/shell hybrids such as CdSe/ZnS. In the absence of stabilizing materials the NCs are prone to aggregation. Therefore NCs must be derivatized to preserve them as single, non-aggregated entities.

Organometallic thermolysis is generally performed in an organic solvent (trioctylphosphine oxide (TOPO) or trioctylphosphine (TOP)) at high temperature [14]. The desired size of the NCs can be adjusted by changing the amount of injected precursor and the time for which they are left to grow in the hot surfactant solvent. The NCs thus obtained are hydrophobic, since they are covered with a surfactant layer.

NCs can also be formed by the so-called reverse micelle method. This technique is based on the natural structures created by water-in-oil mixtures upon the addition of an amphiphilic surfactant such as sodium dioctyl sulfosuccinate (AOT). A series of micelle-protected PbS nanoparticles were synthesized using lead acetate and alkanethiols [15, 16]. A novel approach was developed to prepare thin films of nanosized ZnS-passivated CdS particles via metal-organic chemical vapour deposition (MOCVD) [17]. Molecular beam epitaxy [18] has also been used for the production of nanoparticles.

The potential benefits and use of conjugated NC probes for various applications in biomedicine in general, and in the field of biosensors in particular, has prompted extensive efforts to develop methods to synthesize water-dispersed and biocompatible NCs.

The aim of this paper is to present and highlight various synthetic strategies for obtaining water dispersed CdS NCs, optically characterize these and then develop a simple and direct mode for electrochemical detection.

Herein we describe and discuss the current use of chemical methods such as those based on either reverse micelles or arrested precipitation to prepare water dispersed CdS NCs. Aspects related to crystal characterization as well as the possibility for the direct electrochemical detection of the obtained NCs will be shown. While the electrochemistry of NCs is usually focused on organic solvents such as *N*,*N*-dimethylformamide (DMF) [19], we present here for the first time a simple and efficient method for their direct detection in an aqueous medium.

The obtained material, as well as the novel electrochemical detection strategy, holds great promise for future application in genosensors and immunosensors. The CdS NCs produced as described are just an example. Other heavy metal based NCs such as PbS, ZnS or CuS can be obtained by using similar procedures. Their application in electrochemical coding technology [7] is of great importance for the development of DNA biosensors for the simultaneous detection of several DNA strands.

2. Experimental details

2.1. General methods

Syntheses were carried out using Schlenk techniques under nitrogen. Chemicals and solvents were used as-received from Sigma-Aldrich: cadmium perchlorate, 100% AOT, 98% heptane, 99.9% sodium sulfide hydrate, 98% cystamine, 100% mercaptoethanosulfonate, methanol, hexamethyldisilathiane (HMSDT), 97% thioglycerol, 99% glutathione, tetramethylammoniumhydroxide (TMAH) and ethanol. Pyridine and acetone were from Panreac (Spain).

Electron microscopy (TEM) were performed on a JEOL JEM-2010.

Three methods of synthesis for nanometre scale CdS NCs were used.

2.1.1. Preparation of CdS NCs based on two reverse micelles. Method 1. This method is based on the interaction of two reverse micelle solutions. According to this method [20] an AOT/*n*-heptane water-in-oil microemulsion was prepared by the solubilization of 4 ml of double distilled water in 200 ml *n*-heptane in the presence of 14 g of AOT surfactant.

The resulting mixture was separated into 120 and 80 ml sub-volumes of reverse micelles where 0.48 ml of 1.0 M $Cd(ClO_4)_2$ and 0.32 ml of 1.0 M Na_2S solutions, respectively, were added into the 120 and 80 ml sub-volumes of reverse micelles. The two sub-volumes were then mixed well under nitrogen for 1 h. The appearance of a fluorescent yellow indicates the formation of the CdS NCs. After that, the formed NCs underwent two modifications: (a) modification by adding 0.12 ml of thioglycerol; (b) modification by adding 0.66 ml of 0.32 M mercaptoethanosulfonate and 0.34 ml of 0.32 M cystamine solutions.

In both cases the resulting mixture was then stirred for 24 h under nitrogen. It was subsequently evaporated under vacuum and the residue successively washed with pyridine, *n*-heptane, acetone and methanol by means of ultracentrifugation. Methanol instead of tetrahydrofuran was used in the case of modification by thioglycerol.

Two kind of water-dispersed CdS nanoparticles covered either by cystamine and mercaptoethanosulfonate or by thioglycerol were obtained.

2.1.2. Preparation of CdS NCs based on one reverse micelle. Method 2. In this method HMSDT was used as an organometallic reagent to generate sulfide ions [21] instead of sodium sulfide micelles (method 1). HMSDT was added directly to the $Cd(ClO_4)_2$ micelle solution (prepared as in method 1), avoiding the reaction between two micelle solutions as in method 1.

The $Cd(ClO_4)_2$ reverse micelle solution was prepared in the following way: 13 g AOT, 500 ml *n*-heptane, 1.7 ml of double distilled water and 0.44 ml of 1 M $Cd(ClO_4)_2$ solution were mixed well. A solution of 50 ml *n*-heptane containing 0.05 ml of HMSDT was then quickly added. The resulting mixture was stirred for 1 h under nitrogen. The appearance of a fluorescent yellow indicates the formation of CdS NCs as in method 1. The water dispersed thiol-capped CdS NCs were then prepared as described in method 1.

2.1.3. Preparation of CdS NCs based on arrested precipitation. Method 3. This method is based on arrested precipitation of water dispersed cadmium with sulfide precursors [22]. According to this method 3.228 g glutathione as modifier and 0.799 g $CdCl_2$ were first dissolved in 176 ml water and stirred for 5 min. Subsequently 8.5 ml TMAH and 315 ml ethanol were added and after 10 min the precursor solution was thoroughly degassed. HMDST (0.738 ml) was added quickly to the degassed precursor solution giving a clear (slightly yellow) colloidal solution of glutathione-coated CdS nanoparticles. The mixture was magnetically stirred for 1 h and the prepared particles were precipitated by adding tetrahydrofuran (THF). One day later the supernatant was decanted and the precipitate was dissolved in a water/THF mixture and precipitated again with THF to remove excessive reagents and reaction byproducts. Finally, the supernatant liquid was decanted and the precipitate was dried under vacuum (<1 mbar). The powdery CdS nanoparticles were dissolved again in water, giving a clear colloidal solution.

2.2. Preparation of the working electrode

The working electrode ($Bi(NO_3)_3$-GECE) used for the stripping analysis was based on a graphite–epoxy composite electrode (GECE) mixed with the bismuth nitrate salt. It was prepared as reported previously [23]. The percentage of $Bi(NO_3)_3$ in the prepared composite was 2.0% (w/w) (see also supporting information available at: stacks.iop.org/Nano/17/2553 for more details).

2.3. Electrochemical detection of CdS NCs

The proposed protocol for detection of CdS NCs is similar to the detection protocol for Au nanoparticles described previously by our group [24]. It involves absorption of CdS NCs (glutathione modified CdS obtained according to method 3) onto the surface of the $Bi(NO_3)_3$-GECE and the subsequent electrochemical stripping detection of the adsorbed NCs in a mixture of HCl and HNO_3. The final measurements employed a background subtraction protocol involving storing the response for the blank solution and subtracting it from the analytical signal.

