CONTRIBUTIONS to SCIENCE, **3** (1): 57–66 (2005)
Institut d'Estudis Catalans, Barcelona

Toward nanoanalytical chemistry: case of nanomaterial integration into [bio]sensing systems

A. Merkoçi* and S. Alegret
Grup de Sensors i Biosensors, Departament de Química, Universitat Autònoma de Barcelona

Resum

La nanotecnologia orientada al camp de la química analítica, o química nanoanalítica, ja sigui a escala nanomètrica o a macroescala, s'està convertint en una matèria de gran potencial. Els conceptes associats a aquesta química nanoanalítica són acceptats ràpidament, entre d'altres, pel camp de la biologia molecular. El disseny de noves estructures amb propietats òptiques i electroquímiques especials i la seva integració en sistemes de [bio]detecció representen alguns dels molts aspectes relacionats amb la investigació en aquest nou camp. La química nanoanalítica haurà de proporcionar noves eines per a diferents aplicacions, ja sigui en el camp mèdic, mediambiental o industrial.

Es presenten exemples de química nanoanalítica relacionats amb nanopartícules d'or, *quantum dots* i nanotubs de carboni. Com a primer exemple, es descriurà la detecció de DNA mitjançant ICPMS de nanopartícules d'or, així com la detecció electroquímica d'aquestes mateixes partícules. El segon exemple serà l'ús de *quantum dots* per al disseny dels anomenats «xips de DNA en solució». Finalment, es presentarà la integració de nanotubs de carboni en una matriu de resina epoxi per formar un nou material compòsit amb aplicacions interessants en la tecnologia dels sensors.

Paraules clau: Química nanoanalítica, nanotecnologia, nanopartícules d'or, quantum dots, nanotubs de carboni, ICPMS, anàlisi electroquímica, [bio]sensors

Abstract

Analytical chemistry-oriented nanotechnology, or nanoanalytical chemistry, at nanometer or macroscale, at both nanometer and macroscale dimensions, is on its way to becoming a field of great potential. Nanoanalytical chemistry concepts are finding rapid acceptance in molecular biology and other fields. The design of novel nanostructures with special optical and electrochemical properties and their integration into [bio]sensing systems represent only one aspect of the research in this new field. Analytical chemistry-oriented nanotechnology, or nanoanalytical chemistry, will provide new tools for various applications in fields like medicine, environmental studies, and industry.

Nanoanalytical chemistry examples related to gold nanoparticles, quantum dots, and carbon nanotubes will be presented in the present paper. DNA detection by ICPMS of gold nanoparticle tags as well as direct electrochemical detection of the same nanoparticles will be described here as the first example. The use of quantum dots for the design of so-called "DNA chips in solution" will be the second example. The integration of carbon nanotubes in an epoxy matrix forming a novel composite material with interesting applications in sensor technology will be the paper's final example.

Keywords: Nanoanalytical chemistry, nanotechnology, gold nanoparticles, quantum dots, carbon nanotubes, ICPMS, electrochemical analysis, [bio]sensors

1. Introduction

Nanotechnology has become one of the most highly charged disciplines in science and technology today. The intense interest in nanotechnology is being driven by various interesting fields and is leading to a new industrial revolution. Nanotechnology includes many branches of science and technology, from space exploration, to simple consumer materials. This highly multi-disciplinary field depends upon shared knowledge, tools, techniques and information from many different areas of research and requires input from materials scientists, engineers, physicists, chemists, biologists, and clinical researchers.

To demonstrate that any product or manufacturing process meets a specified functional demand requires quantitative measurements traceable to an agreed metrology scale. In order to apply practical metrology in the field of nanotechnology,

*Author for correspondence: Arben Merkoçi. Grup de Sensors i Biosensors, Departament de Química, Universitat Autònoma de Barcelona. 08193 Bellaterra, Catalonia, EU. Tel. 34 935811976. Fax: 34 935812379. Email: arben.merkoci@uab.es

i.e., to make measurement in the nanometer range traceable to the SI units of quantity of matter, practical measurement standards must be constructed. However, for an accurate analytical chemistry application these standards need consolidation, and novel specific standards must be constructed. Hence, to convert nanotechnology into a successful business, it is necessary to access the relevant metrology tools that give us the ability to measure not only in three dimensions with atomic resolution but also to use other classical tools of analytical chemistry as well as novel ones.

Therefore, nanometrology and analytical chemistry should be seen as indispensable parts of nanotechnology. These disciplines should develop hand in hand with the development of nanoscience and nanotechnology, respectively. These intrinsic analytical chemistry aspects of nanotechnology, which are strongly related to nano-aspects, are giving us the chance to forge a new branch of analytical chemistry, now called nanoanalytical chemistry, a discipline or series of disciplines appearing at the beginning of twenty-first century. Day after day, nanoanalytical chemistry is indeed becoming a crucial factor in the development of nanotechnology.

This analytical chemistry-oriented nanotechnology, or nanoanalytical chemistry, which works at nanometer or macroscale dimensions, is a subject of great potential. In biological cells, in ever smaller semiconductor devices, attention is now being given to chemical analysis within very small dimensions. Currently, no general instrumentation allows a satisfactory spatial resolution for such analysis. And recently the concept of nanotechnology has been broadened to deal with quantities other than length, for instance, very small amounts of substance. This has led to the term nanochemistry.

Nanoanalytical chemistry concepts are finding rapid acceptance in the field of molecular biology and other disciplines. The comparable size scale of nanoparticles and biological materials, such as antibodies and proteins, facilitates the use of these particles for biological and medical applications. DNA arrays in solution, for example, can now be used to monitor gene expression levels, since many genes can be examined simultaneously thanks to the use of quantum dots with special optical and electrochemical properties.

The design of nanostructures with special nano[bio]analytical properties and with entirely new properties represents only one aspect of the ongoing research in this new field. Analytical chemistry-oriented nanotechnology, or nanoanalytical chemistry, will significantly improve treatment of a variety of diseases in the coming years and will provide new tools for medicine, making possible novel and fast diagnosis, drug design and delivery, molecular-scale surgery to repair and rearrange cells, tissue engineering, and other applications, including fields like environmental disciplines or industries.

The integration of nanomaterials in sensing [bio]systems represents only one area of nanoanalytical chemistry. Inorganic nanoparticles, including gold nanoparticles and quantum dots, are particularly attractive building blocks for the generation of a novel generation of biosensing systems, including [bio]sensors. Carbon nanotubes represent other building blocks with various applications of analytical interest.

2. Gold nanoparticles

While the most frequently used labels for electrochemical sensors to date have been enzymes and small molecules like electroactive indicators (dyes, etc.), various kinds of nanoparticles have emerged in the last 5 years as novel labels of biological molecules, gold and silver nanoparticles being the pioneers in bionananosensing technologies, especially with optical detection. It now appears clear that nanoparticles will overcome many of the significant chemical and spectral limitations of molecular fluorophores. Nanoparticles have a chemical behaviour similar to that of small molecules and will be able to be used as specific optical or electroactive labels. Such nanoparticles may be expected to be superior in several ways. Compared to existing labels, they are more stable and cheaper. They 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.

