Analysis & Sensing

Review
doi.org/10.1002/anse.202400017

Chemistry Europe
European Chemical Societies Publishing

www.analysis-sensing.org

Bimetallic Nanoparticles as Electrochemical Labels in Immunosensors for the Detection of Biomarkers of Clinical Interest

David Valero-Calvo,[a, b] Francisco Javier García-Alonso,[b, c] and Alfredo de la Escosura-Muñiz*[a, b]

Electrochemical immunosensors have emerged in the last years as outstanding analytical systems for the detection of analytes of clinical interest. As alternative to the traditional enzymatic labels, the use of nanoparticles and especially bimetallic ones has gained increased attention thanks to their advantages related to the higher simplicity, stability and sensitivity offered. Main routes for the detection of such nanoparticle labels are based on i) dissolution of the nanoparticle into the corresponding metal ions followed by voltammetric detection; ii) taking advantage of the electrocatalytic effect of the metals towards secondary reactions; and iii) taking advantage of their electrochemiluminescence properties.

1. Introduction

Main challenges in immunosensors for clinical diagnostics are still related to the low levels of detection of biomarkers and the selectivity required. Moreover, the time-stability is also crucial for the implementation of immunosensors for routine analysis. In this context, electrochemical immunosensors have been established as one of the main analytical systems for the detection of analytes of clinical interest for diagnosis purposes. The high affinity and specificity between antibodies and target antigens, together with the high stability of the formed immunocomplexes have led the great growth of immunosensors.[1,2] Among different transduction techniques for the detection of the target antigen, electrochemical ones stand out due to the easy use, low-cost and miniaturization possibilities.[3] However, the sensitivity needed for the detection of analytes of clinical interest at low levels makes necessary the development of signal amplification strategies. In this way, the use of enzymatic labels has been widely proved as reliable strategy to achieve the desired sensitivity.[4] Nevertheless, the low thermal stability, risk of denaturation and high cost are important limitations that prevent the use of enzymes as tags in electrochemical immunosensors. In this context, the use of nanoparticles as labels in electrochemical immunosensors has recently emerged due to their excellent performance.[5–7] Among nanoparticles, metallic ones stand out due to the good stability, intrinsic redox activity, the ease production and biocompatibility for further bioconjugation.[8] Different metallic oxides, carbon nanocomposites and other nanocomposites-based electrochemical sensors have also shown a superior performance for the detection of biomarkers of clinical interest due to the high sensitivity, rapid response and selectivity provided, taking advantage of the unique electronic properties of such nanomaterials.[9–13] In particular, growth interest has been found in bimetallic nanoparticles, that together exhibit enhanced red-ox properties and electrocatalytic behavior towards many reactions employed in biosensing.[14]

Bimetallic nanoparticles are combination of two different metals at nanoscale that together show improved properties due to the synergistic effect generated. The combination of two metals has opened the door to new practical applications as enhanced catalytic properties, sensitivity and stability are offered by these new materials compared with monometallic ones.[15] This behavior is mainly attributed to the higher catalytic performance of metals surrounded by second and third elements. As illustrative example, it is well-known that the selective introduction of gold atoms in palladium nanocluster structures enhances the catalytic activity of palladium. This synergy is strongly enhanced when the amount of gold is controlled and occupies the more reactive positions of the cluster, affecting the obtained electrocatalytic activity. Such bimetallic nanoparticles exhibited a remarkable improved catalytic activity towards glucose oxidation and hydrogen peroxide decomposition compared with both bare metals.[16] Moreover, the combination of different metals allows to take advantage of individual properties of each one. For example, one metal can be more advantageous for biofunctionalization while the other may have electrochemical properties ideal for detection. Methods for the synthesis of bimetallic nanoparticles are mainly based on i) co-reduction, ii) successive reduction,

[a] D. Valero-Calvo, A. de la Escosura-Muñiz
NanoBioAnalysis Group-Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain
E-mail: alfredo.escosura@uniovi.es

[b] D. Valero-Calvo, F. J. García-Alonso, A. de la Escosura-Muñiz
Biotechnology Institute of Asturias, University of Oviedo, Santiago Gascon Building, 33006 Oviedo, Spain

[c] F. J. García-Alonso
NanoBioAnalysis Group-Department of Organic and Inorganic Chemistry, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain

iii) reduction of metal complexes and iv) electrochemical formation.[17] Specific properties of bimetallic nanoparticles may also be tailored by the synthesis methodology and reaction conditions due to the dependence of properties on atom distribution and the structure formed after synthesis.[18] Different bimetallic nanoparticle structures have been described, such as crown-jewel structures, hollow heterostructures, core@shell structures and alloyed and porous structures, which specific properties have paved the way to multiple different applications.[19] The specific selection of different metallic combinations, controlling the proportion of each metallic compound also allows the tuning of synthetized bimetallic nanoparticles with different properties in function of the target application.[20] Among different metals described for the synthesis of bimetallic nanoparticles, gold metal is one of the most widely used for the combination with other metals[21] due to its easy well-established synthesis methodology pioneered by Turkevich[22] for obtaining size-controlled and well-dispersed AuNPs that can act as a core and also as a coating on the surface of nanoparticles. Broad variety of combinations of bimetallic nanoparticles with different metals such as gold, platinum, palladium and silver have been used in a wide range of different applications.[23–25]

Different approaches have been proposed for the electrochemical detection of bimetallic nanoparticle tags. First, bimetallic nanoparticles detection can be performed by oxidative dissolution of the nanoparticle into the corresponding metal ions followed by voltammetric detection. Alternatively, many approaches take advantage of the electrocatalytic effect of the metals towards secondary reactions for their sensitive detection without previous oxidative dissolution. Finally, it is worthy to mention that electrochemiluminescence properties of bimetallic nanoparticles have also been approached for their detection in immunosensors.

Therefore, looking at the great interest and continuous research in this field, a review on the use of bimetallic nanoparticles as labels in electrochemical immunosensors is needed. Specifically, this review focus on their application as labels for the detection of biomarkers of interest in clinical diagnostics. A detailed and critical overview of the main approaches found in the literature is provided, ending with future perspectives and prospects in this field.