3. Results and discussion

3.1. Generation of CdS NCs

The methods based on reverse micelles exploit natural geometrical structures created by water-in-oil mixtures upon adding an amphiphilic surfactant such as AOT. By varying the water content of the mixture, it was shown that the size of the water droplets suspended in the oil phase could be varied systematically. The water-in-oil reverse micelles are characterized by ω_0, the molar ratio of water/surfactant (S): $\omega_0 = [H_2O]/[S]$. The size of the water pool at the reverse micelle core can be carefully controlled by adjusting the $[H_2O]/[S]$ ratio [25].

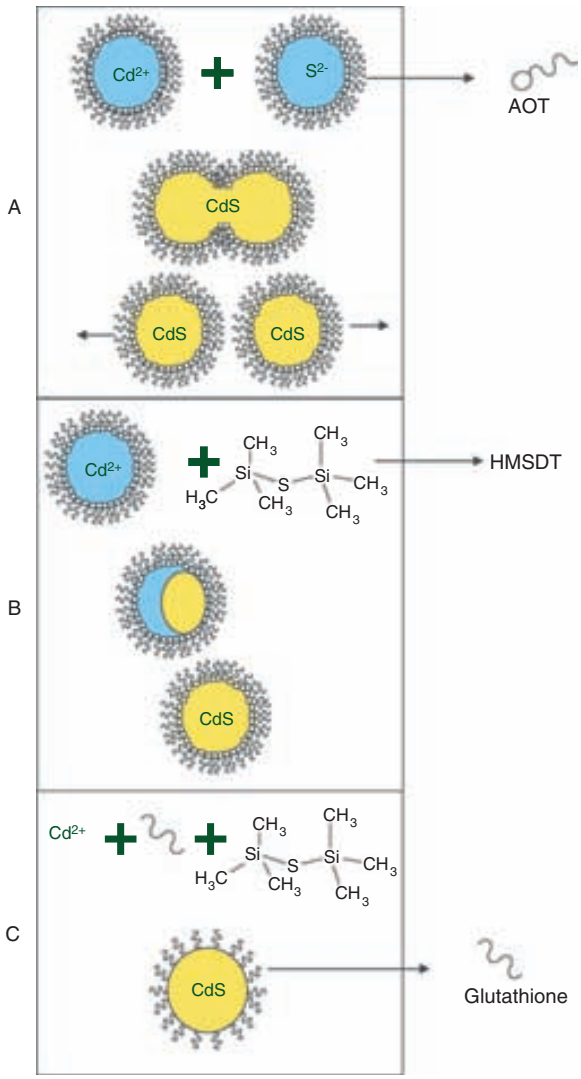

Figure 1. Schematic representation of the mechanism of the generation CdS NCs according to method 1 (A), 2 (B) and 3 (C). The figure shows a schematic of the AOT molecules used in the micelles of methods 1 and 2 along with the molecular formula of the HMSDT used in method 2 and the glutathione modifiers used for CdS NCs obtained according to method 3. The schematics of (A) and (B) correspond to NC formation until the state when the AOT surfactant is still covering the surfaces. The substitution of AOT with thiol protector groups is not shown.

The generation of CdS NCs according to method 1 (see figure 1(A)) happens after the collision between two AOT reverse micelles: the Cd^{2+} one with that of S^{2-}. The collisions between the two micelles result in the formation of a short-lived dimer formed by expulsion of some AOT molecules into the bulk heptane phase (figure 1(A)). During the lifetime of the dimer, the two reverse micelles exchange the contents (S^{2-} and Cd^{2+}) of their aqueous cores before decoalescing, resulting in

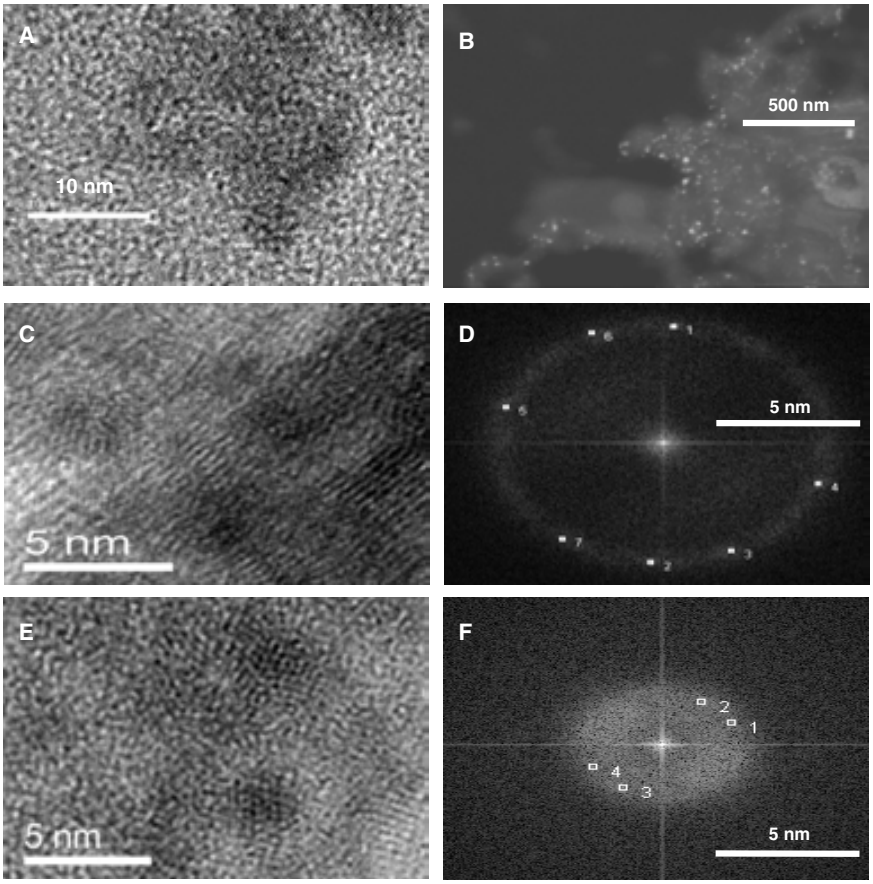

Figure 2. HRTEM (A), (C), (E), bright-field TEM image (B) and Fourier transform images (D), (F) of CdS NCs prepared by methods 1 (A), (B), 2 (C), (D) and 3 (E), (F). CdS NCs prepared as described in the text.

the eventual equilibrium and distribution of all contents. Since the size of the water pools in reverse micelles can be controlled by adjusting the ω_0, the above micelle interaction model corresponds to that of a nanoreactor reported earlier [25]. Nevertheless the reaction dynamics of the microemulsions are still not fully understood. The formed CdS NCs coming from the intermediate (dimer) nanocrystal covered with AOT were than the subject of further surface modification as described in the experimental part (not shown in the schematic of figure 1(A)).

However, in method 2, the addition of the sulfide generator, in that case HMSDT, is substituting for the use of a second micelle. This sulfide generator reacts directly with the AOT micelle of cadmium (II) which serves at the same time as a nanoreactor and mould for the formed CdS NCs.

