Nanoparticles as Electroactive Labels

Subjects: Materials Science, Biomaterials

Contributor: Alfredo De la Escosura-Muñiz

Nanoparticles are emerging materials with outstanding potential for their use as labels in electrochemical immunosensing. Gold, silver and quantum dots are the main components of such particles, thanks to their direct electroactivity (redox properties). Protein biomarkers of a variety of diseases, including tumour cells, are the target analytes on which such electrochemical immunosensors have been mostly applied.

Nanoparticles Labels Electroactivity Electrochemistry Immunosensors Biosensors Nanomaterials

1. Introduction

Some NPs possess redox properties that make them easy to be directly detected with electrochemical techniques, without the need for additional steps/reactions after the immunoreaction in which they act as labels. The most representative examples are detailed below.

2. Gold Nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) stand out from the variety of NPs used as labels in immunosensing due to their simple synthesis, narrow size distribution, optical and electrochemical properties and easy bioconjugation alternatives. The advantageous properties of AuNP-based immuno and DNA electrochemical assays have been extensively exploited in the last few years [[1], [2]]. The first works here were based on the NPs dissolving/destruction in aggressive acidic reagents, followed by the detection of the resulting Au (III) ions by anodic stripping voltammetry (ASV). One of the pioneer works following such strategy was reported by Limoges's group, who also used the AuNPs as tags in an immunoassay for immunoglobulin G (IgG) detection at µg/mL levels [[3]].

After that, Liu and Lin [[4]] introduced the advantage of using magnetic particles as platforms of the immunoreaction for the same analyte determination, lowering the detection limits at ng/mL levels. In addition to the direct determination of IgG proteins, immunoassays for other analytes determination based on the ASV of AuNP tags have also been reported. For example, a disposable microfluidic device for the detection of *Salmonella typhimurium* through a magneto-immunoassay using both magnetic particles and AuNPs linked to specific antibodies (Figure 1A) was reported by de Oliveira et al. [[5]], reaching a limit of detection as low as 7 cells/mL. It also deserves to be highlighted the possibility of tagging AuNPs labels with different metal ions, for the multiplexing of different tumour markers [[6]].

Despite the high sensitivity of the ASV detection of the Au (III) resulting of the NP dissolving/destruction, the need of hazardous reagents in this process has limited its practical application in immunosensors as a reliable alternative to traditional immunoassays based on enzymatic labels. In this sense, Costa-García's group was the pioneer in the development and application of an alternative methodology based on the direct detection of AuNPs without the need for previously dissolving them in highly acidic media [7]. The strategy is based on the electrochemical oxidation of the NPs surface by applying a low oxidative potential in diluted hydrochloric acid, followed by the electrochemical voltammetric reduction back of the Au (III) to Au (0). Later on, Merkoçi's group combined this strategy with the labelling of antibodies with AuNPs and the advantages of using magnetic particle platforms, for the detection of IgG protein at pg/mL levels [8]. In 2011, de la Escosura-Muñiz et al. studied for the first time the effect of the size of AuNPs on the aforementioned direct electrochemical detection when used as electroactive labels in an immunoassay for IgG determination on magnetic particle platforms [9]. Their findings suggest a better performance for small NPs (5 nm AuNPs) instead of the standard Turkevich's ones (20 nm AuNPs) due to their higher surface area, as illustrated in Figure 1B.