2. Bimetallic Nanoparticles as Electroactive Labels

The use of bimetallic nanoparticles as electroactive tags has been proposed due to their unique properties compared with monometallic ones. Typically, a preliminary step is required in which an acidic solution is added to perform an oxidative dissolution of the nanoparticle, releasing the metallic ions that are then voltammetrically detected. The use of sensitive techniques as anodic stripping voltammetry (ASV), in which a preconcentration step is performed, allows the obtaining of very low limits of detection (Table 1).

As representative example, Cu@Au nanoparticles have shown enhanced properties for the detection of *E. coli* in surface water with a detection limit of 30 CFU/mL. The core@shell structure is approached for the conjugation of antibodies that are connected to the gold shell, while the Cu^{2+} ions released from the copper core after acidic treatment are detected by ASV (Figure 1A).[26]

Also interesting is the combination of magnetic properties of metals as iron with red-ox properties of gold. For example, gold-coated magnetic nanoparticles (Fe_3O_4@AuNPs) have been proposed for taking advantage of the magnetic Fe_3O_4 core for separation purposes and of the gold shell for the electrochemical detection. Such nanoparticles have been evaluated for the detection of digoxin in serum samples by monitoring the reduction of $AuCl_4^-$ to Au^0 by differential pulse voltammetry

David Valero-Calvo received his B.Sc. in Chemistry in 2020 at the University of Cádiz (Spain). He is now a Ph.D. student in the NanoBioAnalysis group, Department of Physical and Analytical Chemistry, University of Oviedo (Spain) under a Pre-doctoral Researcher (FPI) grant. His research interests focus on the development of biosensors based on nanomaterials for the detection of biomarkers of clinical interest for diagnosis.

Francisco Javier García-Alonso received his Ph.D. in Chemical Sciences in 1982 from the University of Valladolid (Spain). He is Full Professor in Inorganic Chemistry at the University of Oviedo (Spain) since 2009. His research interests focus on the synthesis of novel inorganic nanomaterials for applications in biosensing.

Alfredo de la Escosura-Muñiz holds a Ph.D. in Chemistry (2006) from the University of Oviedo (Spain). As of December 2023, he holds a position as Associate Professor in the Nanobioanalysis Group, Department of Physical and Analytical Chemistry, University of Oviedo (Spain). His research interests focus on the development of biosensing systems based on nanoparticles and nanochannels for point-of-care diagnostic applications.

Table 1. Approaches based on the use of bimetallic nanoparticles as electroactive labels for the detection of biomarkers of clinical interest.

Bimetallic nanoparticles	Transduction	Analyte	Lineal range	LOD	Real samples	Ref.
Cu@Au	ASV	*E. coli*	50−5.0×10^4 CFU/mL	30 CFU/mL	Surface water	[26]
Fe_3O_4@Au	DPV	Digoxin	0.5−5 ng/mL	0.05 ng/mL	Serum	[27]

Cu@Au: copper coated with gold; Fe_3O_4@Au: magnetite covered with gold; ASV: anodic stripping voltammetry; DPV: differential pulse voltammetry; CFU/mL: colony-forming unit/mL.

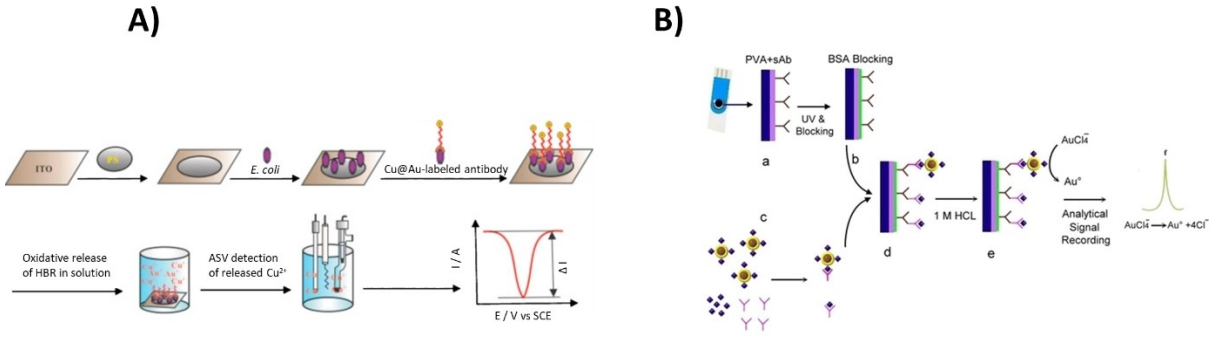

Figure 1. Use of bimetallic nanoparticles as electroactive labels. A) Scheme of an immunoassay for the detection of *E. coli* using Cu@Au bimetallic nanoparticles. After acidic treatment with HBR, Cu^{2+} ions are released and further detected through ASV. Reprinted from Ref. [26] with permission. B) Scheme of a competitive immunoassay for the detection of digoxin using gold-coated magnetic nanoparticles (Fe_3O_4@AuNPs). After acidic treatment in HCl, $AuCl^{4-}$ ions are detected through voltametric reduction (by DPV) to Au^0. Reprinted from Ref. [27] with permission.

(DPV) after previous oxidation of the Fe_3O_4@AuNPs in 1 M HCl, achieving limits of detection at ng/mL levels (Figure 1B).[27]

Despite the high sensitivity achieved, the need of acidic and hazardous agents limits the practical application of this methodology, that has been mostly replaced by detection routes based on the electrocatalytic properties of the bimetallic nanoparticles.

3. Bimetallic Nanoparticles as Electrocatalytic Labels

The high electrocatalytic activity of bimetallic nanoparticles has been the main focus of attraction for their use as tags in electrochemical immunosensing (Table 2). The high surface-volume area, together with the synergistic effect of the metals contained in such nanoparticles highly favor their catalytic properties towards several reactions.

3.1. Detection through the Electrocatalytic Effect Towards the H_2O_2 Reduction

Among different catalytic reactions employed for biosensing purposes, the reduction of H_2O_2 to H_2O has been one of the most employed through a two electron O_2 reduction (Eq. 1).