Controlled or arrested precipitation reactions can yield dilute suspensions of quasi monodispersed particles [26]. This precipitation strategy is followed in method 3. The sulfide generator HMSDT, as in method 2, acts as a precipitant of the cadmium (II) from its chloride solution in the presence of glutathione thiol. The stability of the initially formed small crystallites is influenced by the dynamic equilibrium illustrated by

$$(\mathrm{CdS})_{\mathrm{crystal}} \rightleftharpoons (\mathrm{Cd}^{2+})_{\mathrm{solv}} + (\mathrm{S}^{2-})_{\mathrm{solv}}. \qquad (1)$$

Small crystallites are less stable than larger ones and tend to dissolve into their respective ions. Subsequently, the dissolved ions can recrystallize on larger crystallites which are thermodynamically more stable [26]. The generated CdS NCs are passivated by being covered immediately with the glutathione thiol protective groups, preventing their further growth.

3.2. Characterization by TEM

Figure 2(A) shows a high-resolution transmission electron micrograph (HRTEM) of an assembly of CdS NCs obtained according to method 1. The obtained NCs can be seen to have an average size in the range of 3–4 nm. Each CdS particle shows well-defined lattice fringes which indicate that the particles are highly crystallized even at such a small size. These kinds of fringes were also reported for nanocrystalline semiconducting metal oxides for use in gas sensing [27]. Figure 2(B) is a bright-field image of CdS NCs produced by the same method. The white spots correspond to the formed NCs while the white-grey layer spread over almost the whole CdS NC zone corresponds to an amorphous material that comes from the cystamine and mercaptoethanosulfonate residuals used as thiol protector species.

The HRTEM image (figure 2(C)) of CdS nanoparticles recovered with thioglycerol shows spherical particles of 2–3 nm in size; these are smaller than the CdS produced by method 1 (3–4 nm). The crystallinity of the produced material

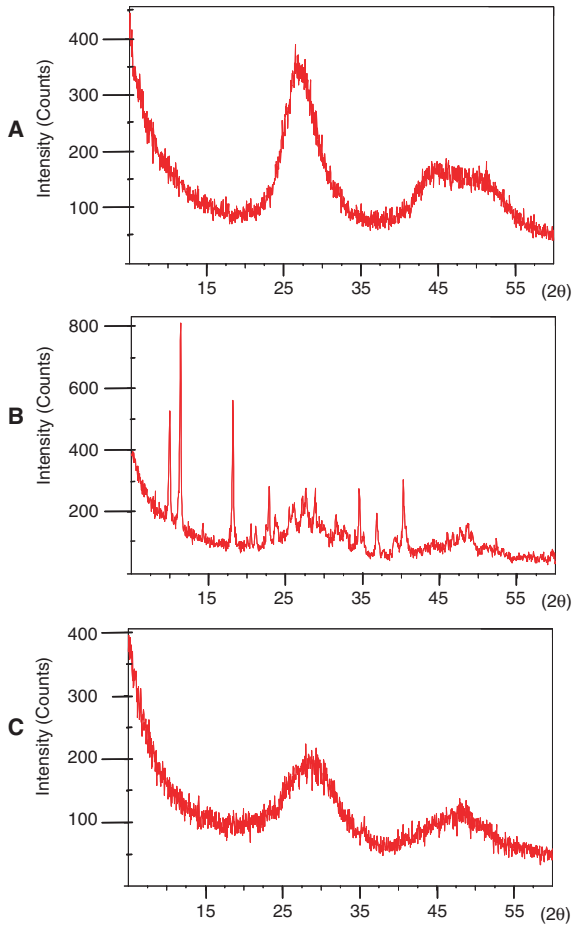

Figure 3. Powder x-ray diffraction patterns of CdS NCs prepared using methods 1 (A), 2 (B) and 3 (C).
(This figure is in colour only in the electronic version)

is also shown by clear lattice fringes of single CdS dots like those produced by method 1 (figure 2(A)).

The HRTEM image of CdS NCs obtained by method 3 (figure 2(E)) shows lattice fringes that are less defined than those observed for NCs obtained by methods 1 (figure 2(A)) and 2 (figure 2(C)). This suggests that the NCs obtained according to method 3 were more amorphous than those obtained with the other methods. The same amorphous aspects were observed for the CdSe synthesized using the thermolytic method [28] with a 10 min particle formation time instead of 90 min that gave a well crystallized and fringed lattice structure.

The Fourier transform (FT) of the experimental images (figures 2(C) and (E)) of the CdS NCs obtained with methods 2 and 3, respectively, are also presented (see figure 2 (D) and (F), respectively). The white dots (1–7 in (D) and 1–4 in (F)) correspond to the crystalline planes of the CdS NCs obtained by methods 2 and 3, respectively. FT data for each dot were calculated (see table 1 in supporting information available at stacks.iop.org/Nano/17/2553). The *d*-spacings of similar order to those obtained by Kadirgan *et al* [29] demonstrate the presence of hexagonal phase cadmium sulfide obtained by methods 2 and 3.

3.3. Characterization by XRD

As already demonstrated, the obtained CdS NCs seem to be closed packed. Assuming this fact, the reflection in the small angle regime can be correlated with lattice planes through the particles' centres of mass [30].

The diffraction patterns (figure 3) of CdS NCs show features typical of nanodimensional particles. Nevertheless, comparing the three patterns of CdS NCs we can conclude that in the three cases the peaks (in the region $d\theta \approx 25$–30) related to the crystalline planes of the cadmium sulfide structure are not observed. The (110), (103) and (112) planes of wurzite CdS [31] are not well defined (see figure 3; note the different scales), appearing as broad peaks. There might be two reasons for the absence of sharp peaks [32]: first, due to the very small size of the particles (4 nm) obtained in each of the three methods, the intensity of reflections (figures 3(A)–(C)) in the wide angle region of the diffraction patterns is broadened; second, due to impurities such as silicon byproducts from HMSDT used in the *in situ* generation of the sulfide ions. Similar results were also obtained by Weller *et al* [33]. According to Weller *et al*, a clear identification of the crystalline structure was not possible, maybe due to the small particle size; the identification was easier for bigger NCs.

3.4. Electrochemical detection of CdS NCs

The $Bi(NO_3)_3$-GECE in an open circuit (figure 4, upper part, step (A)) was immersed in a solution containing an appropriate concentration of CdS NCs for 10 min. After the accumulation step, the electrode was carefully washed with distilled water before being immersed into a voltammetric cell containing 10 ml of 0.1 M HCl and 250 μl of 1 M nitric acid. The electrochemical reduction of CdS NCs to Cd was performed at −1.4 (versus Ag/AgCl) for 120 s in an unstirred solution (figure 4, step (B)). Immediately after the electrochemical reduction step, differential pulse voltammetry (DPV) was performed. During this step (figure 4, step (C)) the potential was scanned from −0.83 to −0.43 V (step potential 10 mV, modulation amplitude 50 mV, scan rate 33 mV s^{-1}; unstirred solution), resulting in an analytical signal due to reoxidation of Cd to Cd^{2+}.