2.1. DNA detection through ICPMS of gold nanoparticle tag

An attempt to measure directly the atomic composition of gold nanoparticles conjugated to oligonucleotides carrying the c-myc peptide via interaction of antibodies through the use of ICPMS has already been reported [1] Two reference method strategies, the classical Dot Blot [2] format and ICPMS, the proposed format, were used to detect the c-myc peptide epitope attached to synthetic oligonucleotides (see Figure 1). In both cases, oligonucleotide-peptide conjugates

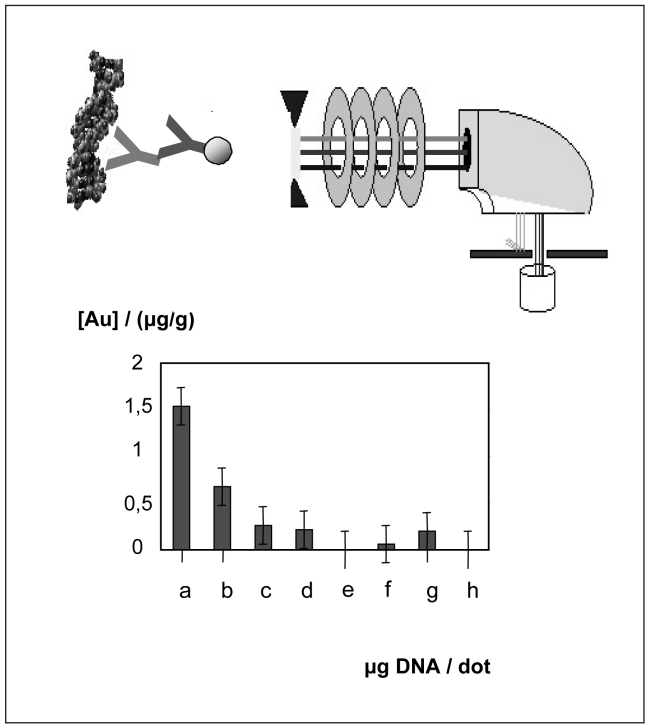

Figure 1. (upper part): Schematic of the DNA labelled with gold nanoparticles via immunoreactions with the peptide sequence inserted in the middle of the DNA chain. (lower part): Gold signal obtained from the positive ICPMS-linked assays with decreasing concentrations of the oligonucleotides: 8 (a); 4 (b); 2 (c); 1 (d); 0.5 (e); 0.25 (f); 0.125 (g); 0 (h) µg oligonucleotide / dot.

were first applied to a nitrocellulose membrane using a manifold attached to a suction device. After immobilization of the oligonucleotide by UV radiation, the samples were incubated with an anti-c-myc monoclonal antibody. In the case of the Dot-Blot [3], the format strategy was followed by incubation with a secondary antibody conjugated to horseradish peroxidase and development with luminol as chemiluminescent substrate. The detection limit was 0,125 μg (8 pmol) oligonucleotide/dot. In the case of the ICPMS strategy, the format was followed by incubation with the secondary antibody (Anti-mouse IgG) conjugated to gold nanoparticles, with ICPMS detection after dissolving. The enhanced DNA signals obtained by ICPMS of gold tags are combined with the high specificity of oligonucleotide-peptide conjugate interaction with anti-c-myc monoclonal antibody, followed by the immunoreaction with the secondary antibody (Anti-mouse IgG) conjugated to gold nanoparticles. Oligonucleotide-peptide conjugates are chimeric molecules made by oligonucleotides covalently linked to peptide sequences. They are produced to transfer some of the biological and/or biophysical properties of peptides to synthetic oligonucleotides. [4,5] The introduction of peptides into oligonucleotide sequences has resulted in the introduction of a higher number of multiple nonradioactive labels [6]. Recently, the introduction of epitope peptide sequences into oligonucleotides as nonradioactive labeling system has also been described [7].

Since colloidal Au nanoparticles with extremely high particle density show strong adsorption activity, it is necessary to determine the extent of the nonspecific binding (NSB) of the goat anti-Mouse– Au colloidal in the assay. Au signals from NSB were almost zero, indicating an excellent NSB level at ICPMS detection.

Taking into consideration that the detection limit of ICPMS is around 20 ng Au / g solution, it was deduced that a 3 ng /dot that corresponds to 0.2 pmols (equal with 200 fmols) of DNA could be detected, which means a quantity around 40 times lower than with the Dot-Blot format.

This ICPMS-linked DNA detection offers several advantages. The tag is directly analyzed, giving rise to the possibility of oligonucleotide quantification even at very low detection limits. It is reasonable to expect that multiple tagged secondary antibodies connected with primary peptide sequence antibodies can be used for simultaneous determination in the same sample. The immediate acidification of the reacted and separated sample allows for long-term storage before analysis and simplifies assay protocols. The stability of nanoparticles under UV and visible light enables a long-term monitoring of the environment, for instance, for biological warfare agents or natural pathogenic organisms such as cholera and Escherichia coli, which represent a critical problem for countries with limited water treatment capabilities.

In a very early stage, the proposed ICPMS-linked DNA assay may have significant potential as an important nonradioactive DNA detection method for the simultaneous determination of various sequences by labelling different kinds of inorganic nanoparticles (and also taking advantage of the recent development of ICPMS technique). This study will also be of interest for the development of novel genosensors and DNA chips based on multiple labelling [8] by according to the specific immunoreactions with the peptide sequences introduced into the DNA probes.

2.2. Electrochemical detection of gold nanoparticles

Lastly, various nanoparticle-based electrochemical DNA hybridisation assays were developed using gold and silver tracers. Homogeneous preparations of gold nanoparticles varying in size from 1.2 to 20 nm can be easily prepared. Various procedures on the preparation of gold nanoparticles are reported [9,10]. DNA strands can be easily coupled to colloidal gold particles and, additionally, they do not appear to lose their biological activity. The attachment of oligonucleotides onto the surface of a gold nanoparticle can be performed by simple adsorption [11] or via biotin-aividin linkage where the avidin is previously adsorbed onto the particle surface [12]. However, the most commonly used method to attach oligonucleotides onto gold nanoparticles is via thiol-gold bonds.

Various strategies for the electrochemical detection of gold tracers have been reported. The major part of these strategies is based on the intrinsic electrochemical signal of the metal nanoparticle observed after dissolving it with HBr/Br_2.[13] The obtained gold(III) ions 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 DNA hybridisation is also used, and an enhanced electrochemical signal due to silver is obtained.[14]

Nevertheless other interesting methods have been reported. Mirkin and colleagues [15] have exploited the silver deposition technique to construct a sensor based on conductivity measurements. In their approach, a small array of microelectrodes with gaps (20μm) 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.

Gold-coated iron nanoparticles have also been used in DNA detection assays. [16] After hybridisation, 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 developed DNA labelling mode offers high sensitivity, a well-defined concentration dependence, and minimal contributions from non-complementary nucleic acids.

The vast majority of these electrochemical methods based on chemical dissolution of AuNPs represent hazardous procedures and therefore methods based on direct electrochemical detection of gold nanoparticle tags, which would replace the chemical oxidation agent, are urgently needed. Costa-García et al. [17] employed a carbon paste electrode and detected Au-NPs either by silver enhancement or directly after accumulation onto the electrode surface.

