Bionanostructures and their integration into electrochemical sensing system. A review of DNA applications

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Current progress in applying nanoparticles as labels in electrochemical DNA detection systems will be shown. Some aspects of the synthesis of nanoparticles are described, especially the applications of gold nanoparticles or QDs as quantitation tags or electrochemical hosts for DNA detection so as to design what are known as 'chips in solution'.

Keywords: Quantum dots; gold nanoparticles; DNA, electrochemical detection; stripping voltammetry; sensors.

Se demuestra el progreso en la aplicación de nanopartículas como marcas en sistemas de detección electroquímica de ADN. Se describen algunos aspectos de síntesis y más en detalles los de aplicación de nanopartículas de oro o de quantum dots como elementos de cuantificación o de identificación para el análisis de ADN para el diseño de los llamados 'chips' en solución.

Descriptores: Puntos cuanticos; nanopartículas de oro; DNA, detección electroquímica; voltametria de redisolución; sensores.

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1. Introduction

Nanoparticles such as quantum dots (QDs) [1] or other colloidal nanocrystals have an enormous potential for being used as labels in bioanalytical systems. [2] The basic concept is to develop smart nanostructures that not only molecular recognition abilities but also built-in codes for rapid target identification. For example, the surface of a QD or of a polymer bead or micelle loaded with QDs can be conjugated to biomolecular probes [3–5] such as oligonucleotides and antibodies, while an identification code is embedded in the bead's interior. By integrating molecular recognition and the electrochemical coding, each nanoparticle could be considered a "chemical lab" that detects and analyzes a unique sequence or compound in a complex mixture. Multiple analyses can be performed by using various QDs along with electrical/electrochemical detection methods. Such encoded beads should find broad application in gene expression studies, high-speed screening, medical diagnostics, and environmental and food analysis.

In the present review, we shall focus only on the use of nanoparticles for DNA electrochemical detection. The current progress in applying QDs to DNA sequence detection based on electrochemical schemes will be shown. It will cover firstly some aspects of the synthesis of nanoparticles and then in more detail applications of gold nanoparticles or QDs as quantitation tags or electrochemical hosts for DNA detection so as to design what are known as 'chips in solution'.

2. QD bionanostructures

Gold nanoparticles as well as quantum dots are the most reported nanostructures applied in the electrochemical analysis of DNA. Synthesis of these nanoparticles and than their modification with DNA will be described in the following sections.

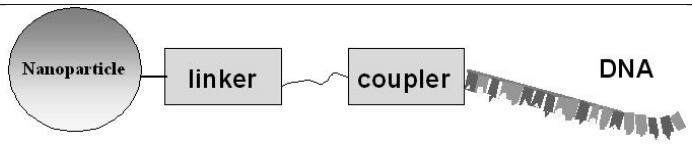
2.1. Synthesis of nanoparticles

Gold nanoparticles. Homogeneous preparations of gold nanoparticles varying in size from 3 to 20 nm can be easily prepared. Various procedures on the preparation of gold nanoparticles are reported [6, 7]. Colloidal gold can be synthesized with high quality in an 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 ($NaBH_4$) [8].

Quantum dots (QDs). Several synthetic methods for the preparation of QDs have been reported [9]. They are based on pattern formation (colloidal self-assembled pattern formation by surfactant micellation), [10–13] organometallic thermolysis [14] or electrochemical deposition [15].

The QDs can be formed also in what is called the "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). By varying the water content of the mixture, the size of the water droplets suspended in the oil phase could be varied systematically. Cadmium, lead and zinc sulphide quantum dot nanoparticles for electroanalytical applications were prepared [16] based on the inverse micelle method (see schematic presentation of the principle in Fig. 1 for PbS quantum dots), slightly modified from literature protocol [17]. The AOT/n-heptane water-in-oil microemulsion was prepared by the solubilization of distilled water in n-heptane in the presence of AOT surfactant. The resulting mixture was separated into reverse-micelle subvolumes where cadmium (or lead or zinc) nitrate and sodium sulphide

TABLE I. Typical methods used to connect gold or quantum dot nanoparticles to DNA.