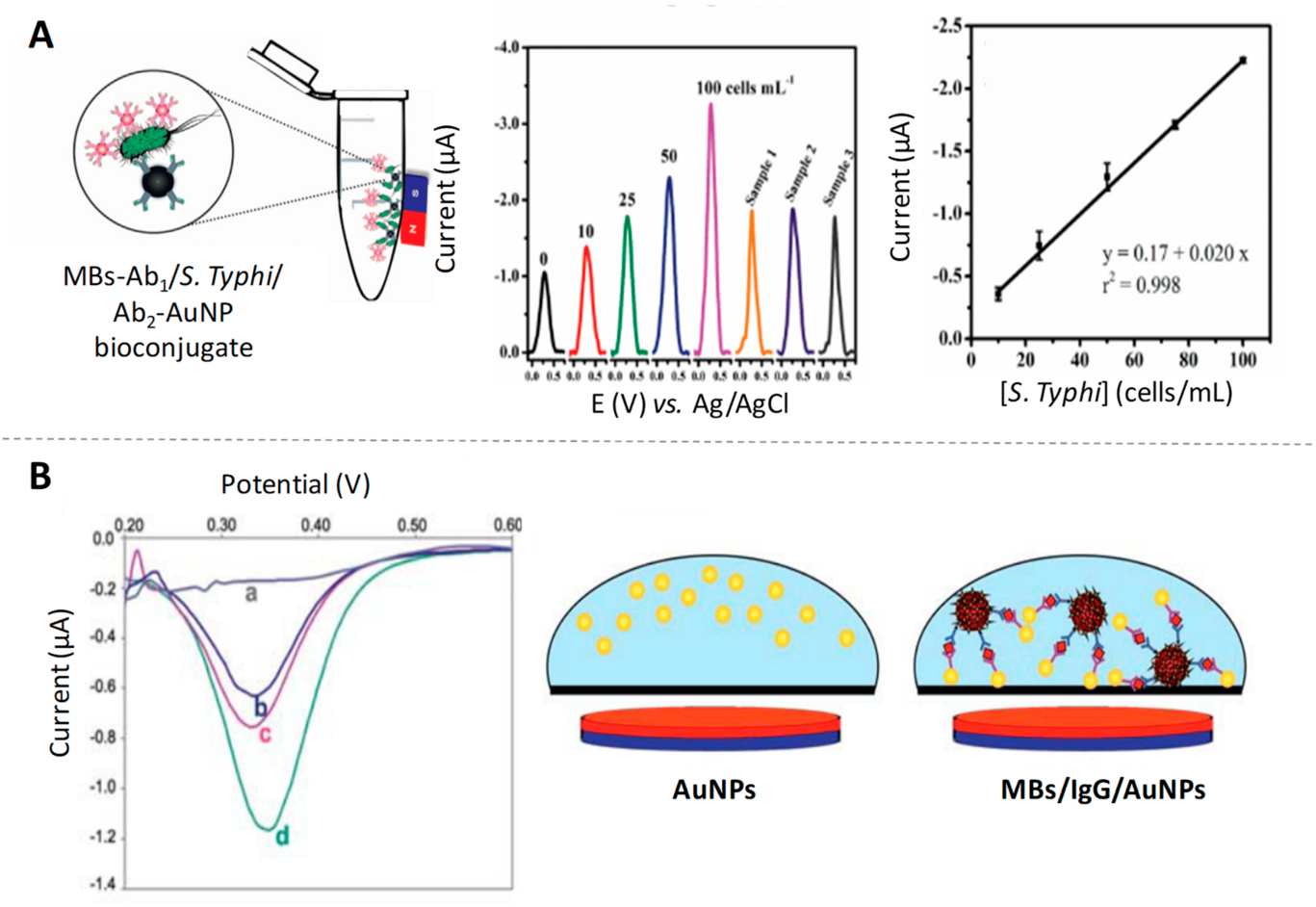

Figure 1. Gold nanoparticles (AuNPs) as electroactive labels. (**A**) Scheme of the magneto-immunoconjugate for the detection of *Salmonella typhimurium* using AuNP tags, together with differential pulse voltammetry (DPV) responses and calibration curve. Adapted from [5] with permission; (**B**) DPV curves obtained for the magnetosandwich immunoassay using AuNPs of different sizes: (a) blank, (b) 80 nm, (c) 20 nm and (d) 5 nm; and scheme of the process occurring on the electrode surface. Adapted from [9] with permission.

3. Silver Nanoparticles (AgNPs)

The excellent electroactivity of silver metal together with the well-defined sharp voltammetric peaks associated to the process of oxidation of Ag (0) to Ag (I) make silver nanoparticles (AgNPs) to be of great potential for analytical applications, as reviewed by Compton's group [10]. Here, the presence of chloride ions in the electrolyte solution is of key relevance for forming the AgCl specie that facilitates the voltammetric oxidation.

Based on that principle, Ting et al. proposed the use of Ag tags in the development of an immunosensor for prostate specific antigen (PSA) detection at fg/mL levels [11]. In the same vein,
an electrochemical biosensor for clenbuterol using melamine functionalized AgNPs was reported by Miao and co-workers [12] (Figure 2A), reaching limits of detection at pg/mL levels. Similarly, antibodies to tick-borne encephalitis virus (TBEV), one endemic flavivirus that can cause serious infections in humans, were detected at 50 IU/mL using this methodology [13].

The high susceptibility of Ag to oxidation makes easy its combination with Au to obtain bimetallic AuAgNPs [14] with such NPs having the benefits of both metals. In this sense, Merkoçi's group reported first the synthesis and electrochemical characterization of AuAgNPs [15] (Figure 2B) and then, applied them for the quantification of *Escherichia coli* and *Salmonella typhimurium* bacteria, taking advantage of the affinity of Ag for cell surface macromolecules [16] (Figure 2C). These findings opened the way for the development of low-cost and quickly electrochemical detection of bacteria as alternatives to traditional culture-based methods.