Another alternative for the detection of AuNPs based on the use of graphite-epoxy composite electrodes is proposed by

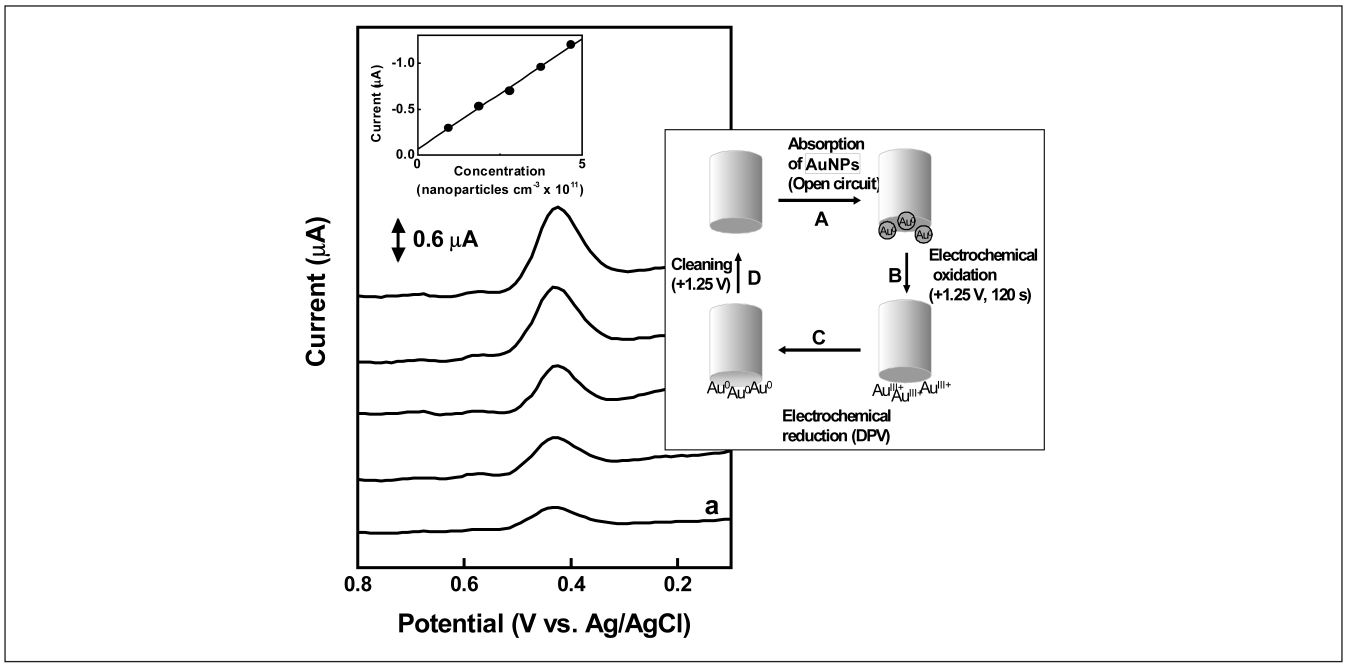

Figure 2. Diagram (right) of the direct DPV detection of gold nanoparticles obtained by using a graphite-epoxy composite. Results (left) obtained for increasing concentrations of gold nanoparticles. Differential pulse voltammograms of AuNP solutions with concentrations of (a) 9.3×10^{10}, (b) 1.9×10^{11}, (c) 2.8×10^{11}, (d) 3.7×10^{11}, (e) 4.7×10^{11} nanoparticles cm^{-3}. The inset is the calibration plot for AuNP concentrations in the range 9.3×10^{10} to 4.7×10^{11} nanoparticles cm^{-3}. Supporting electrolyte: hydrochloric acid (0.1 M); accumulation time: 10 min; dissolution time: 120 s; dissolution potential: +1.25 V; step potential: 10 mV; modulation amplitude: 50 mV. Adapted from reference [18].

our group.[18] AuNP detection protocol (Figure 2, right) involves absorption of AuNPs on the surface of the graphite epoxy composite electrode (GECE). During this step the GECE in an open circuit was immersed in a solution containing an appropriate concentration of AuNPs for 10 min. After the accumulation step, the electrode was carefully washed with distilled water before being immersed into the voltammetric cell containing 0.1M HCl. The electrochemical oxidation of colloidal AuNPs to $AuCl_4^-$ was performed at +1.25 V (vs. Ag/AgCl) for 120 s. Immediately after the electrochemical oxidation step, differential pulse voltammetry (DPV) was performed. During this step the potential was scanned from +1.25 V to 0 V (step potential 10 mV, modulation amplitude 50 mV, non-stirred solution), resulting in an analytical signal due to the reduction of $AuCl_4^-$. After the DPV measurement, a cleaning step was performed consisting of the application of a potential of +1.25 V (vs. Ag/AgCl) for 5 min whilst stirring. A DPV scan was carried out after the cleaning step to confirm the removal of the gold residues. The final measurements employed a background subtraction protocol involving storing the response for the blank solution and subtracting it from the analytical signal.

Various relevant parameters influencing the analytical DPV response of the AuNPs were investigated: (a) the time-dependence of the absorption of gold nanoparticles; (b) the oxidation potential of the AuNPs; (c) the influence of electrochemical oxidation time of the AuNPs upon the DPV signal.

The GECE based protocol for detection of gold nanoparticles produces a defined concentration dependence for the optimised parameters (Figure 2, left). The calibration plot for 10 nm AuNPs was linear over the range from 4.7×10^{8} to 4.7×10^{11} nanoparticle cm^{-3}, with a sensitivity of -2.34×10^{-12} ($\mu A\ cm^3$ $nanoparticles^{-1}$) and intercept of 0.086 μA (correlation coefficient 0.999). The favourable signal-to-nose characteristics observed for the 4.7×10^{8} nanoparticle cm^{-3} colloidal gold solution leads to a detection limit of 1.8×10^{8} nanoparticle cm^{-3} based on a S/N ratio = 3.

This high sensitivity is coupled to a good AuNP detection reproducibility using GECE. Reproducible DPV signals for AuNPs over the entire GECE operation protocol were observed (RSD=5.6%).

3. Quantum dots

In principle, semiconductor nanoparticles, or quantum dots (QDs), provide a novel platform for improving labelling technologies used in the design of DNA sensors (genosensors). QDs are crystalline clusters of a few hundred to a few thousands atoms that have all three dimensions confined to the 1-to-10-nm-length scale [19].

Despite the enormous opportunities clearly offered by DNA sensors, some important hurdles remain. The first difficulties involve the electrode probes themselves and their fabrication into useful arrays. Array sizes on the order of 10 have thus far been demonstrated but, more typically, arrays of 50-100 sequences will be needed for clinical applications. For example, genetic screening for cystic fibrosis carriers requires testing for 25 different mutations plus positive and negative controls.[20] Although it is not difficult to fashion electrode pads with reproducible dimensions of a micron or less, the electrochemical readout requires mechanical connections to each individual electrode. The construction of very large, multiplexed arrays (on the order of 10^3) therefore presents a major engineering challenge. Labelling technology by using nanoparticles may provide a possible solution for this problem.

The current progress in applying QDs to DNA sequence detection based on electrochemical schemes will be shown in the following sections. A brief description of the QD synthesis and modification with DNA strands will be introduced first, and then the application of QDs in single or "DNA chip in solution" electrochemical detectionformats will be shown.

3.1 Preparation and modification with DNA

Three most important qualities are required to make QDs available in electrochemical sensing systems. First, QDs obviously should be easy to detect by any conventional electroanalytical method. Their size distribution should be as narrow as possible so as to ensure enough reproducibility during electrochemical assays where they will be used as biological molecule labels. QDs dispersed in a solvent should be stabilized in a way that prevents agglomeration.

