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Electrochemical Impedance Spectroscopy (bio)sensing through hydrogen evolution reaction induced by gold nanoparticles

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ABSTRACT: A new gold nanoparticles (AuNPs) based detection strategy using Electrochemical Impedance Spectroscopy (EIS) through Hydrogen Evolution Reaction (HER) is proposed. This EIS-HER method is used as an alternative to the conventional EIS based on $[Fe(CN)_6]^{3-/4-}$ or $[Ru(NH_3)_6]^{3+/2+}$ indicators. The proposed method is based on the HER induced by AuNPs. EIS measurements for different amounts of AuNP are registered and the charge transfer resistance (Rct) was found to correlate and be useful for their quantification. Moreover the effect of AuNP size on electrical properties of AuNPs for HER using this sensitive technique has been investigated. Different EIS-HER signals generated in presence of AuNPs of different sizes (2, 5, 10, 15, 20, 50 nm) are observed, being the corresponding phenomena extendible to other nanoparticles and related catalytic reactions. This EIS-HER sensing technology is applied to a magneto immunosandwich assay for the detection of a model protein (IgG) achieving improvements of the analytical performance in terms of a wide linear range (2 – 500 ng mL^{-1}) with a good limit of detection (LOD) of 0.31 ng mL^{-1} and high sensitivity. Moreover, with this methodology a reduction of one order of magnitude in the LOD for IgG detection, compared with chroamperometric technique normally used was achieved.

Keywords: Gold nanoparticles, electrochemical impedance spectroscopy, hydrogen evolution reaction, magneto-immunoassay

1. INTRODUCTION

Gold nanoparticles (AuNPs) possess unique electrochemical and optical properties including and high surface-to-volume ratio and excellent biocompatibility making these interesting material in developing a variety of biosensors (Bonanni et al. 2008).

Hydrogen evolution reaction (HER) induced by AuNPs have been used in a variety of analytical applications for novel and improved sensing and biosensing devices with high specificity and high sensibility. (de la Escosura-Muñiz et al. 2010; de la Escosura-Muniz et al. 2011; Espinoza-Castañeda et al. 2013; Maltez-da Costa et al. 2010; Maltez-da Costa et al. 2012a, b; Saha et al. 2012).

In addition AuNPs are also used in Electrochemical Impedance Spectroscopy (EIS) to either enhance the impedimetric signal or modify electrodes in order to increase the amount of analites adsorbed onto their surface, or to exploit a dendritic signal amplification in various applications. (Bonanni et al. 2008; Derkus et al. 2013; Gao et al. 2013; Ren et al. 2013; Shen et al. 2013)

EIS is a powerful, non-destructive and highly informative technique, which is usually used for interface characterization and kinetic parameter determination for different reactions carried out on interfaces (Bonanni et al. 2011; Katz and Willner 2003; Park and Yoo 2003; Xianzong et al. 2013; Xie et al. 2012). EIS beside other techniques is used to study HER at different pure metals (Pd, Pt, Ni, Re, Au, Ir) and alloys (Pt- Rh, Ni – rare earth) establishing a qualitative relation between metal loading and potential variation (Castro and Milocco 2005; Chen et al. 2012; Khanova and Krishtalik 2011; Ortega-Chavez et al. 2008).

In the current study a new and efficient AuNPs-based detection strategy using EIS and HER in acid solution monitored through carbon screen printed electrodes (SPCE) is described. Chronoamperometry and differential pulse voltammetry (DPV) are commonly used

techniques to detect AuNPs (de la Escosura-Muñiz et al. 2010; de la Escosura-Muniz et al. 2011; Espinoza-Castañeda et al. 2013; Maltez-da Costa et al. 2010; Maltez-da Costa et al. 2012a, b). This EIS-HER method is used as an alternative to the conventional EIS based on the $[Fe(CN)_6]^{3-/4-}$ or $[Ru(NH_3)_6]^{3+/2+}$ indicators (Chang and Park 2010; Rodriguez et al. 2005; Wei et al. 2011). This new detection approach is successfully applied in a magneto immuno sandwich assay for HIgG detection achieving a low limit of detection (0.31 ng mL^{-1}). Furthermore, the effect of size on the EIS signals of AuNPs has been investigated demonstrating the applicability of this techniques to evaluate the particles size. This EIS-HER method is very sensitive toward AuNPs quantification and consequently may be offered for several biosensing applications.

2. MATERIALS AND METHODS

2.1. Chemicals

Magnetic beads (Dynabeads M-280 Tosylactivated) were purchased to Invitrogen™ (Paisley, UK).Gold colloid, gold nanoparticles of different diameters were obtained from British Biocell (BBi, Cardiff, UK). Tetrachloroauric acid, sodium citrate, boric acid, , PBS 10mM pH 7.4, Albumin from Bovine Serum (BSA) in powder, HCl 37% (v/v) , human IgG from serum and antihuman IgG produced in goat were purchased from Sigma Aldrich (SA, St Quentin Fallavier, France). The anti-human IgG produced in rabbit was purchased from Sigma Aldrich (Schnelldorf, Germany). Polyester substrates were purchased from MacDermid Autotype. All the inks, Carbon ink, Ag/AgCl ink and the insulating ink were purchased from AchesonTM, Henkel group.