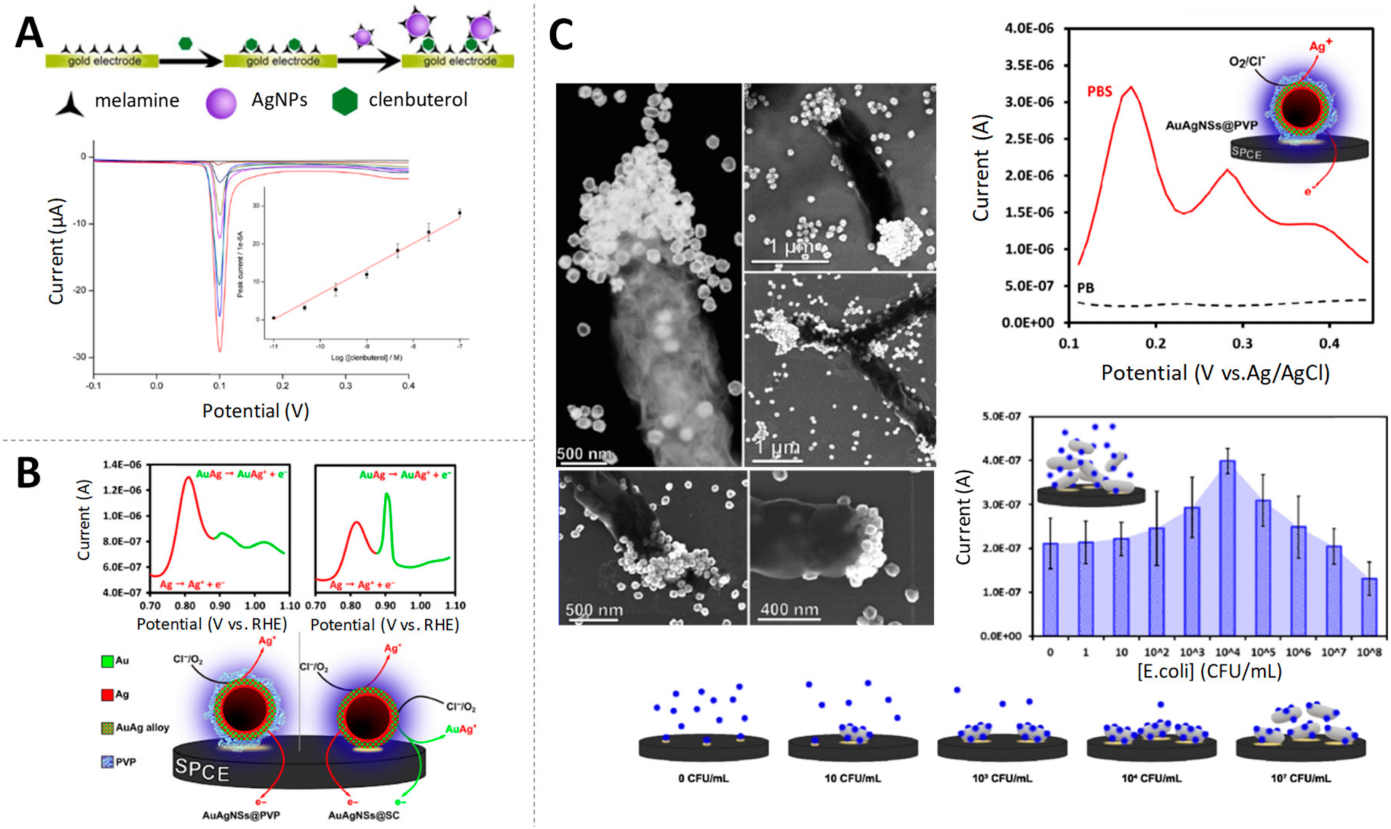

Figure 2. Silver nanoparticles (AgNPs) as electroactive labels (**A**) Schematic representation of the melanine functionalized AgNP-based electrochemical biosensor for the quantification of clenbuterol, linear sweep voltammetry (LSV) responses for increasing concentrations of clenbuterol and, inset, calibration curve of peak current values vs. the logarithm of clenbuterol concentration. Adapted from [12] with permission; (**B**) DPVs of AuAg NPs coated with polyvinyl pyrrolidone (PVP, left) and sodium citrate (SC, right). The analytical peak at +0.8 V corresponds to the stripping oxidation of metallic silver, while the peak at +0.9 V corresponds to the oxidation of the alloyed silver. In the bottom, proposed electrochemical mechanism for the AuAg NPs voltammetric profile. Adapted from [15] with permission; (**C**) Scanning transmission electron microscope (STEM) images of *E. coli* cells with AuAg NPs specifically linked; comparison of DPV curves of AuAgNPs in different buffers and *E. coli* detection through incubation with AuAg NPs and DPV measurements, with bacteria concentration ranging from 10^1 to 10^8 CFU/mL. Adapted from [16] with permission.

4. Quantum Dots (QDs)

Quantum dots (QDs) are semiconductor NPs with spherical shape and a diameter between 1–12 nm. Nowadays, they are one of the most studied nanomaterials, mainly because of their unique optical and semiconductive properties. They were discovered in the early 1980s by Alexey Ekimov during his research on semiconductor nanocrystals [17],[18]. Some of the novel characteristics of QDs are their narrow spectral bands, high photoluminescence emission quantum yields and size-tunable emission profiles. This, altogether, make them as excellent potential labels to be used in bioassays [19]. Apart from their optical properties, valuable information can be provided by their electrochemical behaviour, broadly studied by Bard's group in 2005 [20]. QDs have normally a core@shell structure made of semiconductors. The one in the outer layer is used to protect the core against possible oxidation reactions that could release the inner ions. Different organic capping ligands are also used to control the solubility of QDs and their functionalization, [21] with the aim of using them in bioassays, especially in optical biosensing, because of their size-controlled luminescence [22].

In addition to their well-established optical properties/applications, QDs have also inherent electroactivity coming from their metallic components that make them easy to detect with electrochemical techniques. The typical strategy consists in the QDs dissolution in highly acidic/oxidative media followed by the ASV detection of the metal ions released. Moreover, the use of QDs made of different metals allows to do the simultaneous detection of different targets through the specific potential of re-oxidation of each metal. In this sense, Wang and co-workers were pioneers in the use of QDs of different metals (Pb, Zn, Cd) as labels for the simultaneous detection of different analytes through ASV analysis [23].

ASV detection after QDs labels acidic dissolving has also extensively used in immunosensing in the last decade. As example, PSA, a biomarker for prostate cancer, has been detected using CdS QDs as labels in a sandwich-type immunoassay at clinically relevant levels of pg/mL [24].

Other QDs, CdSe QDs, have been combined with zirconia NPs (ZrO_2) by Lu et al. [25] in the development of a highly selective electrochemical immunosensor for the detection of organophosphorylated butyrylcholinesterase

(OP-BChE), a biomarker of the exposure to toxic organophosphorus agents, at environmental relevant levels of ng/mL. Core@shell CdSe@ZnS QDs, with ASV detection of released Cd, have also been highly used in electrochemical immunosensing. For example, Martín-Yerga et al. proposed the ASV detection of CdSe@ZnS QDs tags in electrode arrays [26], later applied for the detection of celiac disease biomarkers at clinical relevant levels (around 2 U/mL) [27],[28] (Figure 3A). In the same vein, Pinwattana et al. used CdSe@ZnS QDs for the quantification of phosphorylated bovine serum albumin (BSA-OP) at the ng/mL scale [29].