			Reference
Au	streptavidin	biotin-$(CH_2)_6$- DNA	[24,15]
Au	-	HS-$(CH_2)_6$-DNA	[26]
Au	cyclic disulfide linker	(The linker is connected to DNA)	[27]
CdS	Cd^{2+}., HS-$(CH_2)_2$-OH	Adsorption through Coulombic interactions	[28]
CdSe/ZnS	-	HS-$(CH_2)_6$-	[29]

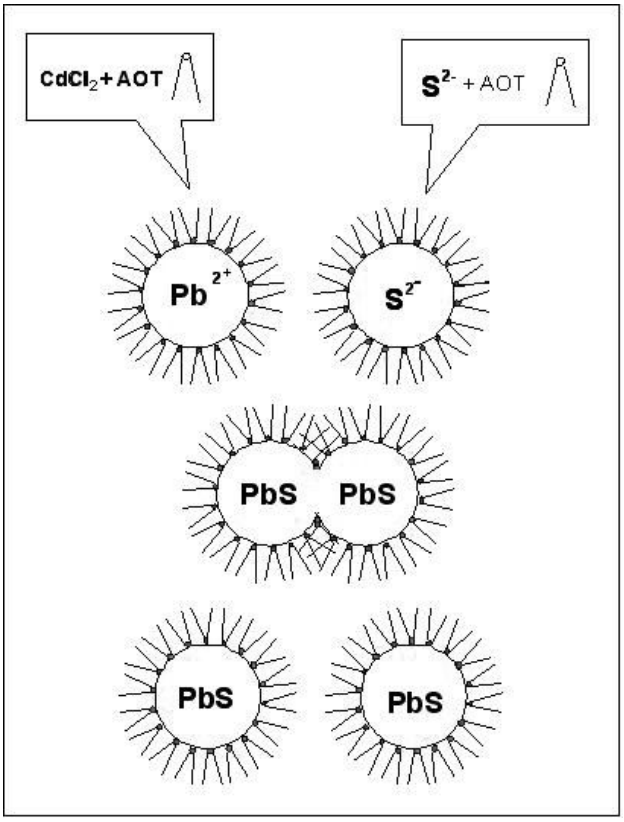


FIGURE 1. Schema of the inverse micelle method used to prepare PbS quantum dots. This technique exploits natural geometrical structures created by water-in-oil mixtures upon adding an amphilic surfactant such as sodium dioctyl sulfosuccinate (AOT). By varying the water content of the mixture, it was shown that the size of the water droplets suspended in the oil phase could be varied systematically. Adding metal salts to the water pools could cause nucleation reactions carried out at room temperature.

solutions were added, respectively. The two subvolumes were mixed and stirred under helium to yield the CdS (or PbS, ZnS accordingly) nanoparticles. Subsequently, cystamine solution and 2-sulfanylethane sulfonic acid were added.

2.2. Modifications with DNA

The n-alkylthiolated DNA has been used extensively in the preparation of DNA functionalized gold and semiconductor nanoparticles (See typical modifications in Table I). As an interesting alternative, DNA oligonucleotides that contain several adenosyl phosphothioate residues at their ends have been used to interact directly with the metal surface of nanoparticles. [18] The use of cyclic disulfide linkers [27] such as leads to nanoparticle capping, which are more stable towards ligand exchange than the corresponding conjugates prepared with the conventional reagents that contain a single thiol group or acyclic disulfide units. The greater stability is likely a result of the anchoring of the ligands to the nanoparticles through two sulfur atoms.

To prepare the DNA conjugates of CdS, PbS and ZnS, an aqueous solution of the each nanoparticle was exposed to the thiolated oligonucleotide probe at room temperature under helium, and was gradually brought to a phosphate buffer. The resulting solution was dialyzed for 48 hrs against 0.2 M NaCl and 0.1 M phosphate buffer (pH 7.4) containing 0.01% sodium azide, so as to remove the excess of DNA strands.

3. DNA quantitation by using nanobiostructured tags

By analogy to fluoresence-based methods, several electrochemical detection methods have been used in which target DNA sequences have been labelled with electroactive QDs. The appearance of the characteristic electrochemical response of the QD reporter therefore signals the hybridization event. The direct attachment of DNA strands, either onto the surface of QDs or onto the surface of polystyrene microbeads loaded with QDs, can be used.

3.1. Quantitation via direct labelling with QDs and stripping detection

A detection method of DNA hybridization based on labelling with CdS QDs tracers followed by the electrochemical stripping measurements of the cadmium have been developed and detailed procedure was described previously [16].