$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \quad (1)$$

The wide availability and ease of monitoring through amperometric techniques have greatly increased the development of sensors based on hydrogen peroxide detection.[28] Bimetallic nanoparticles that mimic peroxidase activity of traditional enzymes have emerged as potential substitutes of enzymatic tags. Moreover, the use of these nanoparticles has been extended for catalytic reactions that occur at neutral pH like the water oxidation reaction (WOR)[29] and oxygen reduction reaction (ORR).[30]

For example, the use of Cu@Ag nanoparticles functionalized with β-cyclodextrin (CD) for the conjugation with adamantine (ADA)-modified secondary antibody through CD-ADA interaction has been proposed for replacing the use of horseradish peroxidase (HRP). The proposed strategy showed an enhanced electrocatalysis of H_2O_2 reduction based on promoted catalytic effects of copper core after loading with silver nanoparticles. The developed immunosensor was assayed for the detection of carcino-embryonic antigen (CEA) in serum samples with a detection limit of 20 fg/mL showing also good selectivity versus interference substances, good reproducibility and an acceptable stability at least for one week.[31] The same detection principle has been approached using Pd@Pt nanoparticles modified with amino groups functionalized with graphene to achieve an increased loading of antibodies. Combination of Pd nanoparticles used as seeds for dendritic growth of Pt nanoparticles lead to a mesoporous core-shell Pd@Pt structure providing a high specific surface area (due to the porosity) offering large catalytic active sites for enzymatic reactions showing a higher scale of sensitivity. The developed immunosensor was applied for the detection of prostate specific antigen (PSA) in human serum samples achieving limit of detection of 3.3 fg/mL (Fig-

Table 2. Approaches based on the use of bimetallic nanoparticles as electrocatalytic labels for the detection of biomarkers of clinical interest.

Bimetallic nanoparticles	Transduction	Analyte	Lineal range	LOD	Real samples	Ref.
Cu@Ag	Amperometry	CEA	0.0001–20 ng/mL	20 fg/mL	Serum	[31]
Pd@Pt nanoparticle	Amperometry	PSA	10 fg mL^{-1}–50 ng mL^{-1}	3.3 fg mL^{-1}	Serum	[32]
Pt@CeO_2	DPV	Dopamine	2–180 nM	0.71 nM	Simulated brain system	[33]
Au@Pd	Amperometry	CA125	0.002 U–20 U mL^{-1}	0.001 U mL^{-1}	Serum	[34]
Au@Ag/Au NPs	Amperometry	SCCA	0.5 pg/mL–40 ng/mL	0.18 pg/mL	Serum	[35]
Au@Ag nanoparticle	Amperometry	AFP	20 fg/mL–100 ng/mL	6.7 fg/mL	Serum	[36]
Au@Pt	Amperometry	AFP	0.1 pg/mL–10 ng/mL	0.05 pg/mL	Serum	[37]
Au-Pd	Amperometry	AFP	0.05–30 ng/mL	0.005 ng/mL	Serum	[38]
Pd-Ni	Amperometry	AFP	0.0001–16 pg/mL	0.03 pg/mL	Serum	[39]
PtCu	Amperometry	PSA	50 fg/mL–40 ng/mL	16.6 fg/mL	Serum	[40]
Pd-Pt	Amperometry	hTPA	0.0050–15 ng/mL	1.2 pg/mL	Serum	[41]
Au-Ag	Amperometry	CEA	0.001–50 ng/mL	0.3 pg/mL	Serum	[42]
CuCo	Amperometry	CEA	0.0001–80 ng/mL	0.031 pg/mL	Serum	[43]
AuPt	Chronoamperometry	Vangl1	0.1–450 pg/mL	0.03 pg/mL	Serum	[44]
Au@Pt/Au	Chronoamperometry	p53 peptide	50–1000 nM	66 nM	Plasma	[45]
Pd-AuNPs	Chronoamperometry	Hyaluronidase	125–4600 ng/mL	50 ng/mL	Wound exudate	[46]

Cu@Ag: copper@silver; Pd@Pt: paladium@platinum; Pt@CeO_2: platinum@cerium oxide; Au@Pd: gold@paladium; Au@Ag/Au NPs: gold@silver/gold nanoparticles; Au@Ag: gold@silver; Au@Pt: gold@platinum; Au–Pd: gold palladium; PdNi: palladium nickel; PtCu: platinum copper; Pd–Pt: palladium-platinum; Au–Ag: gold-silver; CuCo: copper cobalt; AuPt: gold platinum; Au@Pt/Au: gold@platinum/gold; Pd-AuNPs: palladium gold nanoparticles; DPV: differential pulse voltammetry; CEA: carcinoembryonic antigen; PSA: prostate specific antigen; CA125: carbohydrate antigen 125; SCCA: squamous cell carcinoma antigen; AFP: alpha fetoprotein; hTPA: human tissue polypeptide antigen.

ure 2A) with good selectivity and reproducibility and also stability after four weeks.[32] Another bimetallic combination as Pt@CeO_2 showed an enhanced performance for the catalytic towards H_2O_2 reduction compared to monometallic CeO_2 due to the presence of more oxygen vacancies in lattice bimetallic structure, favoring the electrons exchange. The use of these nanoparticles as signal amplifier was approached for the detection of dopamine with a limit of detection at nM levels showing good intra and inter-assay reproducibility, high selectivity and an excellent stability after 90 days.[33] Au@Pd nanoparticles combination has also been approached taking advantage of palladium covalent functionalization with the amino groups of functionalized antibodies and the high electrocatalytic activity of Au@Pd towards the H_2O_2 reduction for the detection of carbohydrate antigen 125 (CA125) in serum samples with a detection limit of 0.001 U/mL featuring also good selectivity in presence of biological interferents, good inter-reproducibility and remaining stable after three weeks.[34] Novel nanoparticles with core@double shell structure of Au/Ag/Au confirmed by transmission electron microscopy (TEM) have been synthetized to improve the electrocatalytic activity of AuNPs by introducing AgNPs. Such structures were used as enzyme-mimetic labels towards H_2O_2 reduction with chronoamperometry response without requiring of red-ox mediator species. The developed immunosensor exhibited improved performance as lower consumption costs, better stability and higher sensitivity, compared with traditional enzymes, applied for the detection of squamous cell carcinoma antigen (SCCA) in human serum with a detection limit at pg/mL levels displaying good reproducibility, high selectivity and good stability after two weeks.[35]

Different bimetallic nanoparticle structure combinations as Au@Ag (Figure 2C),[36] Au@Pt nanodendrites,[37] Au–Pd nanocrystals,[38] and PdNi alloy[39] have also exhibited superior biosensing performance for the detection of alpha fetoprotein (AFP) through the catalytic reduction of H_2O_2, reaching clinically relevant levels in serum samples.