As in the case of the AuNPs, the QDs detection by stripping after dissolving is practically limited by the need of hazardous reagents for the NP dissolution and the metal ions release. In this context, Merkoçi's group was the first in proposing the direct detection of CdS QDs, based on the reduction/re-oxidation of Cd (II) in the surface of the NP, without the need of decompose the QDs [30]. This methodology was later applied in an immunosensor using CdSe@ZnS QDs tags for the detection of apolipoprotein E (ApoE), an Alzheimer's disease biomarker, at clinical relevant levels of ng/mL [31] (Figure 3B). The same authors proposed later a signal amplification strategy based on the use of bismuth-modified electrodes for improving the Cd detection (Figure 3C), which was applied for the determination of human IgG (HIgG) at ng/mL levels in a model immunoassay [32].

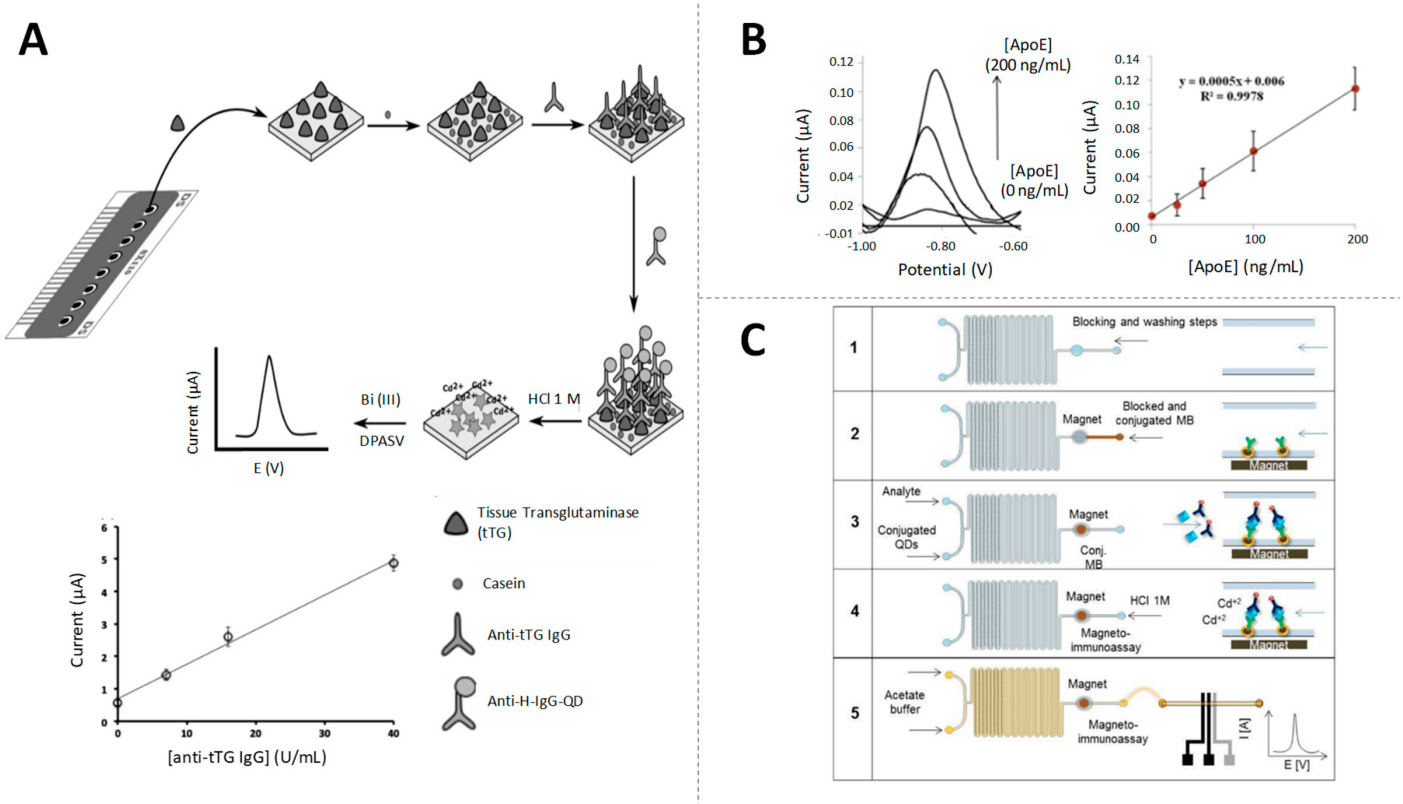

Figure 3. Quantum dots (QDs) as electroactive labels. (**A**) Schematic diagram of the electrochemical biosensor array for the quantification of anti-tissue transglutaminase (anti-tTG immunoglobulin G (IgG)) antibodies, based on the detection of QDs and linear response of the sensor for different concentrations of anti-tTG IgG antibody. Adapted from [27] with permission; (**B**) Performance of an ApoE-magnetoimmunoassay using QDs and calibration

curve of ApoE between 0 and 200 ng/mL. Adapted from [31] with permission; (**C**) Scheme of an in-chip magnetoimmunoassay for human IgG (HIgG) detection using QDs tags. Adapted from [32] with permission.

5. Other Nanoparticles (NPs)

Some other nanoparticles with outstanding electrochemical properties, but not yet been used as labels in biosensors, deserve to be briefly included in this review due to their great potential for such application. That is the case of cerium oxide nanoparticles (CeO_2 NPs) and mercury selenide nanoparticles (HgSe NPs). Copper-based metal nanoparticles (CuNPs), that have been proposed only for DNA hybridization biosensing, are also listed in this section.