Several synthetic methods for the preparation of QDs have been reported.[21] These methods are based on pattern formation (colloidal self-assembled pattern formation by surfactant micellation), [22-,23,24,25]organometallic thermolysis [26] or electrochemical deposition [27].

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). By varying the water content of the mixture, the size of the water droplets suspended in the oil phase can be varied systematically. This leads to the idea of using these self-enclosed water pools as micro-reactors for carrying out nanoscale sustained chemical reactions. A series of micelle-protected PbS nanoparticles were synthesised using lead acetate and alkanethiols [28,29].

Cadmium sulphide nanoparticles for electroanalytical applications were prepared [30] based on the inverse micelle method slightly modified from the protocol found in the literature [31] (see Figure 3). 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 nitrate and sodium sulphide solutions were added respectively. The two subvolumes were mixed and stirred under helium to yield the CdS nanoparticles. Subsequently, cystamine solution and 2-sulfanylethane sulfonic acid were added and the mixture was stirred for 24 hr under helium. It was then evaporated under vacuum and the residue was successively washed with pyridine, n-heptane, acetone, and methanol to yield the cystamine/thioethanesulfonate-capped, watersoluble QD nanoparticles.

To prepare the CdS-DNA conjugate, an aqueous solution of the CdS nanoparticles 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 h 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.

3.2. DNA quantification by using QD as label

QDs can be used in a variety of bioanalytical formats with electrochemical detection. When QDs are used as quantitation tags, an electrical / electrochemical signal emanating from the particles is quantified.

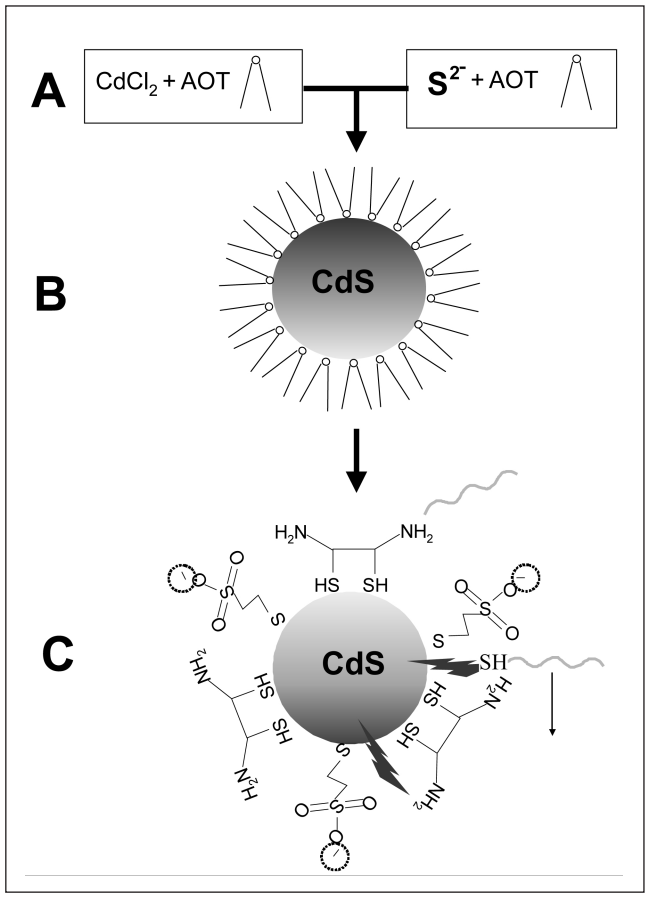

Figure 3. Schematic of the preparation of quantum dots modified with DNA. The whole process (shown is the case of CdS) consists in the preparation of and then the reaction (A) between reverse micelles of Cd^{2+} and S^{2-} in AOT surfacatant. The CdS quantum dots formed (B) still have the AOT as the outer shell. The QDs are washed and transformed so as to be soluble in water and then modified with DNA via thiol or amine groups (C).

By analogy to fluoresence-based methods, several electrochemical detection methods have been pursued in which target DNA sequences have been labelled with electroactive QDs. A detection method of DNA hybridization based on labelling with CdS QDs tracers followed by electrochemical cadmium-based stripping measurements has been developed, the detailed procedure of which has been described previously.[30]

3.3. "DNA chip in solution" by using several QDs

Genomic and proteomic research demands greater information from single experiments, but the potential of current DNA microarray technology represents some limitations. Both the fabrication and read-out of DNA arrays must be miniaturised in order to fit millions 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.

In this context, QDs hold particular promise as the next generation of barcodes for multiplexing 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.

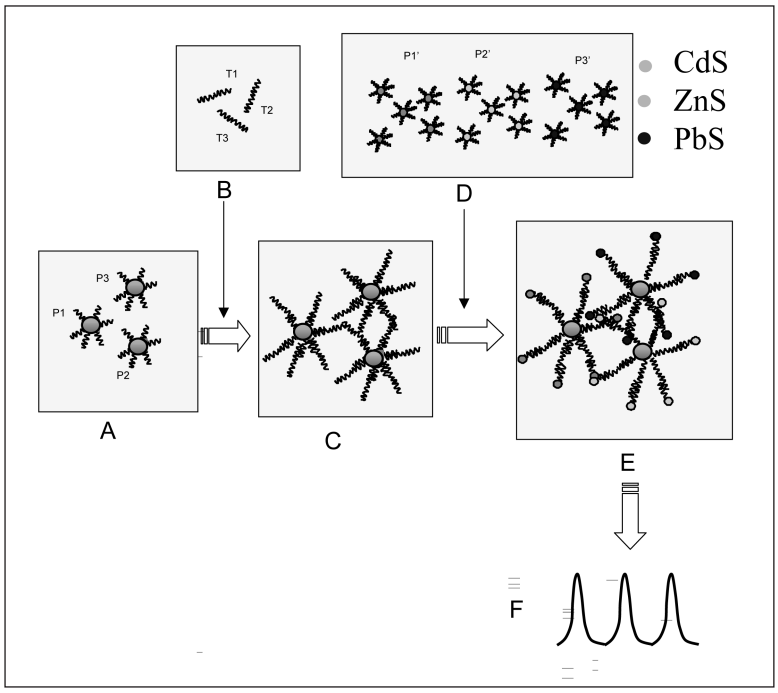

Figure 4. Schematic of the "DNA Chip in solution" assay achieved by using a multi-target DNA detection based on different QD tracers. A) Introduction of the probe modified magnetic beads B) Addition of the target C) Hybrid-conjugated microspheres D) QD-DNA conjugates E) Particle-linked DNA assembly F) Square wave anodic stripping voltammetry (SWASV) measurements of the dissolved QDs were carried. Paramagnetical particles were used as DNA immobilization platform.

Inspired by multicolour optical bioassays [32-, 33,34] an electrochemical coding technology based on labelling of probes bearing different DNA sequences with different QDs has been developed. [35] This novel technology has, for the first time, enabled 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 4 (A-F) represents the schematic of the analytical protocol (experimental details at [35]) of the multi-target electrical DNA detection protocol based on different QD tracers. Three different QDs, ZnS, CdS and ZnS, were first produced. The sandwich assay involved a dual hybridization event. In a first step (A) the probe (P1, P2, P3) –modified magnetic beads were introduced. The corresponding amount of each target (T1, T2, T3) was added (B) into 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 (C) were then washed and the second hybridisation, and with each QD-DNA (P1', P2',P3') conjugates (D) occurred. The resulting particle-linked DNA assembly (E) 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 (F) at an in-situ prepared mercury film electrode giving voltammograms as reported. [35] The DNA connected quantum dots 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). 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.