Other bimetallic Pt–Cu nanoparticles carried by reduced graphene oxide/graphitic carbon nitride (rGO/g-C_3N_4) were also evaluated for the detection of PSA biomarker through H_2O_2 electrocatalysis.[40] The combination of well-known Pt superior catalytic activity with integration of Cu metal have shown a synergistic effect providing a superior stability and higher catalytic efficiency with the use of rGO/g-C_3N_4 as signal label modified with Au-thionine functionalized graphene oxide (Au@Th/GO) as substrate platform, leading to a triple amplification signal showing a limit of detection of 16.6 fg/mL. The use of bimetallic nanoparticle structures as Pd–Pt nanocrystals has also shown an enhanced electrocatalytic activity *versus* bare both metals towards the H_2O_2 reduction for the detection of human tissue polypeptide antigen (hTPA) in serum samples with a limit of detection of 1.2 pg/mL and showing good inter-reproducibility, good selectivity in presence of interfering compounds and good stability of Pd–Pt-antibody conjugate after one month.[41] In this line, a novel approach was proposed by Sun and co-workers that developed a paper-based electrochemical immunosensor taking advantage of enhanced sensitivity of Au–Ag bimetallic nanoparticles. Combination of good

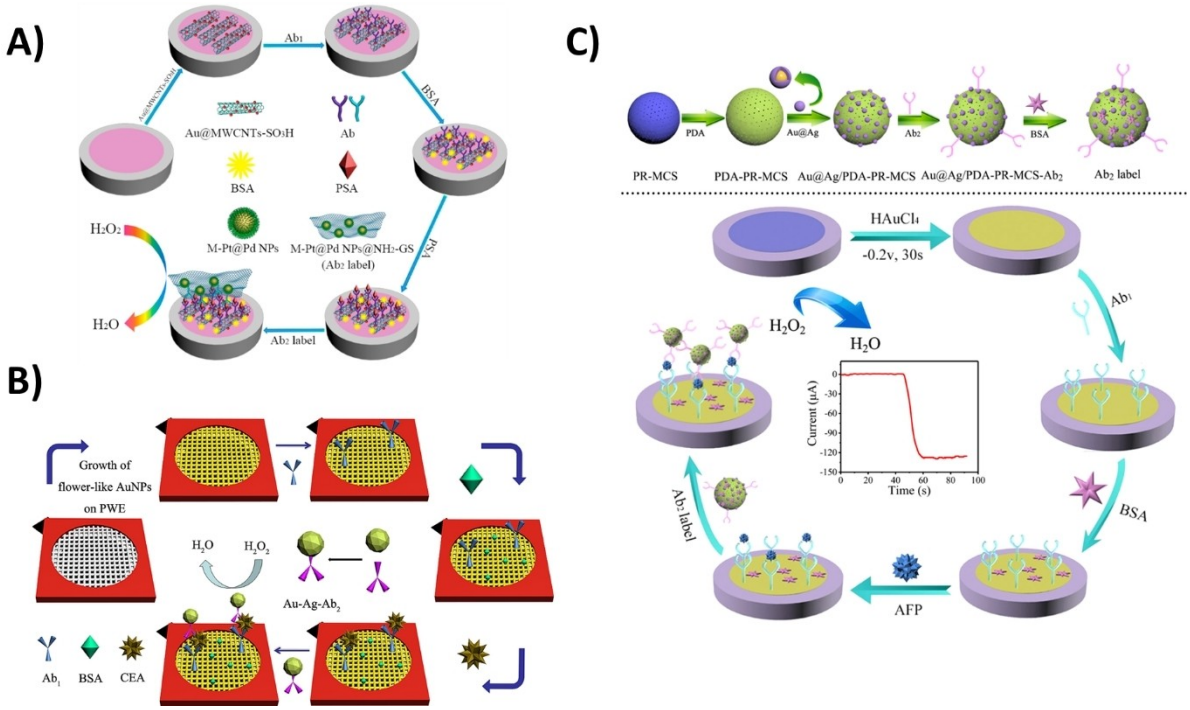

Figure 2. Bimetallic nanoparticles as electrocatalysts of H_2O_2 reduction to H_2O. A) Scheme of an immunoassay for the quantification of PSA based on M–Pt@Pd NPs electrocatalytic labels. Reprinted from Ref. [32] with permission. B) Scheme of a paper-based immunoassay platform for the detection of CEA using Au–Ag-Ab_2-labeled nanoparticles. Reprinted from Ref. [42] with permission. C) Scheme of preparation of the labelling of antibodies with Au@Ag bimetallic nanoparticles and development of electrochemical immunoassay for the detection of AFP through amperometric response of H_2O_2 reduction. Reprinted from Ref. [36] with permission.