There have been relatively few reports on the electrochemical properties of semiconductor NCs [19, 34, 35], being the electrochemistry of NCs in organic solvents such as CdS in DMF [19] the most established system so far. Water dispersed glutathione modified CdS NCs obtained according to method 3 were chosen for study in this work because stable monodisperse particles which are readily dispersed can be prepared relatively easily.

Figure 4 (schematic, lower part) shows the mechanism for the direct detection of CdS NCs using the $Bi(NO_3)_3$-GECE. The built-in bismuth property is the distinctive feature of this $Bi(NO_3)_3$ modified GECE which is used for the generation of bismuth adjacent to the electrode surface. The $Bi(NO_3)_3$-GECE ensures *in situ* generation of bismuth ions and film formation without the need for external addition of bismuth in the measuring solution. The rich microstructure of $Bi(NO_3)_3$-GECE, composed of a mixture of carbon microparticles within the epoxy-resin forming internal microarrays, might have a profound effect not only on the NC adsorption (as in the case of

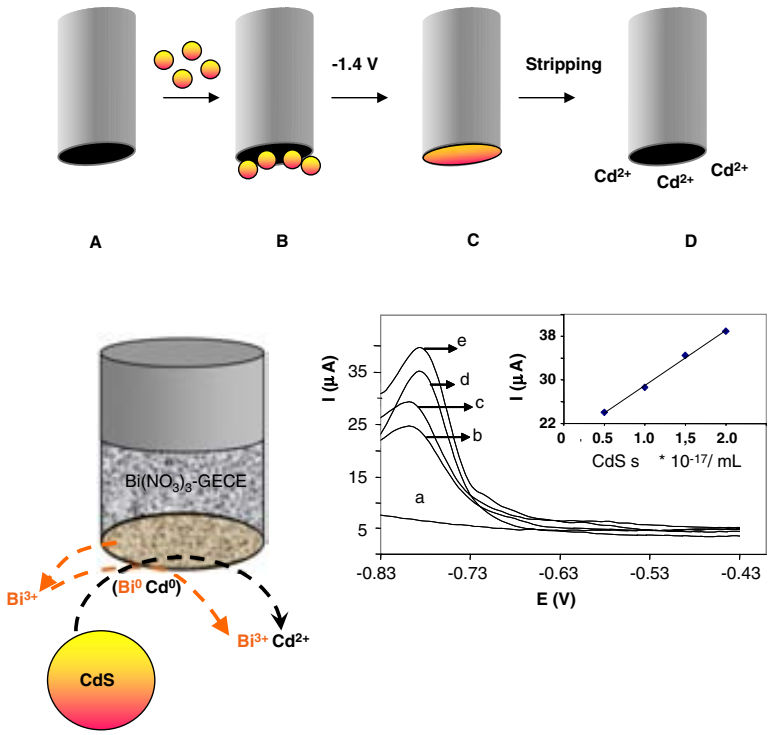

Figure 4. Upper part: schematic of the electrochemical detection scheme used for CdS NCs. The graphite–epoxy composite electrode ($Bi(NO_3)_3$-GECE) (A) is incubated for 10 min in a solution containing CdS NCs. The $Bi(NO_3)_3$-GECE that contains CdS NCs (B) is then polarized at −1.4 V which leads to the formation of a reduced Cd layer (C) which is then reoxidized (D). Lower part (left): a schematic that shows the mechanism of the direct detection of CdS NCs using the $Bi(NO_3)_3$-GECE. Lower part (right): typical DPASV curves along with the calibration curve (inset) of CdS NCs colloidal in the range of concentrations 0.5 (b), 1.0 (c), 1.5 (d) and 2.0 (e) $\times 10^{17}$ CdS NCs ml^{-1}. Also shown is the blank (a). Experimental conditions were as described in the text.

gold nanoparticles [24]) but also upon the formation of Cd film and consequently its stripping, producing a high sensitivity analytical signal [23].

In analogy with the electrochemistry of PbS nanocrystals in aqueous solution [35], a cathodic reduction

$$CdS + 2H^+ + 2e \rightarrow Cd^0 + H_2S \qquad (2)$$

that depends on the pH of the solution occurs. The CdS NCs are reduced in this way to Cd^0 while a potential of −1.4 V is applied for 120 s. After this 'preconcentration' redissolution of the formed Cd^0 occurs, giving a DPV response that depends on the quantity of CdS NCs.

Various relevant parameters influencing the analytical DPV response of the CdS NCs were investigated. The dependence of the adsorption of CdS NCs onto $Bi(NO_3)_3$-GECE over time was studied. The concentration of CdS NCs on the surface of the $Bi(NO_3)_3$-GECE can be increased by increasing the incubation time for which the electrode remains in contact with the colloidal solution of CdS NCs. However, as a trade-off between sensitivity and analysis time, an optimal absorption time of 10 min was selected. Optimization of the reduction potential of the CdS NCs onto the DPV signal was performed by measuring between −1.00 and −1.40 V (versus Ag/AgCl). A potential of −1.4 V (versus Ag/AgCl) was selected as optimal for analytical determination of the CdS NCs. The effect of the electrochemical reduction time of the CdS NCs upon the DPV signal was also studied. The CdS NCs signal displays an increase in response between 30 and 120 s, after which the current levels off. The optimal duration of electroreduction was therefore 120 s.

The $Bi(NO_3)_3$-GECE based protocol for detection of CdS NCs shows a defined concentration dependence. Typical DPASV curves for increasing concentrations of CdS NCs are shown in figure 4. The corresponding calibration plot for CdS NCs is also shown, and is linear over the range from 0.5×10^{16} to 2.0×10^{17} CdS NCs cm^{-3} with a sensitivity of 10^{-16} ($\mu A\ cm^3$/nanoparticle) and an intercept of 18.9 μA (correlation coefficient 0.997). The detection limit, based on a S/N ration of 3, was around 10^{15} CdS NCs cm^{-3}. Favourable signal-to-noise characteristics were observed for whole range of CdS NC concentrations. The obtained sensitivity is coupled to a good CdS NC detection reproducibility using the $Bi(NO_3)_3$-GECE. Reproducible DPV signals for CdS NCs over the entire $Bi(NO_3)_3$-GECE operation protocol were observed (RSD = 10%).

CdS NCs obtained according to methods 1 and 2 were also characterized using the same methods (results not shown) and similar results as those for method 3 were obtained. The developed protocol will be of interest for DNA hybridization studies in connection with paramagnetic particles as the DNA immobilization platforms [36].

4. Conclusions

CdS NCs were synthesized using templates such as reverse micelles or directly by arrested precipitation. Two kinds

of water-dispersed CdS NCs covered either by cystamine and mercaptoethanosulfonate or by thioglycerol have been obtained using the reverse micelle method, while glutathione modified ones were obtained using the arrested precipitation method. All the NCs exhibited great stability and good solubility in water, the glutathione modified NCs (obtained by method 3) being more soluble, although these were more amorphous than the NCs obtained by other methods. The diffraction patterns of CdS NCs show features typical of nanodimensional particles, while the XRD images lacked the crystalline planes due to the small sizes of the NCs as well as some impurities. The obtained results were found to be consistent with those for similar NCs obtained with other methods.