5.1. Cerium Oxide Nanoparticles (CeO_2 NPs)

Among all the metal oxide-based nanoparticles, cerium oxide nanoparticles or nanoceria have attracted significant attention owing to their singular properties, especially as catalysts. Their crystal structure have lots of oxygen vacancy defects, and because of that, they exhibit a very important oxygen storage capacity [33]. For this reason, the oxidation state of cerium at the NP surface can vary easily between +3 and +4, so they can both act as oxidizing and reducing agents [34]. This allows them, apart from acting as catalysts, to mimic the activity of enzymes in biosensors [35]. CeO_2 NPs have been widely used in biosensing based on such mimetic properties. Ispas and colleagues have investigated the electrochemical behaviour of nanoceria towards the oxidation and reduction of hydrogen peroxide, resulting in a highly sensitive H_2O_2 detection technique with very low response times [36] opening the pathway to CeO_2 NPs for their use in biosensing applications. On this basis, Chaturvedi et al. have developed a CeO_2 NPs-Pt-graphene nanocomposite for the detection of glucose and xanthine, adding peroxide-producing oxidase or superoxide-producing oxidase, respectively [37].

With the aim of avoiding the use of enzymes, this peroxidase mimetic activity of CeO_2 NPs has been combined with the catalytic properties of AuNPs to oxidize glucose into gluconate and hydrogen peroxide [38] which was applied for developing a non-enzymatic glucose biosensor with good analytical characteristics [39].

Recently, it has also been developed a novel method for the quantification of CeO_2 NPs based on their oxidative effect towards ferrocyanide redox system, with great potential for its further application in biosensors [40].

5.2. Mercury Selenide Nanoparticles (HgSe NPs)

Mercury selenide (HgSe) is a very interesting material characterized by its high electron mobility and large electron concentration, extensively investigated in the area of optoelectronics [41]. The electrochemical behaviour of HgSe has been studied in mercury electrodes since the early 1960s, concluding that selenious acid is irreversibly reduced to HgSe in an acidic media. The cathodic stripping of HgSe film has been later used in the quantification of selenium [42].

After that, different studies on the optimization of HgSe thin films by electrochemical atomic layer epitaxy have

been reported, with the different films being grown layer by layer making use of surface limited reactions such as under potential deposition (UPD). UPD is a highly interesting process consisting on the deposition of atomic layers on another element at a different potential of the one needed for the deposition on the element on itself [43].

More recently, it has been demonstrated that selenium and mercury have a toxicological antagonism in animals, occurring normally bioaccumulation of both elements [44].
Such co-accumulation may be due to the existence of HgSe NPs in the liver of cetaceans, assuming that they are the final metabolic product of the lifesaving mechanism in different biological systems [45]. In this context, engineered water-stabilized HgSe NPs have been synthesized and characterized by Bouzas-Ramos and co-workers [46]. Using these NPs and exploiting the ability that electrochemical techniques have to pre-concentrate different metals on the surface of the electrode, a rapid, simple and sensitive quantification of HgSe NPs has been carried out for the first time by Iglesias-Mayor et al. [47] In this work, HgSe NPs were quantified within two orders of magnitude, obtaining good reproducibility, repeatability and limit of detection, showing great potential for further application as tags in electrochemical immunosensors.

5.3. Copper-Based Nanoparticles (CuNPs)

Copper-based metal nanoparticles are attracting attention in bioanalysis due to their biocompatibility, low toxicity and outstanding optical properties. Some recent approaches have taken advantage of the in situ generation of CuNPs after DNA amplification, followed by NP dissolving and Cu ions detection by stripping voltammetry. This strategy has been combined with the use of aptamers for the detection of PSA biomarker at fg/mL levels [48]. The strong interaction between glutathione (GSH) and copper ions [49] has also been approached for the detection of GSH after formation of DNA-templated CuNPs and later voltammetric detection, as described above [50]. This analytical signal readout has also been used in the development of a method for quantifying endonuclease activity [51].

On the other hand, DNA-templated copper NPs are also considered as functional probes in bioanalysis [52]. They are synthesized due to the clustering of Cu onto DNA scaffolds [53] in a fast and efficient way [54]. Aptasensors for the detection of microRNA have also been reported using CuNPs, for example, Wang et al. built a biosensor for microRNA 21 based on the combination of the electroactivity of CuNPs and different amplification strategies [55], with an ultra-low limit of detection at levels of ag/mL, and having reliable results in the analysis of real blood samples. A simpler biosensor for microRNA which only uses exonuclease and copper nanoparticles has been reported by Miao and co-workers [56], with high potential for clinical diagnosis.

References

1. Ambrosi, A.; Castañeda, M.T.; de la Escosura-Muñiz, A.; Mekoçi, A. Gold nanoparticles a powerful label for affinity electrochemical biosensors. Biosens. Using Nanomater. 2009, 6, 177–197.