biocompatibility and large surface area of AuNPs its well-known synergy with Ag metal has postulated these bimetallic structures as ideal nanocarriers for a superior electron transfer conductivity. The developed immunosensor was approached towards the electrocatalytic reduction of H_2O_2 for the detection of carcinoembryonic antigen (CEA), obtaining a detection limit of 0.3 pg/mL and showing good intra and inter-assay reproducibility, good selectivity in presence of three potential interfering compounds and good stability after four weeks (Figure 2B).[42] Interestingly, the synergistic electrocatalytic activity of Co/Cu carbon nanocubes towards the H_2O_2 reduction through specific intrinsic redox activity showed an enhanced limit of detection for CEA reaching a detection limit at levels of 0.031 pg/mL. The developed immunosensor showed high selectivity in presence of potentially interfering compounds, good precision after five electrodes measurement and high stability after five weeks. The catalytic process was also confirmed by X-ray photoelectron spectroscopy (XPS) measurements, revealing the interesting behavior of bimetallic nanoparticles in the reaction mechanism.[43] Another interesting approach consists in the use of C_{60} nanomaterial as carrier of antibodies labeled with bimetallic Au–Pt nanoparticles. The prepared immunosensor was applied for the detection of Vangl1 in serum samples through enhanced electrocatalytic activity of C_{60}-Au–Pt tag towards the H_2O_2 reduction showing a detection limit of 0.03 pg/mL. The selectivity studies featured the high specificity for Vangl1 *versus* potential interference substances, while stability was evaluated showing that the catalytic activity remained after 20 days with a 90% of the initial signal. The reproducibility was evaluated for five intra-assays showing good precision.[44]

3.2. Detection through the Electrocatalytic Effect Towards WOR and ORR

WOR and ORR are simple reactions occurring at a neutral pH, easily monitored by electrochemical techniques. The WOR involves a four-electron transfer process ($2H_2O \rightarrow O_2 + 4H^+ + 4e^-$), also being known as oxygen evolution reaction (OER), while the ORR involves a two-electron transfer process ($O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$). Of special interest is the fact that such reactions occur at a neutral pH, so nanoparticles able to catalyze such reaction would be detected in buffer media without the need of additional reagents. This is the case of some bimetallic nanoparticles.

As example, bifunctional Au@Pt/AuNPs were used for the detection of unfolded p53 peptide Alzheimer's disease biomarker in plasma samples, taking advantage of their electrocatalytic activity towards the WOR, reaching a limit of detection of 66 nM. The formation of a core@shell structure displaying Au protuberances growth on the nanoparticle surface enhances the bioconjugation of the bimetallic nanoparticle with p53 antibodies while the high catalytic surface of Pt is approached for the catalysis of WOR at neutral pH without requiring the addition of external agents (Figure 3A). The selectivity of the

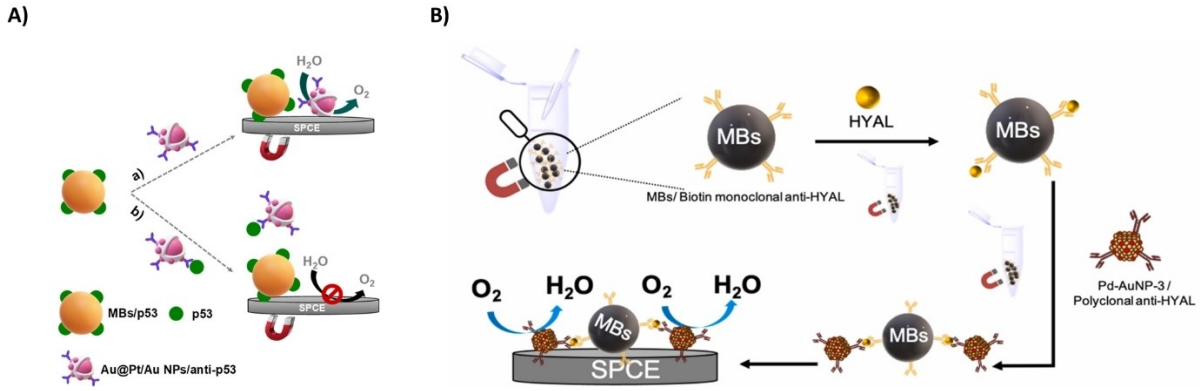

Figure 3. Use of bimetallic nanoparticles as electrocatalysts of the WOR and the ORR. A) Scheme of a magnetoimmunoassay for the detection of p53 antigen using Au@Pt/Au NPs as electrocatalytic tag for WOR. Reprinted with permission from Ref. [45]. Copyright 2020, American Chemical Society. B) Scheme of a magnetoimmunoassay for the detection of hyaluronidase using Pd-AuNPs as electrocatalytic tags for ORR. Reprinted with permission from Ref. [46].

electrochemical immunoassay was demonstrated using human IgG as negative control, not showing any variation from the blank signal. In addition, the method featured good reproducibility with a relative standard deviation (RSD) below 5% for three repetitive assays.[45]

The electrocatalytic activity of bimetallic Pd-AuNPs towards the ORR has also been applied for immunosensing purposes. Selective introduction of gold atoms on a Pd cluster was evaluated for further functionalization with anti-hyaluronidase, facilitated by well-known affinity of Au to cysteine residues present in the antibody structure. Different compositions of Pd and Au in the nanoparticles were also evaluated in order to maximize the synergy between both metals and enhance their electrocatalytic activity. Such labels and detection mode have been applied for the detection of hyaluronidase, a wound infection biomarker, achieving a limit of detection of 50 ng/mL, being also able to discriminate between infected and sterile wound exudates without requiring any pre-treatment of the sample (Figure 3B). In addition, the selectivity of the Pd-AuNPs based immunosensor was successfully assayed against compounds present in human samples as BSA, IgG and lysozyme, while the developed methodology showed a good reproducibility with a RSD below 8%.[46]

4. Bimetallic Nanoparticles as Electrochemiluminescence Labels

Electrochemiluminescence (ECL) based on nanoparticles has become a powerful bioanalytical tool.[47,48] Of special relevance is the use of bimetallic nanoparticles as advantageous tags in ECL (Table 3), providing highly sensitive analytical systems with low back-ground signal and also specific measurements.[49]

For example, Au@Pt/Au nanoparticles have been evaluated as electrochemiluminescence acceptor probe showing an enhancement performance of ECL-resonance energy transfer (RET) due to the effect of the shape and size of such bimetallic nanoparticles. The combination of the bimetallic nanoparticles with luminophore $[Ru(bpy)_3]^{2+}$ showed a stronger localized surface plasmon resonance (LSPR) absorption. The developed ECL-RET immunosensor was applied for the rapid detection of N-protein of SARS-CoV-2 virus in saliva featuring a detection limit of 1.27 pg/mL (Figure 4A). The response of the immunosensor was also assayed *versus* potential protein interferences demonstrating the feasibility to discriminate between N and S protein while the RSD found for five different developed immunosensors was below 3%. The stability was evaluated after 45 days storage in a humid chamber showing just a loss of 3% in the ECL initial signal.[50] The higher catalytic activity of bimetallic nanoparticles towards H_2O_2 decomposition as alternative to conventional enzymes like HRP has been also

Table 3. Approaches based on the use of bimetallic nanoparticles as electrochemiluminescence tags for the detection of biomarkers of clinical interest.