Direct electrochemical detection of the obtained CdS NCs was achieved due to the physical absorption of metal nanoparticles onto the surface of a graphite epoxy composite electrode modified with bismuth nitrate salt. The electrochemical study revealed a linear dependency of the stripping current on the CdS NC concentration. The well-defined peak at around −0.78 V is attributed to the oxidation of the cadmium layer arising from the previous cathodic reduction of CdS NCs. These observations indicate that there is the possibility of directly detecting CdS NCs by using the built in bismuth precursor electrode which combines the NC adsorption properties of graphite–epoxy electrode with the stripping ability of bismuth film.

The small label size, established bioconjugation chemistry and the unusual optical and electrical properties of CdS NCs make them unique tools for DNA detection. Other NCs such as ZnS, PbS etc can be obtained and detected in a similar way. The use of these water dispersed NCs in electroanalytical genomics and proteomics is still under study in our laboratories. Study of the effect of the physical properties of the NCs on their electrochemical properties, so as to better select these as labels for DNA or antibodies, will be subject of future work.

Acknowledgments

This work was financially supported by the Spanish 'Ramón Areces' foundation (project 'Bionanosensores'), MEC (Madrid) (projects MAT2005-03553, BIO2004-02776,) and the 'Ramón y Cajal' programme of the Ministry of Science and Technology (Madrid) that supports A Merkoçi. M Pumera is grateful for the support from the Marie Curie Intra-European Fellowship from European Community under 6thFP (MEIF-CT-2004-005738). The authors would like to thank to Mr Onofre Castell (*Servei de microscopia,* UAB) for the HRTEM and bright-field TEM images along with their Fourier transforms.

Supporting information available

Table with the FT data of positions shown at image (D) (see figure 2 in the text) and image (F) (see figure 2 in the text) as well as details on the procedure of the preparation of the $Bi(NO_3)_3$-GECE can be found in the supporting information available at stacks.iop.org/Nano/17/2553.

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Supporting information

CRYSTAL AND ELECTROCHEMICAL PROPERTIES OF WATER SOLUBLE CDS QDS OBTAINED VIA REVERSE MICELLE AND ARRESTED PRECIPITATION

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Contents

Table 1S. FT of positions shown at image D (see figure 2 in the text) and image F (see figure 2 in the text).

FT of imge D (see Figure 2 in the text) that corresponds to method 2 CdS QDs.				
Spot#	d-Spacing (nm)	Rec. Pos.(1/nm)	Degrees to Spot 1	Degrees to x-axis
1	0.3378	2.960	0.00	86.16
2	0.3313	3.018	179.78	- 94.05
3	0.3350	2.985	152.07	- 65.90
4	0.3384	2.955	106.40	- 20.24
5	0.3402	2.940	75.95	162.11
6	0.3261	3.067	28.22	114.38
7	0.3322	3.010	147.48	- 126.36

FT of image F (see Figure 2 in the text) that corresponds to method 3 CdS QDs.				
Spot#	d-Spacing (nm)	Rec. Pos.(1/nm)	Degrees to Spot 1	Degrees to x-axis
1	0.3349	2.986	0	24.13
2	0.3521	2.840	32.49	56.62
3	0.3490	2.866	145.65	-121.52
4	0.3383	2.956	178.48	-154.35

Preparation of the $Bi(NO_3)_3$GECE

$Bi(NO_3)_3$GECE was prepared using graphite powder with a particle size of 50 μm (BDH, UK), Epotek H77 (epoxy resin), hardener (both from Epoxy Technology, USA) and $Bi(NO_3)_3$ (Aldrich). Graphite powder and $Bi(NO_3)_3$ salt were first mixed together. The obtained dried mixture was mixed well with epoxy resin (mixed with hardener) in a ratio of 1:4 (w/w) as described in a previous work [1,2].

The resulting $Bi(NO_3)_3$ containing graphite epoxy paste was placed into a PVC cylindrical sleeve body (6 mm i.d.), which has an inner electrical copper contact, to a depth of 3 mm. The conducting composite material glued to the copper contact was cured at 40ºC during a week. Before each use, the surface of the electrode was wet with doubly distilled water and then thoroughly smoothed, first with abrasive paper and then with alumina paper (polishing strips 301044-001, Orion).

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Calculation of CdS NC suspension concentration.

A mean diameter of CdS NCs of around 3nm (according to TEM data) was used to calculate the volume of one particle considered a sphere. The density of CdS is considered to be 4,82 g/mL (according to www.chemfinder.com). By this way we can calculate the weight of one CdS NC. Having the weight of the dried CdS NCs product

(after the synthesis) the number of CdS NCs can be determined (dividing the total weight of CdS NCs by the weight of a single sphere NC) and consequently the concentration of the suspension by knowing the volume of suspension water.

Detection of cadmium sulphide nanoparticles by using screen-printed electrodes and a handheld device

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Detection of cadmium sulphide nanoparticles by using screen-printed electrodes and a handheld device

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Abstract
A simple method based on screen-printed electrodes and a handheld potentiostatic device is reported for the detection of water soluble CdS quantum dots modified with glutathione. The detection method is based on the stripping of electrochemically reduced cadmium at pH 7.0 by using square wave voltammetry. Various parameters that affect the sensitivity of the method are optimized. QD suspension volumes of 20 μl and a number of around 2×10^{11} CdS quantum dots have been able to be detected. The proposed method should be of special interest for bioanalytical assays, where CdS quantum dots can be used as electrochemical tracers.

S Supplementary data files are available from stacks.iop.org/Nano/18/035502

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Inorganic compounds with nanometre dimensions—nanoparticles or quantum dots—are important because of their photovoltaic, photoelectrochemical, and electroluminescent applications including sensors and biosensors [1–4].

Recent advances in nanobiotechnology, and particularly the development of functionalized nanoparticles combined with advances in molecular biology research, provide the impulsion for the present explosion in DNA sensors and immunosensors among other fields. These devices are playing a growing role in various analytical applications where an accurate, low cost, fast and on-line measuring system is required.

To improve the assay sensitivity and to achieve a better and more reliable analysis there is a great demand for labels with higher specific activity. The most used labels for DNA and immunosensors to date have been enzymes as well as small fluorescent molecules like dyes etc. It is well known, despite the high detection limits and well known assay protocols, that enzymes suffer from stability problems and high cost as well as difficulties or near impossibility to carry out simultaneous detections. Fluorescent dye labels also are expensive, photobleach rapidly, and the equipment required to project the image of the dyes on an array surface is expensive and unwieldy [5].

In principle nanoparticles provide a novel platform for improving specific activity of a label as well as affinity to the tracer molecules (DNA probes or other biomolecules) [6, 7]. Nanosized particles have a chemical behaviour similar to small molecules and can be used as specific labels for DNA strands or antibodies. Nanoparticles in general and quantum dots [8] (QDs) in particular may be expected to be superior in several ways. Compared to existing labels, nanoparticles in general and QDs especially are more stable and cheaper. They allow more flexibility, faster binding kinetics (similar to those in

a homogeneous solution), high sensitivity and high reaction rates for many types of multiplexed assays, ranging from immunoassays to DNA analysis.