2. Govindhan Maduraiveeran; Manickam Sasidharan; Vellaichamy Ganesan; Electrochemical sensor and biosensor platforms based on advanced nanomaterials for biological and biomedical applications.. *Biosensors and Bioelectronics* **2017**, *103*, 113-129, 10.1016/j.bios.2017.12.031.
3. Murielle Dequaire; Chantal DeGrand; Benoît Limoges; An electrochemical metalloimmunoassay based on a colloidal gold label.. *Analytical Chemistry* **2000**, *72*, 5521-5528, 10.1021/ac000781m.
4. Guodong Liu; Yuehe Lin; A renewable electrochemical magnetic immunosensor based on gold nanoparticle labels.. *Journal of Nanoscience and Nanotechnology* **2005**, *5*, 1060-1065, 10.1166/jnn.2005.178.
5. Tássia Regina De Oliveira; Diego Henrique Martucci; Ronaldo Censi Faria; Simple disposable microfluidic device for Salmonella typhimurium detection by magneto-immunoassay. *Sensors and Actuators B: Chemical* **2018**, *255*, 684-691, 10.1016/j.snb.2017.08.075.
6. Teng Xu; Xinle Jia; Xia Chen; Zhanfang Ma; Simultaneous electrochemical detection of multiple tumor markers using metal ions tagged immunocolloidal gold. *Biosensors and Bioelectronics* **2014**, *56*, 174-179, 10.1016/j.bios.2014.01.006.
7. M.B. González García; A. Costa García; Adsorptive stripping voltammetric behaviour of colloidal gold and immunogold on carbon paste electrode. *Bioelectrochemistry and Bioenergetics* **1995**, *38*, 398-395, 10.1016/0302-4598(95)01813-t.
8. Adriano Ambrosi; Maria Teresa Castañeda; Anthony J. Killard; Malcolm R. Smyth; Salvador Alegret; Arben Merkoçi; Double-Codified Gold Nanolabels for Enhanced Immunoanalysis. *Analytical Chemistry* **2007**, *79*, 5232-5240, 10.1021/ac070357m.
9. Alfredo De La Escosura-Muñiz; Claudio Parolo; Flavio Maran; Arben Mekoçi; Size-dependent direct electrochemical detection of gold nanoparticles: application in magnetoimmunoassays. *Nanoscale* **2011**, *3*, 3350-3356, 10.1039/c1nr10377f.
10. Kristina Tschulik; Christopher Batchelor-McAuley; Her-Shuang Toh; Emma J. E. Stuart; Richard G. Compton; Electrochemical studies of silver nanoparticles: a guide for experimentalists and a perspective. *Physical Chemistry Chemical Physics* **2014**, *16*, 616-623, 10.1039/c3cp54221a.
11. Boon Ping Ting; Jie Zhang; Majad Khan; Yi Yan Yang; Jackie Y. Ying; The solid-state Ag/AgCl process as a highly sensitive detection mechanism for an electrochemical immunosensor. *Chemical Communications* **2009**, *no*, 6231, 10.1039/b910730d.
12. Peng Miao; Kun Han; Haixuan Sun; Jian Yin; Jing Zhao; Bidou Wang; Yuguo Tang; Melamine Functionalized Silver Nanoparticles as the Probe for Electrochemical Sensing of Clenbuterol. *ACS Applied Materials & Interfaces* **2014**, *6*, 8667-8672, 10.1021/am501473m.
13. Yekaterina Khristunova; Elena Korotkova; Bohumil Kratochvil; Jiri Barek; Elena Dorozhko; Vlastimil Vyskocil; Evgenii Plotnikov; Olesya Voronova; Vladimir Sidelnikov; Preparation and

Investigation of Silver Nanoparticle–Antibody Bioconjugates for Electrochemical Immunoassay of Tick-Borne Encephalitis. *Sensors* **2019**, *19*, 2103-2113, 10.3390/s19092103.

14. Yiqun Zheng; Jie Zeng; Aleksey Ruditskiy; Maochang Liu; Younan Xia; Oxidative Etching and Its Role in Manipulating the Nucleation and Growth of Noble-Metal Nanocrystals. *Chemistry of Materials* **2013**, *26*, 22-33, 10.1021/cm402023g.
15. Lorenzo Russo; Victor Puntes; Arben Merkoçi; Tunable electrochemistry of gold-silver alloy nanoshells. *Nano Research* **2018**, *11*, 6336-6345, 10.1007/s12274-018-2157-y.
16. Lorenzo Russo; Juan Leva Bueno; Jose Francisco Bergua; Monica Costantini; Marco Giannetto; Victor Puntes; Alfredo De La Escosura-Muñiz; Arben Merkoçi; Low-Cost Strategy for the Development of a Rapid Electrochemical Assay for Bacteria Detection Based on AuAg Nanoshells. *ACS Omega* **2018**, *3*, 18849-18856, 10.1021/acsomega.8b02458.
17. Ekimov, A.I.; Onushchenko, A.A.; Quantum size effect in 3D microscopic semiconductor crystals. *JETP Lett* **1982**, *34*, 345–348, http://www.jetpletters.ac.ru/ps/1517/article_23187.shtml.
18. no; Nanocrystals in their prime. *Nature Nanotechnology* **2014**, *9*, 325-325, 10.1038/nnano.2014.101.
19. A. P. Alivisatos; Semiconductor Clusters, Nanocrystals, and Quantum Dots. *Science* **1996**, *271*, 933-937, 10.1126/science.271.5251.933.
20. Allen J. Bard; Zhifeng Ding; Noseung Myung; Electrochemistry and Electrogenerated Chemiluminescence of Semiconductor Nanocrystals in Solutions and in Films. *Semiconductor Nanocrystals and Silicate Nanoparticles* **2005**, *118*, 1-57, 10.1007/b137239.
21. Markus Grabolle; Jan Ziegler; Alexei Merkulov; Thomas Nann; Ute Resch-Genger; Stability and Fluorescence Quantum Yield of CdSe-ZnS Quantum Dots-Influence of the Thickness of the ZnS Shell. *Annals of the New York Academy of Sciences* **2008**, *1130*, 235-241, 10.1196/annals.1430.021.
22. Antonio R. Montoro Bustos; Laura Trapiella-Alfonso; Jorge Ruiz Encinar; Jose Manuel Costa-Fernandez; Rosario Pereiro; Alfredo Sanz-Medel; Elemental and molecular detection for Quantum Dots-based immunoassays: A critical appraisal. *Biosensors and Bioelectronics* **2012**, *33*, 165-171, 10.1016/j.bios.2011.12.046.
23. Joseph Wang; Guodong Liu; Ronen Polsky; Arben Merkoçi; Electrochemical stripping detection of DNA hybridization based on cadmium sulfide nanoparticle tags. *Electrochemistry Communications* **2002**, *4*, 722-726, 10.1016/s1388-2481(02)00434-4.
24. Minghui Yang; Alireza Javadi; Shaoqin Gong; Sensitive electrochemical immunosensor for the detection of cancer biomarker using quantum dot functionalized graphene sheets as labels. *Sensors and Actuators B: Chemical* **2011**, *155*, 357-360, 10.1016/j.snb.2010.11.055.