Bimetallic nanoparticles	Transduction	Analyte	Lineal range	LOD	Real samples	Ref.
Au@Pt/Au	ECL	N-protein SARS-CoV-2	2.5–250 pg/mL	1.27 pg/mL	Saliva	[50]
PdIr	ECL	Laminin	1 pg/mL–120 ng/mL	0.27 pg/mL	Serum	[51]
AuPd	ECL	CEA and AFP	0.005–200 ng/mL	–	MCF-7 cell surface	[52]
Au@Pd	ECL	CEA	10 fg/mL–100 ng/mL	3 fg/mL	Serum	[53]
Pd@Au	ECL	Col IV	1 pg/mL–10 ng/mL	0.3 pg/mL	Serum	[54]

Au@Pt/Au: gold@platinum/gold; PdIr: palladium iridium; AuPd: gold palladium; Au@Pd: gold@palladium; Pd@Au: palladium@gold; ECL: electrochemiluminescence; CEA: carcinoembryonic antigen; AFP: alpha fetoprotein; Col IV: colistin IV.

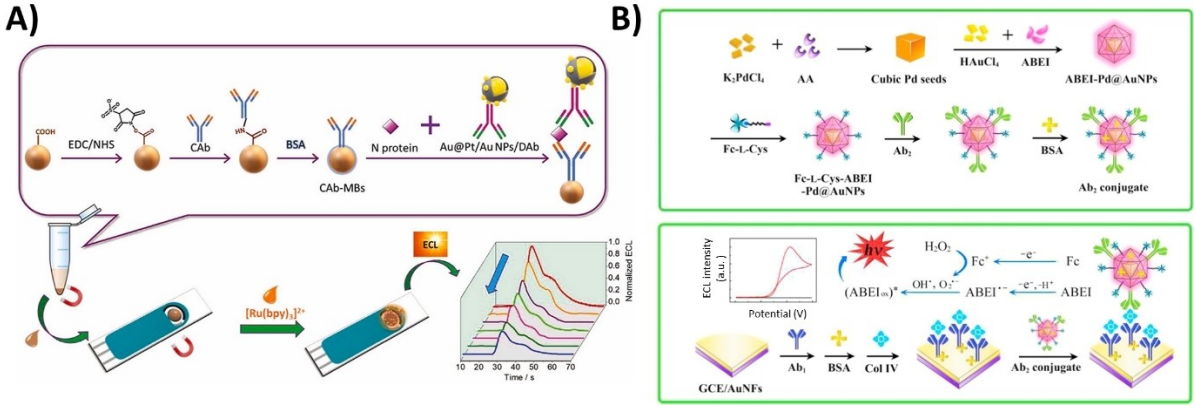

Figure 4. Use of bimetallic nanoparticles as electrochemiluminiscence labels. A) Schematic representation of an magnetoimmunoassay using Au@Pt/Au NPs as ECL signalling probe for the detection of N-protein of SARS-CoV-2 virus. Reprinted from Ref. [50] with permission. B) Scheme of preparation of bimetallic nanoparticles of Pd@Au for further use as electrochemiluminescence tag for the detection of Col IV. Reprinted from Ref. [54] with permission.

exploited for electrochemiluminescence biosensing. The generation of radicals from H_2O_2 that react with ECL luminophores promotes the ECL reaction and enhances the luminescence signal. For example, an interesting approach was developed using PdIr cubes as signal amplifier replacing the use of conventional HRP for the catalysis of H_2O_2, used as coreactant of the N-(aminobutyl)-N-(ethylisoluminol) (ABEI)-H_2O_2 ECL developed immunosensor. The amplification strategy was assayed for the detection of laminin in human serum showing a detection limit of 0.27 pg/mL.[51] This strategy using bimetallic nanoparticles as signal amplifier of ECL through electrocatalytic activity of bimetallic nanoparticles has also been studied using different structures of AuPd nanoparticles[52–54] (Figure 4B).

5. Conclusions and Perspectives

Bimetallic nanoparticles have completely emerged in the field of electrochemical immunosensing, as advantageous tags alternative to traditional enzymatic labels. Such nanoparticles, especially those made of combination of gold, silver, platinum and palladium have stood out because of their outstanding properties. The functionalization ability and electroactivity have allowed their voltammetric analysis, detecting the released metals after acidic dissolution. However, the need of hazardous reagents for such metals releasing has limited the implementation of these methodologies, that have been practically replaced by those based on taking advantage of the electrocatalytic activity of bimetallic nanoparticles. The high surface area of nanoparticles together with the synergy between the different metals play a relevant role here. Among the electrocatalytic routes, the effect exerted towards the reduction of H_2O_2 has been the most exploited one. Moreover, the electrocatalytic effect exerted towards the WOR and the ORR has been shown as outstanding alternative for their detection, since such reactions take place in the same medium in which the immunocomplex formation occurs, without the need of adding any additional reagent. This represents an important advance for the development of point-of-care analytical systems in which all the assay is totally integrated. In a minor extent, electrochemiluniscence properties of bimetallic nanoparticles have also been exploited for their detection in immunosensing.