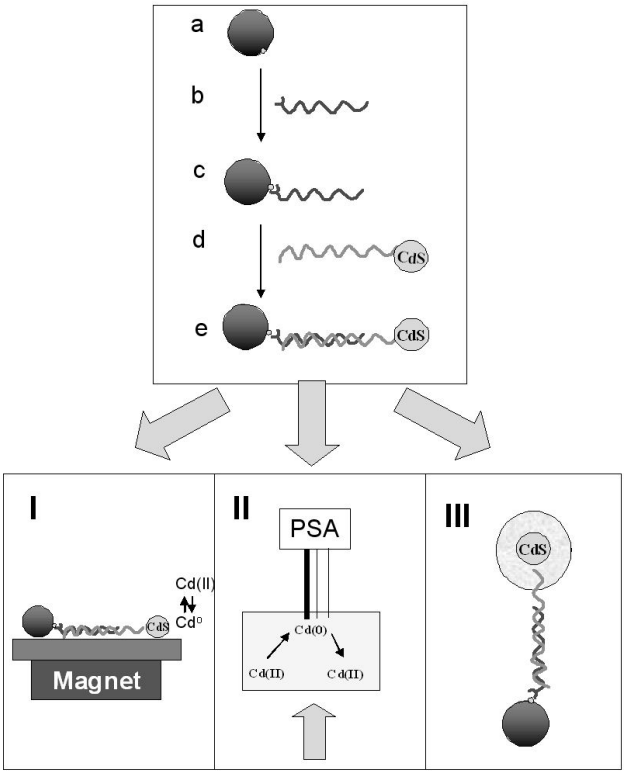


FIGURE 2. Schematic representation of the analytical protocol. The streptavidin coated magnetic beads (a) are connected to the biotinylated target (b) forming the DNA target modified magnetic beads (c). After this step, the hybridiation event to a CdS-labeled probe (d) occurred. The magnetic beads connected to the CdS labelled hybrid (e) are than separated and treated via 3 strategies. I) Direct collection onto a magnet/screen-printed electrode and direct detection with PSA. II) Dissolution with HNO3 so as to release cadmium ions and then detection by PSA. (III) Enhancement of CdS tags and detection, as in strategy II.

The use of CdS QDs as tracer for DNA detection was achieved by using three different protocols (see Fig. 2). These three protocols were based on a common previous analytical protocol that consisted in five steps. (a-e, Fig. 2). Target modified magnetic beads were prepared firstly by using a MCB 1200 Biomagnetic Processing Platform using a modified procedure recommended by Bangs Laboratories. The prepared streptavidin-coated magnetic beads were then washed and the corresponding biotinylated target was connected via a biotin-streptavidin mechanism. Then the hybridization with CdS–DNA probe was performed and the resulting hybrid-conjugated microspheres, after proper washing, were treated following three different protocols (see I,II,III at Fig. 2).

DNA detection using protocol #1. In addition to measurements of the dissolved cadmium, according to this protocol, solid-state measurements were demonstrated following a 'magnetic' collection of the 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%). The response mechanism for the stripping signal obtained is related to the direct oxidation of the CdS QDs at the surface of the electrode. A detailed study of such phenomena was also made by Bard *et al.* [19]According to cyclic voltammetry studies of metallic particles, and in light of the irreversibility for oxidation and reduction of CdS QDs, they propose a multielectron transfer process where by the electrons are consumed by fast coupled chemical reactions due to the decomposition of the CdS cluster.

DNA detection using protocol #2. According to the first protocol, the hybrid conjugated magnetic beads (washed accordingly) were resuspended in a 1 M HNO3 solution. Dissolution of the CdS tag proceeded for 3 min using magnetic stirring. Following a magnetic separation, a measured volume of HNO_3 solution (containing the dissolved cadmium) was transferred into the acetate buffer (pH 5.2) measuring solution. Chronopotentiometric stripping measurements of the dissolved cadmium ion were performed at a mercury-film electrode (prepared on a polished glassy carbon electrode) using a 2 min deposition at -0.90 V in stirring conditions. Subsequent stripping was carried out after a 10 sec rest period (without stirring) using an anodic current of +1.0 μA.

DNA detection using protocol #3. A nanoparticle-promoted cadmium precipitation, by using a fresh cadmium solution hydroquinone, is used to enlarge the nanoparticle tag and amplify the stripping DNA hybridization signal. Cadmium catalytic precipitation experiments were performed by a 20 min incubation of the sample (following hybridization) in a solution containing a standard solution of cadmium nitrate and hydroquinone. The reduction of cadmium ions onto CdS nanoparticles occurs. The enlarged nanoparticles connected with the hybrid were washed again. 'Magnetic' collection experiments were conducted using a mercury-coated screen-printed carbon electrode at -1.10 V in a 0.1 M HCl solution containing mercury by placing a magnet directly under the working electrode to anchor the particle-DNA assembly.

4. Multiple detection of DNA. "Chips in solution"

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.

Inspired by multicolor optical bioassays [20–22] an electrochemical coding technology based on the labelling of probes bearing different DNA sequences with different QDs has been developed [23]. This novel technology, for the first time, made possible the simultaneous detection of more than one target by using an electrochemical detection method. The multiple detection of various DNA targets is based on the use of various QD tags with diverse redox potentials.