25. Nglai Lu; Jun Wang; Limin Wang; Dan Du; Charles Timchalk; Richard Barry; Yuehe Lin; A Novel Nanoparticle-Based Disposable Electrochemical Immunosensor for Diagnosis of Exposure to Toxic Organophosphorus Agents. *Advanced Functional Materials* **2011**, *21*, 4371-4378, 10.1002/adfm.201100616.
26. Daniel Martín-Yerga; Maria Begoña González-Garcia; Agustín Costa-García; Biosensor array based on the in situ detection of quantum dots as electrochemical label. *Sensors and Actuators B: Chemical* **2013**, *182*, 184-189, 10.1016/j.snb.2013.03.004.
27. Daniel Martín-Yerga; Maria Begoña González-Garcia; Agustín Costa-García; Electrochemical immunosensor for anti-tissue transglutaminase antibodies based on the in situ detection of quantum dots. *Talanta* **2014**, *130*, 598-602, 10.1016/j.talanta.2014.07.010.
28. Daniel Martín-Yerga; Agustín Costa-García; Towards a blocking-free electrochemical immunosensing strategy for anti-transglutaminase antibodies using screen-printed electrodes. *Bioelectrochemistry* **2015**, *105*, 88-94, 10.1016/j.bioelechem.2015.05.014.
29. Kulwadee Pinwattana; Jun Wang; Chiann-Tso Lin; Hong Wu; Dan Du; Yuehe Lin; Orawon Chailapakul; CdSe/ZnS quantum dots based electrochemical immunoassay for the detection of phosphorylated bovine serum albumin. *Biosensors and Bioelectronics* **2010**, *26*, 1109-1113, 10.1016/j.bios.2010.08.021.
30. Arben Merkoçi; Luiz Humberto Marcolino-Junior; Sergio Marín; Orlando Fatibello-Filho; Salvador Alegret; Detection of cadmium sulphide nanoparticles by using screen-printed electrodes and a handheld device. *Nanotechnology* **2007**, *18*, 035502, 10.1088/0957-4484/18/3/035502.
31. Mariana Medina-Sánchez; Sandrine Miserere; Eden Morales-Narváez; Arben Merkoçi; On-chip magneto-immunoassay for Alzheimer's biomarker electrochemical detection by using quantum dots as labels. *Biosensors and Bioelectronics* **2014**, *54*, 279-284, 10.1016/j.bios.2013.10.069.
32. Mariana Medina-Sánchez; Sandrine Miserere; Miquell Cadevall; Arben Merkoçi; Enhanced detection of quantum dots labelled protein by simultaneous bismuth electrodeposition into microfluidic channel. *ELECTROPHORESIS* **2015**, *37*, 432-437, 10.1002/elps.201500288.
33. Charles T. Campbell; Charles H F Peden; Chemistry. Oxygen vacancies and catalysis on ceria surfaces.. *Science* **2005**, *309*, 713-714, 10.1126/science.1113955.
34. Daniel Andreescu; Gonca Bulbul; Rifat Emrah Özel; Akhtar Hayat; Naimish Sardesai; Silvana Andreescu; Applications and implications of nanoceria reactivity: measurement tools and environmental impact. *Environmental Science: Nano* **2014**, *1*, 445-458, 10.1039/c4en00075g.
35. Ivana Celardo; Jens Z. Pedersen; Enrico Traversa; Lina Ghibelli; Pharmacological potential of cerium oxide nanoparticles. *Nanoscale* **2011**, *3*, 1411-1420, 10.1039/c0nr00875c.
36. Cristina Ispas; John Njagi; Meghan Cates; Silvana Andreescu; Electrochemical Studies of Ceria as Electrode Material for Sensing and Biosensing Applications. *Journal of The Electrochemical*

Society **2008**, *155*, F169, 10.1149/1.2936178.