Despite the excellent sensitivity and performance in real samples exhibited, some important issues should be solved for the implementation of electrochemical immunosensors based on bimetallic nanoparticles in routine analysis. Although the response of bimetallic nanoparticles is in principle more stable than that of enzymes, the nanoparticle suspension stability is an important issue that is not addressed in most of the reviewed approaches. The polydispersity of the nanoparticle suspension and the efficiency of the conjugation with antibodies are also scarcely studies in many of the approaches. Such basic studies are of key relevance and are strongly required in the near future. Many efforts are also expected in multiplexing analysis, which is of paramount relevance in clinical diagnosis. Dual-detection modes combining electrical, optical or electrocatalytic properties are also expected in the upcoming years.

The design of new bimetallic nanoparticle structures should also open new modes of detection, taking advantage of electrical/optical properties of each metallic component. Among them the bimetallic nanosized metal–organic frameworks (MOFs) deserve a special attention. MOFs are crystalline networks of metals or metal-cluster joined together by multifunctional organic ligands with a very high variety of properties and applications.[55] In fact, although MOFs have already been used satisfactorily in immunoassays, bimetallic MOFs possessing two different metal ions in the same network have revealed synergistic effects, which resulted to be very useful in the field of electrochemical biosensors.[56] On the other hand, the MOFs porous structure allow metal nanoparticles (MNPs) to be anchored on the surface or encapsulated inside their cavities. The resultant MOF/MNPs bimetallic composites can also be excellent labels as far as they have demonstrated outstanding sensing properties due to their enhanced catalytic, and conductive properties. While, up to now, bimetallic MOFs have been scarcely used as labels in electrochemical biosensors,[57] it

is expected that in the next future also bimetallic nano-MOFs will be used as routine tags in these systems.

Acknowledgements

This work has been supported by the MCI-21-PID2020-115204RBI00 and RED2022-134120-T projects from the Spanish Ministry of Science and Innovation (MICINN) and the SV-PA-21-AYUD/2021/51323 project from the Asturias Regional Government. D. Valero-Calvo thanks the MICINN for the award of FPI Grant (PRE2021-097567).

Conflict of Interests

The authors declare no conflict of interest.

Keywords: bimetallic nanoparticles · immunosensors · electrochemical · diagnosis · labels