Other attractive nanocrystal tracers for creating a pool of nonoverlapping electrical tags for such bioassays are InAs, and GaAs semiconductor particles in view of the attractive stripping behaviour of their metal ions.

4. Carbon nanotubes

4.1. General overview

Since their discovery in 1991 [36], carbon nanotubes (CNTs) have generated great interest for various applications based on their field emission and electronic transport properties [37], their high mechanical strength [38], and their chemical properties. From this arises an increasing potential for use as field emission devices [39], nanoscale transistors [40], tips for scanning microscopy [41], or components for composite materials.[42]

CNTs are one of the most commonly used building blocks of nanotechnology. With one hundred times the tensile strength of steel, thermal conductivity better than all but the purest diamond, and electrical conductivity similar to copper, but with the ability to carry much higher currents, CNTs seem to be a wonder material.

CNTs include both singlewalled and multiwalled structures. Single-wall carbon nanotubes (SWCNTs) are comprised of a cylindrical graphite sheet of nanoscale diameter capped by hemispherical ends. The closure of the cylinder is the result of a pentagon inclusion in the hexagonal carbon network of the nanotube walls during the growth process. SWCNT have diameters typically ~1nm with the smallest diameter reported to date of 4^0A. This corresponds to the theoretically predicted lower limit for stable SWCNT formation based on consideration of the stress energy built into the SWCNT cylindrical structure.

Multiwall carbon nanotubes (MWCNTs) comprise several to tens of incommensurate concentric cylinders of these graphitic

shells with a layer spacing of 3-4^{0}A. MWCNTs tend to have diameters in the range 2-100nm. The MWCNT can be considered as a mesoscale graphite system, whereas the SWCNT is truly a single large molecule.

Distinctive properties of CNT, such as a high surface area, accumulation, branched conductivity, minimization of surface fouling and electrocatalytic activity are very attractive for electrochemical sensing.[43,44] Recent studies have demonstrated that CNT exhibits strong electrocatalytic activity for a wide range of compounds, such as neurotransmitters [45-48], NADH [49-52], hydrogen peroxide [45-53], ascorbic [45-47] and uric acid [45], cytochrome c [54], hydrazines [55], hydrogen sulphide [56], amino acids [57] and DNA. [58] It has been suggested that electrocatalytic properties originate from the ends of CNT. [52,59]

4.2. CNT composites for electrochemical sensors

Most of the CNT-based electrodes for electroanalytical applications involve physical adsorption of CNT onto electrode surfaces, usually glassy carbon [45–,46,47,49,52-,53,54,56,57,59]. However, it is important to note that CNT dispersed in mineral oil [51,58] or consolidated into Teflon [50] have been recently used.

A novel carbon nanotube epoxy-composite (CNTEC) electrode has been fabricated and characterized by our group. [60] Epoxy resin and hardener were mixed in a 20:3 ratio (w/w). CNTEC electrodes (see Figure 5) have been produced by loading the epoxy resin, before curing, with MWCNTs of different lengths (0.5-2 μm and 0.5-200 μm) (Figure 5B). Based on electrochemical reversibility and sensitivity studies, it was found that electrodes containing 20% (w/w) CNT represent the optimal composition. Figure 5 shows SEM micrographs of MWCNT before (B) and after (C) being included in the epoxy resin. Good dispersion of CNT in the polymer matrix with a sponge-like topography of the surface can be observed in this figure. The behaviour of CNTEC electrodes has been compared with that of conventional graphite epoxy composite (GEC) electrode. It was found that long-MWCNT (0.5-200 μm) based epoxy composite electrodes show strong electrocatalytic activity towards NADH

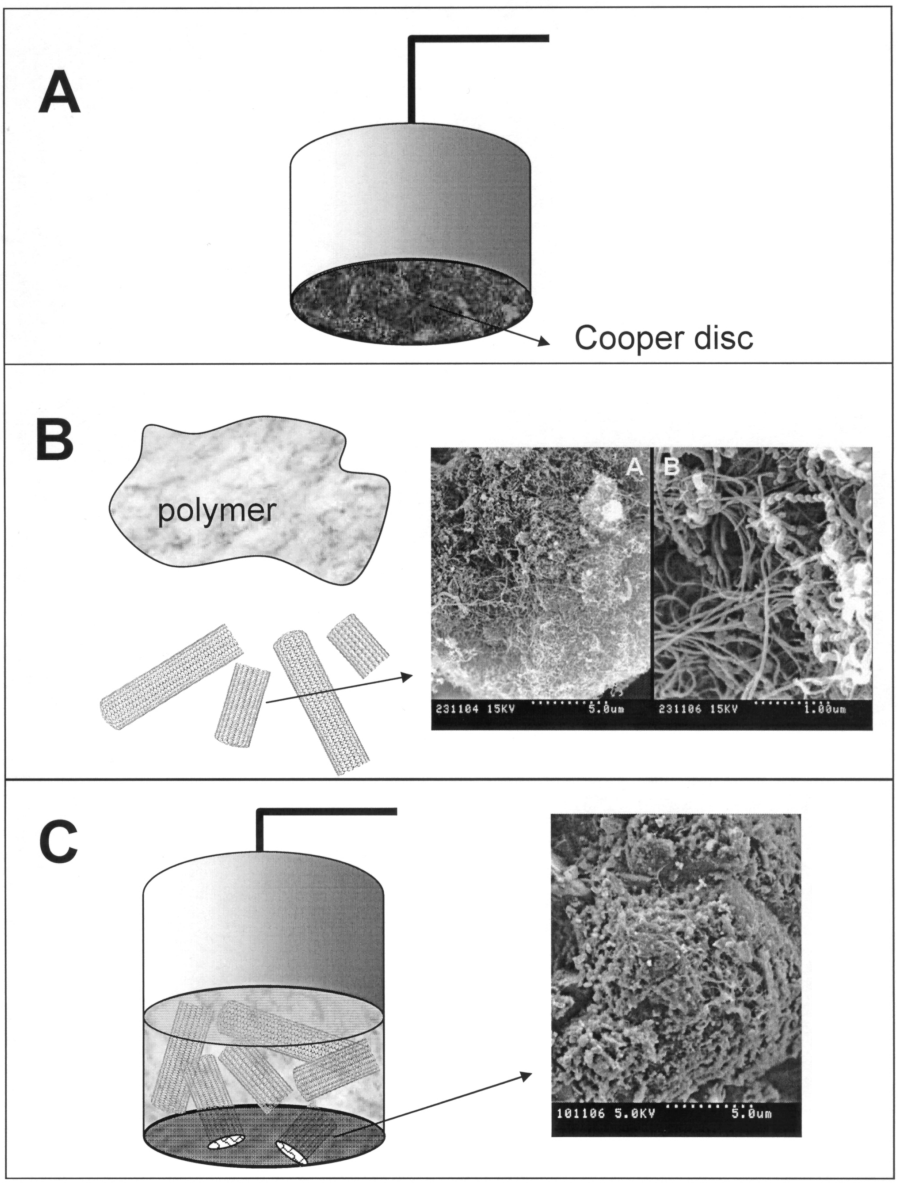

Figure 5. Preparation of CNT composite based sensor. An electrode body (A) with a cooper disc is used as platform for CNT composite application. The CNT composite is prepared by mixing (B, left) CNTs with an epoxy polymer. Also shown is (B, right) the SEM pictures (two resolutions) of the CNT before mixing with the polymer. The prepared paste is then applied onto the electrode (C left). Shown is the SEM image of the electrodes' surface (C right) after curing and polishing the composite. Adapted from reference [60].