37. P. Chaturvedi; D.C. Vanegas; M. Taguchi; S.L. Burrs; P. Sharma; E.S. McLamore; A nanoceria–platinum–graphene nanocomposite for electrochemical biosensing. *Biosensors and Bioelectronics* **2014**, *58*, 179-185, 10.1016/j.bios.2014.02.021.
38. Weijie Luo; Changfeng Zhu; Shao Su; Di Li; Yao He; Qing Huang; Chunhai Fan; Self-Catalyzed, Self-Limiting Growth of Glucose Oxidase-Mimicking Gold Nanoparticles. *ACS Nano* **2010**, *4*, 7451-7458, 10.1021/nn102592h.
39. Lei Jiang; Qi Xue; Chuanjie Jiao; Hanyun Liu; Yan Zhou; Hongchao Ma; Qingli Yang; A non-enzymatic nanoceria electrode for non-invasive glucose monitoring. *Analytical Methods* **2018**, *10*, 2151-2159, 10.1039/c8ay00433a.
40. Alba Iglesias Mayor; Lucía Fernández-Murillo; Francisco Javier García-Alonso; Alfredo De La Escosura-Muñiz; Agustín Costa-García; Nanoceria quantification based on its oxidative effect towards the ferrocyanide/ferricyanide system. *Journal of Electroanalytical Chemistry* **2019**, *840*, 338-342, 10.1016/j.jelechem.2019.04.013.
41. Mkhulu K. Mathe; Steve M. Cox; Venkatram Venkatasamy; Uwe Happek; John L. Stickney; Formation of HgSe Thin Films Using Electrochemical Atomic Layer Epitaxy. *Journal of The Electrochemical Society* **2005**, *152*, C751, 10.1149/1.2047547.
42. Gunnar Mattsson; Leif Nyholm; Åke Olin; Cathodic stripping voltammetry of HgSe. *Journal of Electroanalytical Chemistry* **1994**, *377*, 149-162, 10.1016/0022-0728(94)03456-7.
43. Venkatram Venkatasamy; Mkhulu K. Mathe; Stephen M. Cox; Uwe Happek; John L. Stickney; Optimization studies of HgSe thin film deposition by electrochemical atomic layer epitaxy (EC-ALE). *Electrochimica Acta* **2006**, *51*, 4347-4351, 10.1016/j.electacta.2005.12.012.
44. Mohammad A.K. Khan; Feiyue Wang; MERCURY–SELENIUM COMPOUNDS AND THEIR TOXICOLOGICAL SIGNIFICANCE: TOWARD A MOLECULAR UNDERSTANDING OF THE MERCURY–SELENIUM ANTAGONISM. *Environmental Toxicology and Chemistry* **2009**, *28*, 1567, 10.1897/08-375.1.
45. Mohammad A. K. Khan; Feiyue Wang; Reversible Dissolution of Glutathione-Mediated HgSexS1−xNanoparticles and Possible Significance in Hg−Se Antagonism. *Chemical Research in Toxicology* **2009**, *22*, 1827-1832, 10.1021/tx900234a.
46. Diego Bouzas-Ramos; Alfredo Sanz-Medel; Mario Menéndez-Miranda; Jose Manuel Costa-Fernandez; Jorge Ruiz Encinar; Precise determination of the nanoparticle concentration and ligand density of engineered water-soluble HgSe fluorescent nanoparticles. *RSC Advances* **2016**, *6*, 19964-19972, 10.1039/C5RA26268B.
47. Alba Iglesias Mayor; Olaya Amor-Gutiérrez; Diego Bouzas-Ramos; Jorge Ruiz Encinar; José M. Costa-Fernández; Alfredo De La Escosura-Muñiz; Agustín Costa-García; Simple and rapid

electrochemical quantification of water-stabilized HgSe nanoparticles of great concern in environmental studies.. *Talanta* **2019**, *200*, 72-77, 10.1016/j.talanta.2019.03.038.

48. Ye Zhu; Huijuan Wang; Lin Wang; Jing Zhu; Wei Jiang; Cascade Signal Amplification Based on Copper Nanoparticle-Reported Rolling Circle Amplification for Ultrasensitive Electrochemical Detection of the Prostate Cancer Biomarker. *ACS Applied Materials & Interfaces* **2016**, *8*, 2573-2581, 10.1021/acsami.5b10285.
49. Zhanguang Chen; Zhen Wang; Junhui Chen; Xi Chen; Alizarin red S/copper ion-based ensemble for fluorescence turn on detection of glutathione with tunable dynamic range. *Biosensors and Bioelectronics* **2012**, *38*, 202-208, 10.1016/j.bios.2012.05.030.
50. Zhuxin Wang; Peng Han; Xiaoxia Mao; YongMei Yin; Ya Cao; Sensitive detection of glutathione by using DNA-templated copper nanoparticles as electrochemical reporters. *Sensors and Actuators B: Chemical* **2017**, *238*, 325-330, 10.1016/j.snb.2016.07.078.
51. Xifeng Chen; Dawei Yang; Yuguo Tang; Peng Miao; DNA-templated copper nanoparticles for voltammetric analysis of endonuclease activity. *The Analyst* **2018**, *143*, 1685-1690, 10.1039/c8an00005k.
52. Zhihe Qing; Ailing Bai; Shuohui Xing; Zhen Zou; Xiaoxiao He; Kemin Wang; Ronghua Yang; Progress in biosensor based on DNA-templated copper nanoparticles.. *Biosensors and Bioelectronics* **2019**, *137*, 96-109, 10.1016/j.bios.2019.05.014.
53. Alexandru Rotaru; Subrata Dutta; Elmar Jentzsch; Kurt Gothelf; Andriy Mokhir; Selective dsDNA-Templated Formation of Copper Nanoparticles in Solution. *Angewandte Chemie International Edition* **2010**, *49*, 5665-5667, 10.1002/anie.200907256.
54. Fengzhou Xu; Hui Shi; Xiaoxiao He; Kemin Wang; Dinggeng He; Qiuping Guo; Zhihe Qing; Lv'An Yan; Xiaosheng Ye; Duo Li; et al.Jinlu Tang Concatemeric dsDNA-Templated Copper Nanoparticles Strategy with Improved Sensitivity and Stability Based on Rolling Circle Replication and Its Application in MicroRNA Detection. *Analytical Chemistry* **2014**, *86*, 6976-6982, 10.1021/ac500955r.
55. Yijia Wang; Xinying Zhang; Lang Zhao; Ting Bao; Wei Wen; Xiuhua Zhang; Shengfu Wang; Integrated amplified aptasensor with in-situ precise preparation of copper nanoclusters for ultrasensitive electrochemical detection of microRNA 21. *Biosensors and Bioelectronics* **2017**, *98*, 386-391, 10.1016/j.bios.2017.07.009.
56. Peng Miao; Tian Zhang; Jianhua Xu; Yuguo Tang; Electrochemical Detection of miRNA Combining T7 Exonuclease-Assisted Cascade Signal Amplification and DNA-Templated Copper Nanoparticles. *Analytical Chemistry* **2018**, *90*, 11154-11160, 10.1021/acs.analchem.8b03425.