[1] E. Gizeli, C. R. Lowe, *Curr. Opin. Biotechnol.* **1996**, *7*, 66–71.
[2] J. Tang, D. Tang, *Microchim. Acta* **2015**, *182*, 2077–2089.
[3] W. Zhang, R. Wang, F. Luo, P. Wang, Z. Lin, *Chin. Chem. Lett.* **2020**, *31*, 589–600.
[4] X.-M. Li, X.-Y. Yang, S.-S. Zhang, *TrAC Trends Anal. Chem.* **2008**, *27*, 543–553.
[5] A. Iglesias-Mayor, O. Amor-Gutiérrez, A. Costa-García, A. de la Escosura-Muñiz, *Sensors* **2019**, *19*, 5137.
[6] A. de la Escosura-Muñiz, A. Ambrosi, A. Merkoçi, *TrAC Trends Anal. Chem.* **2008**, *27*, 568–584.
[7] A. de la Escosura-Muñiz, C. Parolo, A. Merkoçi, *Mater. Today* **2010**, *13*, 24–34.
[8] V. Mody, R. Siwale, A. Singh, H. Mody, *J. Pharm. BioAllied Sci.* **2010**, *2*, 282.
[9] P. Kannan, G. Maduraiveeran, *Curr. Med. Chem.* **2023**, *30*.
[10] P. Kannan, G. Maduraiveeran, *Biosensors (Basel)* **2023**, *13*, 542.
[11] G. Maduraiveeran, *J. Anal. Sci. Technol.* **2022**, *13*, 35.
[12] G. Maduraiveeran, *Anal. Methods* **2023**, *15*, 6620–6630.
[13] M. Arivazhagan, P. Kannan, G. Maduraiveeran, *Biosensors (Basel)* **2022**, *12*, 1128.
[14] G. Sharma, A. Kumar, S. Sharma, M. Naushad, R. Prakash Dwivedi, Z. A. Alothman, G. T. Mola, *J. King Saud Univ. Sci.* **2019**, *31*, 257–269.
[15] R. Stephanie, M. W. Kim, S. H. Kim, J.-K. Kim, C. Y. Park, T. J. Park, *TrAC Trends Anal. Chem.* **2021**, *135*, 116159.
[16] H. Zhang, T. Watanabe, M. Okumura, M. Haruta, N. Toshima, *Nat. Mater.* **2012**, *11*, 49–52.
[17] N. Toshima, T. Yonezawa, *New J. Chem.* **1998**, *22*, 1179–1201.
[18] K. Loza, M. Heggen, M. Epple, *Adv. Funct. Mater.* **2020**, *30*, 10.1002/adfm.201909260.
[19] X. Liu, D. Wang, Y. Li, *Nano Today* **2012**, *7*, 448–466.
[20] B. Zhang, H. Yang, Y. Wang, S. Dou, H. Liu, *Adv. Energy Mater.* **2018**, *8*, 10.1002/aenm.201703597.
[21] S. Ali, X. Chen, S. Ahmad, M. Almehmadi, A. Amer Alsaiari, M. Allahyani, Z. Gul, A. Ullah, H. Hussain, L. Li, X. Chen, *Arab. J. Chem.* **2023**, *16*, 104997.
[22] J. Turkevich, P. C. Stevenson, J. Hillier, *Discuss. Faraday Soc.* **1951**, *11*, 55.
[23] M. Zhang, X. Guo, *Coord. Chem. Rev.* **2022**, *465*, 214578.
[24] T. Ghodselahi, S. Arsalani, T. Neishaboorynejad, *Appl. Surf. Sci.* **2014**, *301*, 230–234.
[25] E. Pizzutilo, O. Kasian, C. H. Choi, S. Cherevko, G. J. Hutchings, K. J. J. Mayrhofer, S. J. Freakley, *Chem. Phys. Lett.* **2017**, *683*, 436–442.
[26] X. Zhang, P. Geng, H. Liu, Y. Teng, Y. Liu, Q. Wang, W. Zhang, L. Jin, L. Jiang, *Biosens. Bioelectron.* **2009**, *24*, 2155–2159.
[27] A. Ahmadi, H. Shirazi, N. Pourbagher, A. Akbarzadeh, K. Omidfar, *Mol. Biol. Rep.* **2014**, *41*, 1659–1668.
[28] A. Lupu, P. Lisboa, A. Valsesia, P. Colpo, F. Rossi, *Sens. Actuators B* **2009**, *137*, 56–61.
[29] R. Sen, S. Das, A. Nath, P. Maharana, P. Kar, F. Verpoort, P. Liang, S. Roy, *Front. Chem.* **2022**, *10*, 10.3389/fchem.2022.861604.
[30] J. Zhou, P. Qin, W. Liu, Y. Liu, *Int. J. Electrochem. Sci.* **2022**, *17*, 221152.
[31] J. Gao, Z. Guo, F. Su, L. Gao, X. Pang, W. Cao, B. Du, Q. Wei, *Biosens. Bioelectron.* **2015**, *63*, 465–471.
[32] M. Li, P. Wang, F. Li, Q. Chu, Y. Li, Y. Dong, *Biosens. Bioelectron.* **2017**, *87*, 752–759.
[33] C. Fu, Y. Sun, C. Huang, F. Wang, N. Li, L. Zhang, S. Ge, J. Yu, *Talanta* **2021**, *223*, 121719.
[34] A. Guo, D. Wu, H. Ma, Y. Zhang, H. Li, B. Du, Q. Wei, *J. Mater. Chem. B* **2013**, *1*, 4052.
[35] Y. Wang, Y. Zhang, Y. Su, F. Li, H. Ma, H. Li, B. Du, Q. Wei, *Talanta* **2014**, *124*, 60–66.
[36] X. Zhang, Y. Li, H. Lv, J. Feng, Z. Gao, P. Wang, Y. Dong, Q. Liu, Z. Zhao, *Biosens. Bioelectron.* **2018**, *106*, 142–148.
[37] L. Jiao, Z. Mu, C. Zhu, Q. Wei, H. Li, D. Du, Y. Lin, *Sens. Actuators B* **2016**, *231*, 513–519.
[38] L. Zhao, S. Li, J. He, G. Tian, Q. Wei, H. Li, *Biosens. Bioelectron.* **2013**, *49*, 222–225.
[39] N. Li, H. Ma, W. Cao, D. Wu, T. Yan, B. Du, Q. Wei, *Biosens. Bioelectron.* **2015**, *74*, 786–791.
[40] J. Feng, Y. Li, M. Li, F. Li, J. Han, Y. Dong, Z. Chen, P. Wang, H. Liu, Q. Wei, *Biosens. Bioelectron.* **2017**, *91*, 441–448.
[41] Y. Wang, Q. Wei, Y. Zhang, D. Wu, H. Ma, A. Guo, B. Du, *Nanotechnology* **2014**, *25*, 055102.
[42] G. Sun, Y. Ding, C. Ma, Y. Zhang, S. Ge, J. Yu, X. Song, *Electrochim. Acta* **2014**, *147*, 650–656.
[43] X. Wang, X. Liao, B. Zhang, M. Zhang, L. Mei, F. Wang, S. Chen, X. Qiao, C. Hong, *Microchim. Acta* **2021**, *188*, 1–11.
[44] Q. Chen, C. Yu, R. Gao, L. Gao, Q. Li, G. Yuan, J. He, *Biosens. Bioelectron.* **2016**, *79*, 364–370.
[45] A. Iglesias-Mayor, O. Amor-Gutiérrez, A. Novelli, M.-T. Fernández-Sánchez, A. Costa-García, A. de la Escosura-Muñiz, *Anal. Chem.* **2020**, *92*, 7209–7217.
[46] C. Toyos-Rodríguez, A. Adawy, F. J. García-Alonso, A. de la Escosura-Muñiz, *Biosens. Bioelectron.* **2022**, *200*, 113926.
[47] Y. Jia, Y. Du, Z. Ru, D. Fan, L. Yang, X. Ren, Q. Wei, *Anal. Chem.* **2023**, *95*, 6725–6731.
[48] Y. Jia, X. Ren, X. Zhang, D. Wu, H. Ma, Y. Li, Q. Wei, *Anal. Chem.* **2023**, *95*, 9139–9144.
[49] S. Mahadevarao Premnath, M. Zubair, *Electrochemiluminescence Method* **2024**. May 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 37603619.
[50] A. M. Villa-Manso, T. Guerrero-Esteban, F. Pariente, C. Toyos-Rodríguez, A. de la Escosura-Muñiz, M. Revenga-Parra, C. Gutiérrez-Sánchez, E. Lorenzo, *Talanta* **2023**, *260*, 124614.
[51] X. Jiang, H. Wang, H. Wang, Y. Zhuo, R. Yuan, Y. Chai, *Nanoscale* **2016**, *8*, 8017–8023.
[52] M. Su, H. Liu, S. Ge, N. Ren, L. Ding, J. Yu, X. Song, *RSC Adv.* **2016**, *6*, 16500–16506.
[53] Y. Zhang, X. Pang, D. Wu, H. Ma, Z. Yan, J. Zhang, B. Du, Q. Wei, *Analyst* **2016**, *141*, 337–345.
[54] Y. Liu, H. Wang, C. Xiong, Y. Yuan, Y. Chai, R. Yuan, *Biosens. Bioelectron.* **2016**, *81*, 334–340.
[55] H. Hu, Y. Wang, *TrAC Trends Anal. Chem.* **2024**, *171*, 117520.
[56] M. Kang, S. Huang, M. Wang, O. Oderinde, M. Wang, Z. Zhang, *Microchim. Acta* **2023**, *190*, 358.
[57] Y. Zhang, Y. Xu, N. Li, W. Ma, M. Yang, C. Hou, D. Huo, *Int. J. Hydrogen Energy* **2023**, *48*, 24548–24558.

Manuscript received: February 26, 2024
Revised manuscript received: May 6, 2024
Accepted manuscript online: May 17, 2024
Version of record online: June 7, 2024