Electrical methods for the detection of nanoparticles have been extensively used. Park *et al* [9] reported a conductivity-based DNA detection method utilizing oligonucleotide-functionalized Au nanoparticles that provides an alternative to existing detection methods (see [6, 11] in the mentioned paper) and presents a straightforward approach to high-sensitivity and selectivity, multiplexed detection of DNA.

Although conventional optical probes based on the fluorescence of nanoparticles have been successfully used, those based on electrochemical properties are still under research. The importance of electrochemical methods in nanoparticle based bioassays is extensively revised in the last review of *Chem. Rev.,* dedicated to Nanostructures in Biodiagnostics [10] where it is emphasized among other things that 'Electrical detection methods offer the possibility of portable assays that could be used in a variety of point-of-care environments'.

The use of quantum dots as electrochemical labels for DNA and immunosensing has several advantages. The related technique—stripping voltammetry—compared to optical ones is cheaper, faster and easy to use in field analysis. Moreover, it offers the possibility for simultaneous detection of several biological molecules in the same sample and using a unique sensor owing to the distinctive voltammetric wave produced by different electrochemical tracers. An electrical immunoassay coding protocol for the simultaneous measurements of multiple proteins based on the use of different inorganic nanocrystal tracers have been developed. The concept is demonstrated for a simultaneous immunoassay of β2-microglobulin, IgG, bovine serum albumin, and C-reactive protein in connection with ZnS, CdS, PbS, and CuS colloidal crystals, respectively [11]. The designed assay based on the nanocrystal dissolving shows the efficient coupling of the multiprotein electrical detection to the amplification feature of electrochemical stripping transduction yielding *fmol* detection limits.

The offered advantages along with the possibility to be used in several biosensing systems based on electrochemical techniques require the development of novel nanoparticle detection strategies. Several works on DNA or immunoanalysis based on gold nanoparticle [12–18] or quantum dot [19] detection by using stripping techniques have been reported. The majority of these electrochemical methods have used chemical dissolutions of gold nanoparticles (in a hydrobromic acid/bromine mixture) or quantum dots (with nitric acid) followed by accumulation and stripping analysis of the resulting metal ion solutions. The HBr/Br_2 solution is highly toxic and therefore methods based on direct electrochemical detection of gold nanoparticle tags have been developed [20, 21] and even applied for DNA analysis [22]. Regarding the direct electrochemical detection of quantum dots so as to achieve a full integration of DNA electrochemical sensors there is still much work to be done.

The following work shows a simple method for the direct detection of cadmium sulphide quantum dots in neutral solution medium (pH $\sim$ 7.0) without the need for chemical dissolution. It is based on dropping a few microlitres of CdS QD suspension on the surface of a screen-printed electrode and the subsequent square wave voltammetry detection based on the reduced cadmium formation and stripping, giving a well shaped and sensitive analytical signal. The proposed method can be easily extended for other quantum dots based on other heavy metals, offering new opportunities for applications in electrochemical DNA genosensors.

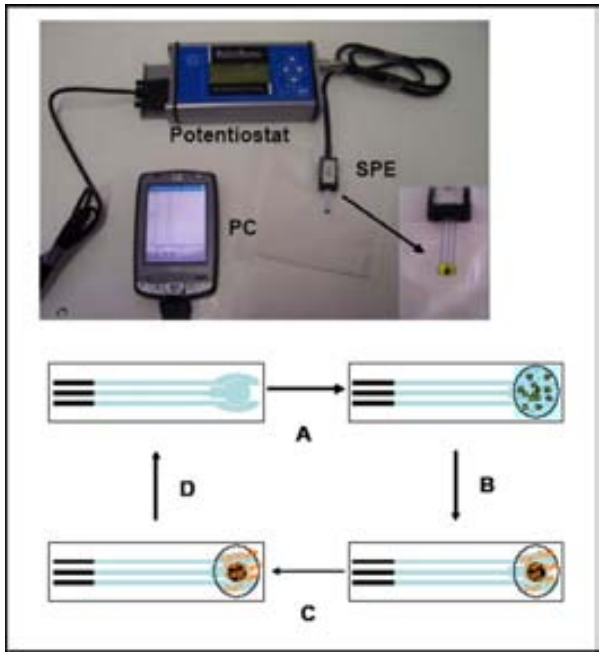

Figure 1. Upper part: image of the hand held device system used for CdS QD detection. The principal components are the potentiostat, the palmtop PC and the screen-printed electrodes (SPE). Lower part: schematic diagram of the direct voltammetric detection of the CdS QDs using an SPE.

2. Experimental details

2.1. Apparatus

All voltammetric experiments were performed using a PalmSens (Palm Instrument BV, Houten, The Netherlands) that consists of a portable potentiostat interfaced with a palmtop PC (155 mm $\times$ 85 mm $\times$ 35 mm) (see figure 1, upper part).

Electrochemical experiments were carried out using a screen-printed electrode (SPE) (Palm Instrument BV, Houten, The Netherlands). The screen-printed electrochemical cell consists of a graphite working electrode (diameter 3 mm), a graphite counter-electrode and a silver pseudo-reference electrode. Electron microscopy (TEM) of CdS QDs was performed on a JEOL JEM-2010.

2.2. Reagents

Synthesis of CdS QDs was carried out using Schlenk techniques under nitrogen. Chemicals and solvents were used as received from Sigma-Aldrich: cadmium perchlorate, hexamethyldisilathiane (HMSDT), and tetramethylammoniumhydroxide (TMAH).

All solutions were prepared in doubly distilled water. Potassium dihydrogenphosphate, phosphoric acid, and sodium

hydroxide were purchased from Sigma-Aldrich; hydrochloric acid (37% m/m) was purchased from PanReac (Barcelona, Spain). Appropriate dilutions from this stock solution were also prepared in 0.1 mol l^{-1} phosphate buffer solution (pH 7.0) prior to each set of measurements.

2.3. Preparation of CdS quantum dot suspension

The preparation method is based on arrested precipitation of water dispersed cadmium with sulphide precursors [23]. According to this method 3.228 g glutathione as modifier and 0.799 g $CdCl_2$ were first dissolved in 176 ml water and stirred during 5 min. Subsequently 8.5 ml TMAH (tetramethylammoniumhydroxide) and 315 ml ethanol were added and after 10 min the precursor solution was thoroughly degassed. 0.738 ml HMDST is added quickly onto the degassed precursor solution, giving a clear (slightly yellow) colloidal solution of glutathione-coated CdS nanoparticles. The mixture was magnetically stirred for 1 h and the prepared particles were precipitated by adding tetrahydrofuran (THF). One day later the supernatant was decanted and the precipitate was dissolved in water/THF mixture and precipitated again with THF to remove excessive reagents and reaction by-products, respectively. Finally, the supernatant liquid was decanted and the precipitate was dried under vacuum (<1 mbar). The powdery CdS nanoparticles were dissolved again in water, obtaining a clear colloidal solution. A stock solution of 1.86×10^{19} CdS QDs ml^{-1} in 0.1 mol l^{-1} phosphate buffer solution (pH 7.0) was prepared and used in further experiments.