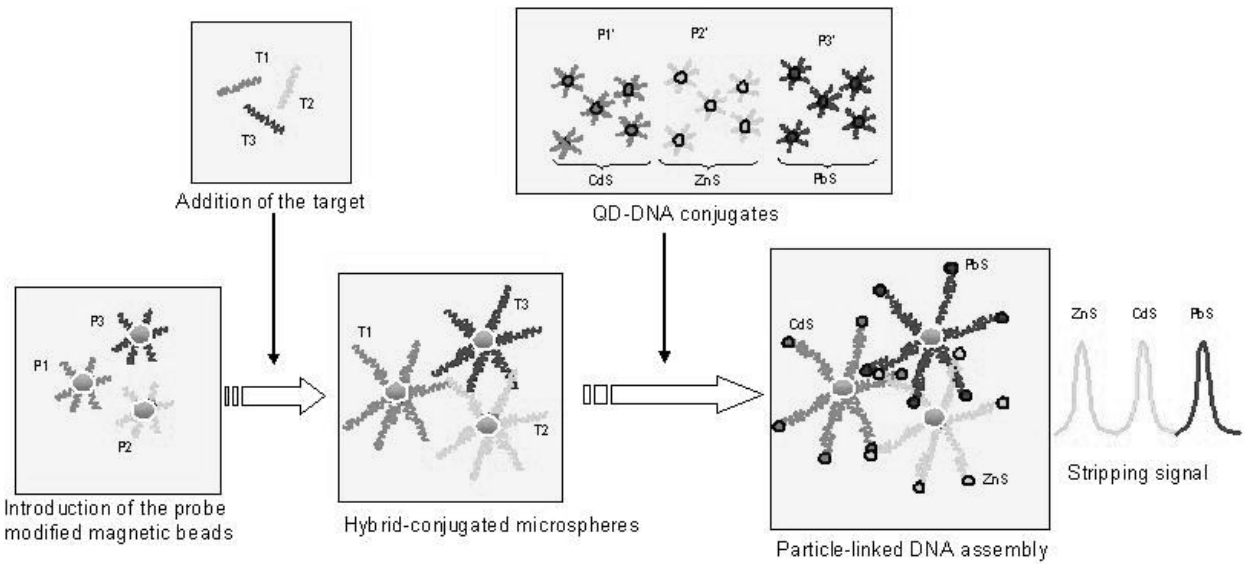


FIGURE 3. Schema of the analytical protocol of the multi-target electrical DNA detection protocol based on different QD tracers (CdS, ZnS and PbS), modified with the corresponding probe P1', P2' and P3'. The detection is performed using square wave anodic stripping voltammetry (SWASV)

Figure 3 represents the schema of the analytical protocol of the multi-target electrical DNA detection protocol based on different QD tracers. Three different QDs, ZnS, CdS and ZnS, were first produced as mentioned in §2. The sandwich assay involved a dual hybridization event. In a first step, the probe (P1, P2, P3)-modified magnetic beads were introduced. The corresponding amount of each target (T1, T2, T3) was added to the hybridization buffer containing the three probe-coated magnetic beads. The first hybridization thus proceeds under magnetic mixing for 20 min. The resulting hybrid-conjugated microspheres were then washed and the second hybridization with each QD-DNA (P1', P2',P3') conjugate occurred. The resulting particle-linked DNA assembly was washed again and resuspended in a 1 M HNO_3 solution. Dissolution of the QD tags thus proceeded for 3 min using magnetic stirring. Following a magnetic separation, the acid solution (containing the dissolved QDs) was transferred into the acetate buffer (pH 5.6) measuring solution containing mercury ion. Square wave anodic stripping voltammetry (SWASV) measurements of the dissolved QDs were carried out at an in-situ prepared mercury film electrode giving voltammograms as reported earlier [23]. The DNA connected quantum dots yielded well-defined, 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). Such encoding technology using QDs offer a voltammetric signature with distinct electrical hybridization signals for the corresponding DNA targets. The number of targets that can be readily detected simultaneously (without using high level multiplexing) is controlled by the number of voltammetrically distinguishable metal markers.

5. Conclusions

The electrochemical properties of QD nanocrystals make them extremely easy to detect using simple instrumentation. QDs nanocrystals are made of a series of metals easily detected by high sensitive techniques such as stripping methods. In addition, these electrochemical properties may make it possible to design simple and inexpensive electrochemical systems for detection of the ultra-sensitive, multiplexed assays.

These novel DNA detection systems may revolutionize the existing enzyme labelling technology in developing new user-friendly bioanalytical systems. Nanoparticles, compared to existing labels, are more stable, allow more flexibility, faster binding kinetics (similar to those in a homogeneous solution), high sensitivity and high-reaction speeds for many types of multiplexed assays, ranging from immunoassays to DNA analysis.

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