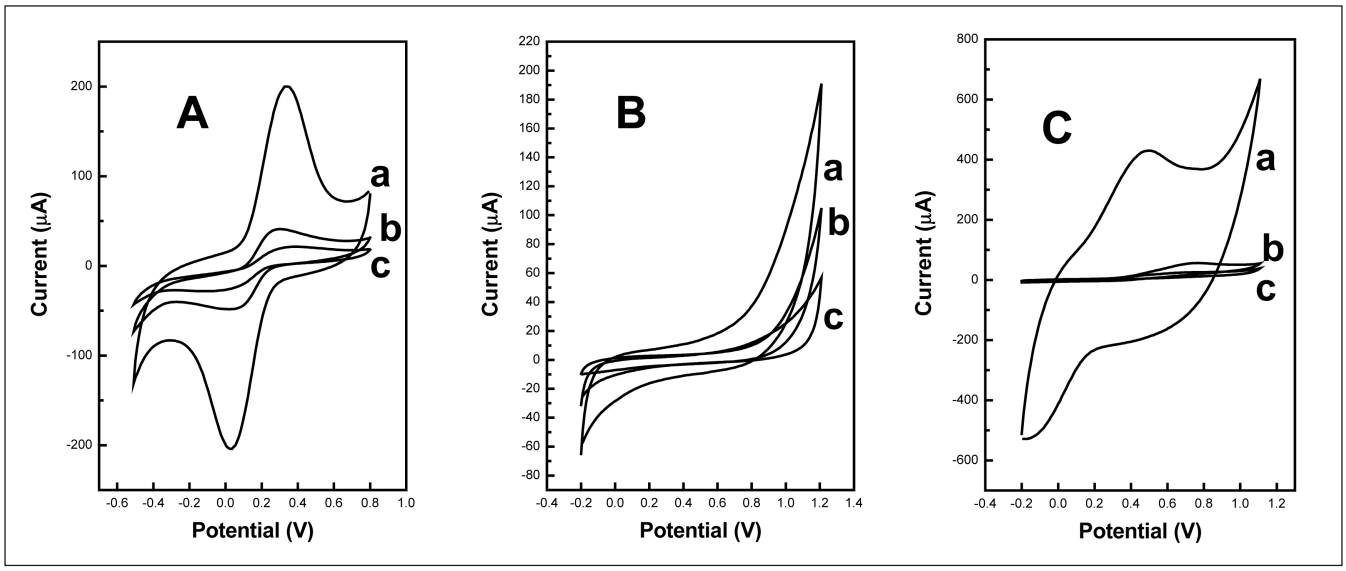

Figure 6. Cyclic voltammograms for (A) 1 mM ferricyanide (B) 1 mM hydrogen peroxide (C) 1 mM NADH. (a) CNT-200-EC, (b) CNT-2-EC and (c) GEC electrodes. 50 mM phosphate buffer, pH 7.4; scan rate 0.1 V s-1; carbon/epoxy ratios, 20:80 (w/w). Adapted from reference [60].

and hydrogen peroxide while short-MWCNT (0.5-2 μm) based epoxy composites show similar oxidation potential as GEC electrode for both NADH and H_2O_2 (see Figure 6). In both cases, CNTEC electrodes provide better reversibility, peak shape, sensitivity and stability when compared to GEC electrode.

The stability of response of the CNTEC electrodes for NADH was evaluated. Figure 7 (A) compares stability of oxidation of NADH at +0.55 V for CNT-200-EC (a), CNT-2-EC (b) and GEC (c) electrodes. The results indicate that both CNT epoxy composite electrodes provide a good stability towards oxidation of NADH. They show 96% (CNT-200-EC) and 93% (CNT-2-EC) of original response even after 45 minutes, while response of GEC electrode decreases by 33% in the same timescale.

The amperometric response (calibration curves) of CNTEC and GEC electrodes towards NADH was assessed at +0.55 V (see Figure 7B). Nanotube-based electrodes display a well-defined concentration dependence over concentration range 0.0-1.0 mM (0.1 mM increments), with sensitivities 72.1 and 29.7 μA mM^{-1} for CNT-200-EC and CNT-2-EC electrodes, respectively (correlation coefficients 0.995 and 0.994), while sensitivity of GEC electrode was 5.0 μA mM^{-1} (correlation coefficient 0.997). The calibration curves of hydrogen peroxide (Figure7C) were obtained at +0.95 V and these were found to be linear over concentration range 0.0-2.0 mM (0.2 mM increments) with sensitivities 18.0, 12.9 and 1.9 μA mM^{-1} for CNT-200-EC, CNT-2-EC and GEC electrodes, respectively (correlation coefficients 0.995, 0.994 and 0.997). The hydrogen peroxide response was not affected by regenerating (polishing) the surface. Series of eight successive measurements, each recorded on a newly polished surface, yielded RSD=5%. Reproducibility of different electrodes prepared from the same CNTEC paste showed similar reproducibility (RSD=6%, n=3).

The CNTEC material is more robust in regard to mechanical strength when compared to the carbon nanotube paste or the