2.4. Electrochemical detection of CdS QDs

The measurements were performed suspending a volume of 20 μl under the sensor stripped in the horizontal position, to ensure electrical contact (complete circuit).

Each SPE was pretreated, before using, by applying −1.1 V for 300 s, and then square wave voltammetric (SWV) scans were carried out until a low and stable background was obtained.

SWV experiments were performed to evaluate the electrochemical behaviour of the screen-printed electrode (SPE) for CdS QD detection. The proposed protocol (figure 1, lower part) involves the introduction of CdS QDs on the surface of the SPE (figure 1(A)). During this step a drop of 20 μl containing an appropriate concentration of CdS QDs (ranging from 0.5 to 14.0×10^{16} QDs ml^{-1}) was suspended onto the SPE for 60 s and a potential of 0 V was applied. The second step was the accumulation step. In this step (figure 1(B)) a deposition potential of −1.1 V for 120 s was applied to promote the electrochemical reduction of Cd^{2+} ions contained in the CdS QD structure to Cd^0. After the accumulation step, SWV was performed. During this step (figure 1(C)) the potential was scanned from −1.1 to −0.7 V (step potential 10 mV, modulation amplitude 30 mV and frequency 15 Hz), resulting in an analytical signal due to the oxidation of Cd^0. After the SWV measurement the SPE was manually cleaned (figure 1(D)) with a 0.1 mol l^{-1} phosphate buffer solution (pH 7.0).

A blank subtraction method was performed. The blank was measured using a separate blank solution (0.1 mol l^{-1} phosphate buffer solution, pH 7.0). After this, using the same sensor, a determined volume of the QD suspension is added and measurements (SWV) performed by using the 'sample' option. In this way the subtracted curve was obtained.

No special activation of the electrode surface was used for further experiments with the same sensor (up to six or seven measurements).

3. Results and discussion

3.1. Detection of cadmium ion solution dropped onto SPE surface

SWV of cadmium ion solution, before CdS QD detection, was previously studied by using the SPE. Two detection methods were performed. According to the first one the SPE was immersed into 20 ml of a 0.1 mol l^{-1} HCl solution and the SWV performed following the classical procedure: accumulation of cadmium ions under stirring conditions and than stripping in a quiescent solution. The results obtained (not shown) were similar to those reported by Palchetti *et al* [24]. Besides this method we pushed the research to the detection of small sample volume so as to achieve later on detection of lower QDs solution volumes. According to this second method a volume as low as 20 μl of cadmium solution dropped onto the SPE surface has been able to be detected. (Supporting information, figure 1S, available at stacks.iop.org/Nano/18/035502). A linear range of response from 10 to 1000 ppb with a detection limit of around 5 ppb cadmium ion was obtained.

3.2. Detection of CdS QDs

The direct detection of CdS QD suspension on SPE represents a novel approach with special interest for electrochemical characterization of QDs and further applications in biosensing based on labelling.

During the deposition time, applying a potential of −1.1 V, reduced cadmium, necessary for further stripping, could have been obtained via two mechanisms (see figure 2).

First mechanism. The cadmium ions in equilibrium with CdS nanoparticles can be directly reduced according to

$$(CdS)Cd^{2+} + 2e \rightarrow (CdS)Cd^0. \quad (1)$$

Second mechanism. The direct reduction of CdS nanoparticles:

$$CdS + 2H^+ + 2e \rightarrow Cd^0 + H_2S \quad (2)$$

in analogy with the cathodic reduction of PbS nanoparticles in water solution [25], that depends on solution pH. (This is also confirmed by the pH effect studies. See the following sections.) The CdS QDs are reduced in this way to Cd^0 while applying a potential of −1.1 V for 120 s. After this 'preconcentration' the redissolution of the Cd^0 formed occurs, giving the SWV response that depends on the number of CdS QDs.

To get further insight into the response mechanism of CdS QD detection linear sweep voltammetry (LSV) was also performed. The plot of the corresponding peak current versus the square root of the scan rate (Supporting information

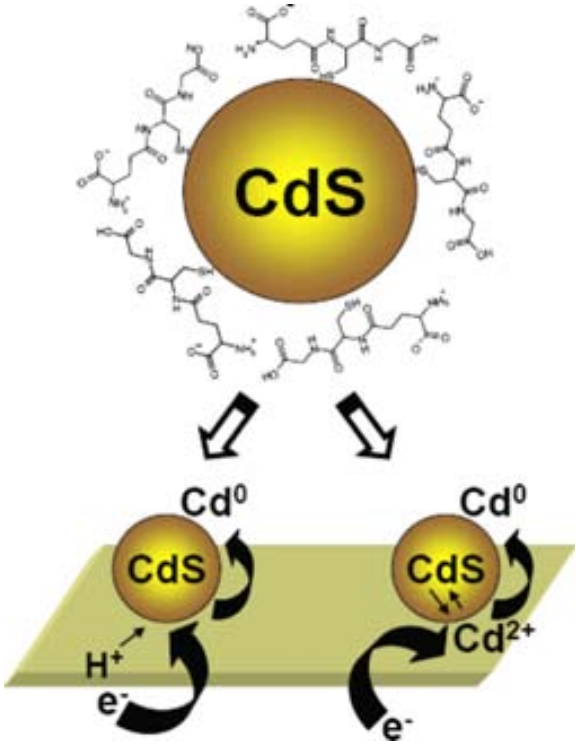

Figure 2. Schematic diagram of the CdS nanoparticle detection mechanisms either through reduced cadmium formation in acidic (left) or neutral (right) medium.

figure 2S, available at stacks.iop.org/Nano/18/035502) did not show a reversible system with diffusion control of the cadmium redox reaction. Instead a mixed phenomenon (probably coming from QDs adsorption) could have occurred, which can be supported by the obtained plot curvature. This is due also to the rate of mass transport (diffusion) for CdS QD nanoparticle redox systems, which seems to be slower than that of the cadmium ions, similar to that for Fe_2O_3 nanoparticles [26].

The effect of pH on the SWV of CdS QD suspension was studied. The results obtained (figure 3(A)) show that the peak current decreases on increasing the pH, being more significant from pH 7.0 to 3.0. This general augmentation of response is related to the increase of the reduced cadmium produced by an enhanced CdS reduction by decreasing the pH (according to equation (2)). The results obtained show that even at pH 7.0 the SWV signal was around half of that obtained at pH 3.0 and so sufficient for further analytical use. Moreover, the use of pH 7.0 is of interest, taking into consideration future applications in DNA sensing, being the usual medium pH in hybridization procedures.