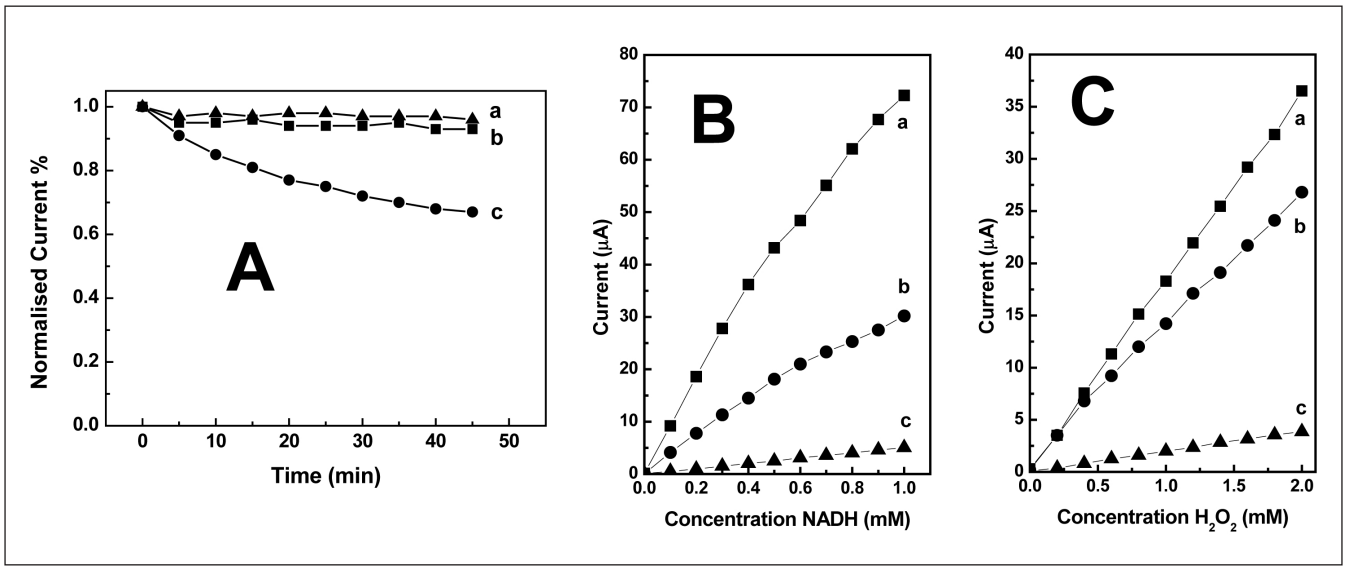

Figure 7. Stability of response (A) and calibration curves for NADH (B) and H2O2 (C). CNT-200-EC, (b) CNT-2-EC and (c) GEC electrodes. Operation potential, +0.55 V (A,B) and +0.95 (C). Solution stirring ca. 400 rpm. 50 mM phosphate buffer, pH 7.4.; carbon:epoxy ratio 20:80 (w/w). Adapted from reference [60].

Teflon composite reported previously. The new carbon nanotube composite indicates that it may become a new class of smart material with unique properties and applications. The resulting CNTEC electrode may offer great promise for biosensing by incorporating biomolecules, such as enzymes, antibodies, or DNA in the CNT/epoxy composite. Research in this direction is in process in our laboratory.

5. Conclusions and future prospects

Nanoanalytical chemistry represents an important challenge of nanotechnology. Today, we face a possible new industrial revolution with nanotechnology which opens enormous perspectives for various fields and especially for analytical chemistry, this last-named field having an active role in nanotechnology development. The integration of nanotechnology with biology and electroanalytical chemistry is just one example of nanoanalytical chemistry. This sort of integration is expected to produce major advances in the field of electrochemical sensors.

Recent progress has led to the development of functional nanoparticles that can be easily linked to biological molecules such as peptides, proteins and nucleic acids. QDs can be used as nonisotopic biolabels, by binding proteins, antibodies, etc. to the surface of the QD. Oligonucleotide derivatized QDs have been used as building blocks to form extended networks that leads to novel DNA hybridisation detection.

Nanoparticles show great promise for electroanalytical applications Especially promising is the potential for detecting single molecule interactions by detecting individual gold colloid label, which opens the way towards new detection limits. The electrochemical detection of these labels using stripping methods allows the detailed study of various biomolecule interactions. Although such single molecule studies are essential for understanding the mechanisms of the analytical methods, they prevent the high parallelization needed for pharmaceutical or medical applications. The developed electrochemical coding is expected to open new opportunities for DNA diagnostics and for bioanalysis in general.

On the other hand, the exploitation of CNTs in the design of electrochemical sensors, although it has shown progress and enthusiasm, is still in its beginnings. Future efforts should aim at better understanding the structural-electrochemical reactivity of CNT-modified electrodes and the factors that govern the electron-transfer kinetics of these attractive devices so as to avoid the risk of precipitated conclusions in attributing electrocatalytic properties to nanotubes without conducting the appropriate control experiments.

To date, even though nanomaterials are incorporated into biosensor applications, there are no biosensors produced entirely at the nanoscale. One of the long-term goals of nanoanalytical chemistry should be the creation of devices that can be used inside a living patient to perform diagnostic tasks. An inherent part of that goal is the integration of nanoscale detection reagents with nanoscale signal transduction elements and electronics. The process of integration will be expensive and risky, since neither the function nor the adoption of the technology is guaranteed.

6. Acknowledgements

This work was financially supported by (1) the Ministry of Education and Culture (MEC) of Spain (Projects BIO2004-02776 and MAT2004-05164 (2) the Ramón Areces Foundation (project 'Bionanosensores'); (3) the "Ramón y Cajal" program of the MEC, which supports Dr. A.Merkoçi.

References

[1] A. Merkoçi, M. Aldavert, Gema Tarrasón, Ramon Eritja, S. Alegret, Analytical Chemistry, 77 (2005) 6500-6503.
[2] H. Akhaven-Tafti, K. Sugioka, Z. Arghavani, Z. Clin Chem. 41 (1995) 1368.
[3] M. Escarceller, F. Rodriguez-Frias, R. Jardi, B. San Segundo, R. Eritja, Anal. Biochem. 206 (1992) 36.
[4] C.H. Tung, S. Stein, Bioconjugate Chem. 11 (2000) 605.
[5] R. Eritja, in 'Solid-Phase Synthesis. A practical guide', Eds. S.A. Kates and F. Albericio, Marcel Dekker, Inc., New York. 2000, p. 529.
[6] G. Tong, J.M. Lawlor, G.W. Tregear, J. Haralambidis, J. Am. Chem. Soc. 117 (1995) 12151.
[7] (a) D. Gottschling, H. Seliger, G. Tarrasón, J. Piulats, R. Eritja, Bioconjugate Chem. 9 (1998) 831. (b) M. Frieden, A. Avino, G. Tarrason, M. Escorihuela, J. Piulats, R. Eritja, Chemistry & Biodiversity. 1 (2004) 930.
[8] J. Wang, G. Liu, A. Merkoçi, Journal of American Chemical Society. 125 (2003) 3214.
[9] W.J. Parak, D. Gerion, T. Pellegrino, D. Zanchet, C. Micheel, S.S. Williams, R. Boudreau, M.A. Le Gros, C.A. Larabell, A.P. Alivisatos, Nanotechnology. 14 (2003) R15
[10] G. Bauer, J. Hassmann, H. Walter, J. Haglmüller, C. Mayer, T. Schalkhammer, Nanotechnology. 14 (2003) 1289.
[11] L.A, Gearheart, H.J. Ploehn, C.J. Murphy, J. Phys. Chem. B. 105 (2001) 12609.
[12] W.L. Shaiu, D.D. Larson, J. Vesenka, E. Henderson, Nucl. Acids Res. 21 (1993) 99.
[13] J. Wang, D. Xu, A.N. Kawde, R. Polsky, Anal. Chem. 73 (2001) 5576.
[14] J. Wang, R. Polsky, D. Xu, Langmuir, 17 (2001) 5739
[15] S.J. Park, T.A.Taton, Mirkin, C.A., Science. 295 (2002) 1503.
[16] J.Wang, G.Liu, A.Merkoçi, Analytica Chimica Acta. 482 (2003) 149.
[17] (a) M.B. Gonzales-Gracia, A. Costa Garcia, Bioelectr. Bioenerg. 38 (1995) 389; (b) Hernández-Santos, M. B. González-García, A. Costa-García, Electrochim. Acta. 46 (2000) 607.
[18] M. Pumera, M. Aldavert, C. Mills, A. Merkoçi, S. Alegret, Electrochimica Acta, 50 (2005) 3702-3707.
[19] C.J. Murphy, Analytical Chemistry. 1 (2002) 520A.
[20] T.G. Drummond, M.G. Hill, J.K. Barton, Nature biotechnology. 21 (2003) 1192.
[21] T. Trindade, P. O'Brien, N.L. Pickett, Chem. Mater. 13 (2001) 3843.
[22] Tonucci R.J, et alters; Science. 258 (1992) 783.

[23] Haverkorn et alt.; Philips Technol. Rev. 40 (1982) 287.
[24] E. Caponetti, L.Pedone, D. Chillura Martino, V. Pantò, V. Turco Liveri, Materials Science and Engineering. 23 (2003) 531.
[25] Dolan G.J.; Appl. Phys. Lett. 31 (1997) 337.
[26] C.B. Murray, D.J. Norris, M.G. Bawendi, J. Am. Chem. Soc.115 (1993) 8706.
[27] Reginald M Penner, Anal. Chem. Res. 33 (2000) 78.
[28] M.P. Pileni, C. Motte, C. Petit, Chem. Mater. 4 (1992) 338.
[29] S. Chen, L.A. Truax, J.M. Sommers, Chem. Mater. 12 (2000) 3864.
[30] J. Wang, G. Liu, R. Polsky, A. Merkoçi, Electrochemistry Communications. 4 (2002) 722.
[31] I. Willner, F. Patolsky, J. Wasserman, Angew. Chem. Int. Ed. 40 (2001) 1861.
[32] M. Han, X. Gao, J. Su, S. Nie, Nat. Biotechnol. 19 (2001) 631.
[33] T.A. Taton, G. Lu, C.A. Mirkin, J. Am. Chem. Soc. 123 (2001) 5164.
[34] Y.W. Cao, R. Jin, C.A. Mirkin, Science. 297 (2002) 1536.
[35] J. Wang, G. Liu, A. Merkoçi, J. Am. Chem. Soc. 125 (2003) 3214.
[36] S. Iijima, Nature. 354 (1991) 56.
[37] (a) M.S. Dresselhaus, G. Dresselhaus, P.C. Eklund, Science of Fullerenes and Carbon Nanotubes, Academic, New York. (1996). (b) W.Z. Li, S.S. Xie, L.X. Qian, B.H. Chang, B.S. Zou, W.Y. Zhou, R.A. Zhao, and G. Wang, Science. 274 (1996) 1701. (c) Z.F. Ren, Z.P. Huang, J.W. Xu, J.H. Wang, P. Bush, M.P. Siegal, P.N. Provencio, Science. 282 (1998) 1105. (d) H. Murakami, M. Hirakawa, C. Tanaka, H. Yamakawa, Appl. Phys. Lett. 76 (2000) 1776. (e) T.W. Ebbesen, H.J. Lezec, H. Hiura, J.W. Bennet, H.F. Ghaemi, T. Thio, Nature. 382 (1996) 54.
[38] M.M. Treachy, T.W. Ebbesen, J.M. Gibson, Nature. 381 (1996) 678.
[39] (a) W.A. de Heer, A. Chatelaine, D. Ugarte, Science. 270 (1995) 1179. (b) W.B. Choi, D.S. Chung, J.H. Kang, H.Y. Kim, Y.W. Jin, I.T. Han, Y.H. Lee, J.E. Jung, N.S. Lee, G.S. Park, J.M. Kim, Appl. Phys. Lett. 75 (1999) 3129. (c) J.M. Bonard, Solid State Electron. 45 (2001) 893.
[40] (a) S.J. Tans, A.R.M. Verschueren, C. Dekker, Nature. 393 (1998) 49. (b) R. Martel, T. Schmidt, H.R. Shea, T. Hertel, P. Avouris, Appl. Phys. Lett. 73 (1998) 2447.
[41] H. Dai, J.H. Hafner, A.G. Rinzler, D.T. Colbert, R.E. Smalley, Nature. 384 (1996) 147.
[42] M.S. Shaffer, X. Fan, A.-H. Windle, Carbon 36 (1998) 1603.
[43] Wang, J.; Liu, G.; Jan, M.R. J. Am. Chem. Soc. 126 (2004) 3010.
[44] Patolsky, F; Weizmann, Y; Willner, I. Angew. Chem. Int. Ed. 43 (2004) 2113.
[45] Luo, H.; Shi, Z.; Li, N.; Gu, Z.; Zhuang, Q. Anal. Chem. 73, (2001), 915.
[46] Wang, J.; Li, M.; Shi, Z.; Li, N. Electroanalysis 14 (2002) 225.
[47] Wang, Z.H.; Liu, J.; Liang, Q.L.; Wang, T.M. Luo, G. Analyst. 127 (2002) 653.
[48] Rubianes, M.D.; Rivas, G.A. Electrochem. Commun. 5 (2003) 689.
[49] Musameh, M.; Wang, J.; Merkoci, A.; Lin, Y Electrochem. Commun. 4 (2002) 743.
[50] Wang, J.; Musameh, M. Anal. Chem. 75 (2003) 2075.
[51] Valentini, F.; Amine, A.; Orlanducci, S.; Terranova, M.L.; Palleschi, G. Anal. Chem. 75 (2003) 5413.
[52] Moore, R.R.; Banks, C.E.; Compton, R.G. Anal. Chem. 76 (2004) 2677.
[53] Hrapovic, S.; Liu, Y.; Male K.B.; Luong, J.H.T. Anal. Chem. 76 (2004) 1083.
[54] Wang, J.; Li, M.; Shi, Z.; Li, N. Anal. Chem. 2002, 74, 1993.
[55] Zhao, Y; Zheng, W.D.; Chen, H.; Luo, Q.M. Talanta. 58 (2002) 529.
[56] Lawrence, N.; Deo, R.P.; Wang, J. Anal. Chim. Acta. 517 (2004) 131.
[57] Wang, J.X.; Li, M.X.; Shi, Z.J.; Li, N.Q.; Gu, Z.N. Electroanalysis. 16 (2004) 140.
[58] Pedano, M.L.; Rivas G.A. Electrochem. Commun. 6 (2004) 10.
[59] Banks, C.E.; Moore, R.R.; Davies, T.J.; Compton, R.G. Chem. Comm. 2004. 1804.
[60] M. Pumera, A. Merkoçi, S. Alegret, Sensors & Actuators, 2005, Printed in the web; doi:10.1016/j.snb.2005.07.010.

About the authors

Arben Merkoçi was awarded the Ph.D. in chemistry from the University of Tirana, Albania, in 1991 and then did postdoctoral research in Greece, Hungary, Italy, Spain, and the USA. His main interests have been electroanalytical methods in several applications in sensors and biosensors. Currently, he is "Ramon y Cajal" researcher and professor at the Sensor & Biosensor Group, Chemistry Department, Autonomous University of Barcelona. His main research interests involve the design of composites, biocomposites and nanobioconjugate materials for enzyme, immuno and DNA based electrochemical sensors. He is a member of the Catalan Chemical Society of the Institut d'Estudis Catalans*.*

Salvador Alegret was made professor of Analytical Chemistry from the Universitat Autònoma de Barcelona in 1991. He is head of the Sensor & Biosensor Group in the Chemistry Department. Currently, he is working on the development of electrochemical chemo- and biosensors based on amperometric, potentiometric and ISFET transducers in chemical, enzymatic, immunological and DNA recognising systems. The resulting sensor devices are being applied in automated analytical systems based on bio- or biomimetic instrumentation concepts for monitoring and process control in different fields, such as biomedicine, environment studies, and the chemical industry. Currently he serves as vice-president of the Institut d'Estudis Catalans.