The SWV parameters were optimized so as to obtain the highest peak signal for CdS QD detection. Figures 3(B)–(E) show the optimization results for a 20 μl drop of QD suspension at pH 7.0 phosphate buffer. A maximum response at 25 Hz was obtained while changing the frequency from 15 to 35 Hz (figure 3(B)), being the modulation amplitude maximum at 30 mV at an operation range from 10 to 40 mV (figure 3(C)). The effect of deposition potential upon QD detection was also studied. As expected, the current response was increased by decreasing the deposition potential from

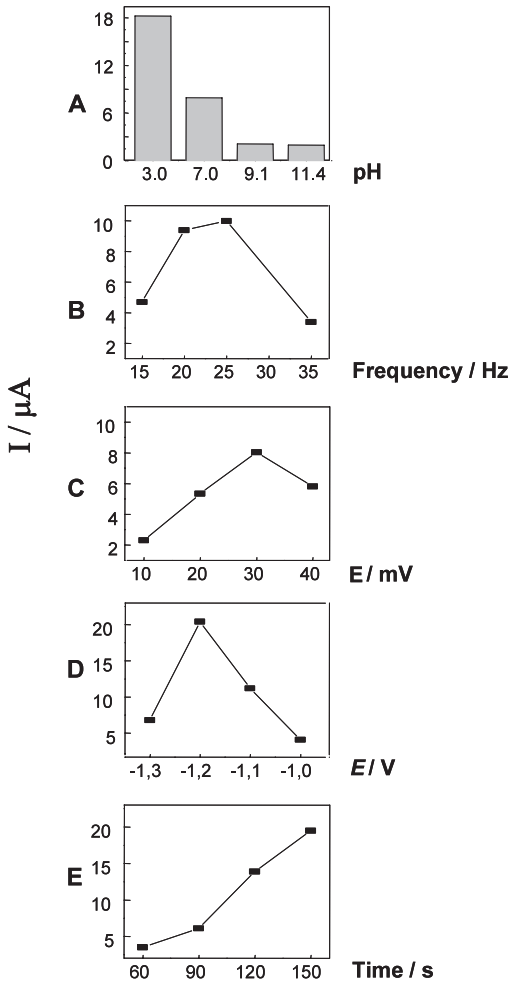

Figure 3. (A) Effect of pH; (B) SW frequency; (C) modulation amplitude; (D) deposition potential; (E) deposition time to the SPE response for CdS QDs (4.65×10^{17} mol l^{-1}) in a solution of 0.1 mol l^{-1} phosphate buffer (pH 7.0). Square-wave voltammetric scan with frequency of 25 Hz, step potential 10 mV and amplitude of 30 mV. Deposition potential of −1.1 V during 120 s.

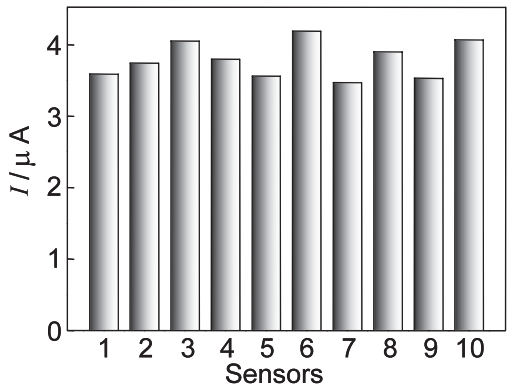

Figure 4. Performance of ten different sensors during measurements with a 14×10^{16} QDs ml^{-1} concentration suspension. Others experimental conditions as in the figure 3.

−1.0 to −1.2 V. An unexpected decrease was obtained on decreasing the potential to −1.3 V (see figure 3(D)). This QD current decrease is related to an inhibition of the response at

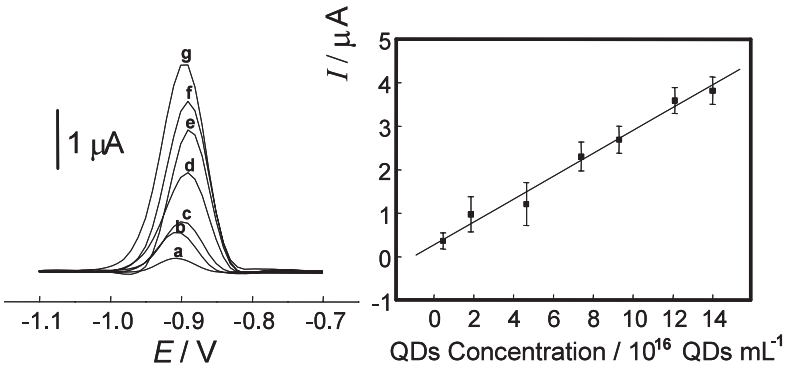

Figure 5. Square-wave stripping voltammograms after blank subtraction for increasing concentration of QDs: (a) 0.5, (b) 2.0, (c) 4.7, (d) 7.4, (e) 9.3, (f) 12.1, (g) 14.0×10^{16} QDs ml^{-1}. Also shown is the corresponding calibration plot (right) over the range 0.5–14.0×10^{16} QDs ml^{-1}. The measuring solution was 0.1 mol l^{-1} phosphate buffer, pH 7.0. Square-wave voltammetric scan with frequency of 25 Hz, step potential 10 mV and amplitude of 30 mV. Deposition potential of −1.1 V for 120 s.

more negative potential values, probably due to an opposite effect coming from glutathione. The increase of deposition time better supports the QD detection due to the increase of the quantity of the reduced cadmium coming from the cadmium ions in equilibrium with CdS QDs at the given conditions.

The response of diverse SPEs to the same drop of a 14×10^{16} CdS QD concentration suspension was also studied (figure 4). The results obtained show an RSD of 6.73%. The stability of the response for the same SPE was also studied (Supporting information figure 2S, available at stacks.iop.org/Nano/18/035502). For this case, an RSD of 11.8% was obtained for up to six measurements and it was almost doubled (22.2%) for four consecutive measurements. These results show that better responses are obtained by using one SPE for each measurement.

Figure 5 shows typical square wave stripping voltammograms after the blank subtraction for increasing concentration of CdS QDs up to 10^{16} QDs ml^{-1}. The corresponding calibration plot including the error bars for a set of three parallel measurements is also shown. CdS QDs as low as 10^{16} ml^{-1} have been possible to detect. Taking into consideration the CdS QD drop volume introduced (20 μl), this corresponds to a detection limit of around 2×10^{14} CdS QDs.

4. Conclusions

A direct detection technique for CdS QDs, that can be extended for other similar QDs, that avoids the chemical dissolving as in the previously reported electrochemical detection methods is achieved. The hypothesis for the detection is explained in the light of the experimental results obtained. Moreover, the optimization of the detection procedure is achieved, which will be of interest for further applications of the proposed techniques in developing electrochemical biosensors.

The detection of CdS QDs is simple, low cost, and based on a sensitive electrochemical method. It is based on the square wave voltammetry of the CdS QD suspension dropped onto the surface of a screen printed electrode. In the first step a drop of 20 μl containing an appropriate concentration of CdS QDs is introduced onto the surface of the SPE and maintained for 60 s while applying a potential of 0 V. In the second step a deposition potential of −1.1 V for 120 s was applied, during which the electrochemical reduction of CdS QDs to Cd^0 occurs. After the accumulation step SWV was performed by scanning from −1.1 to −0.7 V, resulting in an analytical signal due to the oxidation of Cd^0.

The analytical signal used for the CdS QDs quantification comes from a mixed phenomenon detection which depends on the medium pH. The analytical protocols have been optimized to give results which display a wide, linear response range as well as a CdS QD detection limit that is of interest for various applications ranging from DNA analysis to immunoassays. The proposed technique represents a lower cost alternative to optical methods and will be of interest for fast screening as well as in field analysis.

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