2.2. Apparatus

For electrochemical measurement Autolab302 potentiostat/galvanostat/ frequency-response analyzer PGST30, controlled by GPES/FRA Version 4.9 was used. Transmission electron microscope (TEM) images were taken with a JEM-2011 (Jeol, Ltd., Japan).

2.3. Gold Nanoparticles synthesis

Gold nanoparticles were made following the procedure reported before (Turkevich et al. 1951) . A solution of 0.01% (w/v) of tetrachloroauric acid is brought to boiling point under vigorous stirring, then 1.25 mL of sodium citrate at 1% (w/v) are added quickly maintaining the same conditions of stirring and the temperature for 15 more minutes until the colour of the solution turned to deep red. It was left to cool down keeping the stirring. The concentration of gold nanoparticles in the resulting product is 3nM. For the study, different concentrations of gold nanoparticles were used; 3 nM, 300 pM, 30 pM, 3 pM and 0 pM. For the characterization of gold nanoparticles at different sizes, the commercial gold nanoparticles of different diameters (2, 5, 10, 15, 20, 50 nm) were used at a concentration of 3 nM. The initial concentration of the gold nanoparticles in the commercial samples was given by the supplier.

2.4. Screen printed electrodes fabrication

Screen printed electrodes (SPCE) consists of counter electrode, reference electrode of Ag/AgCl and working Electrode. They are fabricated on sheets of polyester substrate by the sequential deposition of different ink layers, first the graphite ink layer on the polyester substrate for working and counter electrodes, next the Ag/AgCl ink for the reference electrode and finally the isolating ink. Each layer is printed using a screen printing machine

with a stencil specific for each layer, which contains the patterns of the electrodes. After its deposition each layer was cured by keeping the printed sheet at 120 ºC for 30 minutes.

2.5. Magneto immunosandwich formation

The magneto immunosandwich were prepared according to previously reported procedures (Ambrosi et al. 2007; de la Escosura-Muñiz et al. 2010; Maltez-da Costa et al. 2010) To prepare the immunosandwich assembly magnetic beads (Dynabeads M-280) were used as support (3 mg/ml per sample). The magnetic beads are washed in borate buffer pH 9.2, and incubated overnight (37 ºC and agitation of 400 rpm) with Anti- human IgG, at a final concentration of 40 µg/ml in the mixture. After the incubation the samples were washed with PBS tween 20 at 0,5 % (v/v). In order to perform a blocking step, the conjugated magnetic beads were resuspended in a solution of PBS and 5 % (w/v) BSA for 1 hour. After the blocking step the samples were washed with PBS. Then the blocked conjugate is incubated with the antigen (in this specific case human IgG) for 1 hour at 25ºC and 400 rpm.

For the gold nanoparticle conjugation to the anti-human IgG, first the pH of the gold nanoparticle is adjusted to 9.2 using borate buffer. Then the gold nanoparticles are incubated with the antibody at a final concentration of 5 µg/ml for 1 hour at 25 ºC and 400 rpm. After the incubation a solution of BSA in milliQ water was added to reach a final concentration of 0,15 mg/ml and incubated for 1 hour at 650 rpm and 25 ºC. Next, the sample was centrifuged at 14000 rpm for 20 min at 4 ºC. The pellet was reconstituted to the initial volume in PBS.

The last incubation consisted in mixing the magnetic bead assembly with the conjugated gold nanoparticles at a ratio of 1:1 (v/v). The incubation was done for 30 minutes long under 600rpm agitation and 25 ºC. After the incubation different washing steps with PBS tween 20 at 5% (v/v), PBS and milliQ water were carried out. The assembly was finally resuspended in milliQ water.

2.6. Electrochemical measurements

In the EIS-HER measurements a first oxidation step for 60 s at +1.35 V was applied followed by a signal composed of DC of -1 V plus AC of 0.05 V_{RMS}. The range of frequencies went from 0.1 to 10000 Hz. The resulting data was fitted in a regular Randless circuit to extract the value of charge transfer resistance (Rtc).

The magneto immunosandwich assay for HIgG detection using EIS-HER was done. A small magnet is placed under the working electrode of the SPCE to direct the magnetic beads towards it. A mixture of 25µl of the magnetoimmunosandwich and 25µl of a 2M HCl solution is placed onto the surface of the electrode.

3. RESULTS AND DISCUSSION

AuNPs, prepared by the Turkevich method (Turkevich et al. 1951) were characterized by Transmission Electron Microscopy (TEM). Figure 1 (inset) shows a typical TEM image of AuNPs. Highly regular and uniform nanoparticles in the size range of 21 ± 3 nm can be observed. In order to explore the HER of AuNPs by EIS reading, a fixed concentration of AuNPs solution (1500 pM) were measured by holding the working electrodes (where AuNPs were deposited) at a constant potential of +1.35 V for 1min (electro-oxidizing Au to Au^{+3}) followed by applying a composed signal of high negative DC potential (polarization potential) plus 0.05 V of AC potential in a range frequency from 0.1 Hz to10 KHz . Different DC potentials were evaluated; Figure 2 shows the Nyquist representation.

For interface modelling, equivalent circuit (corresponds to Randles model) which includes the solution resistance (Rs), double layer capacitance (Cdl) and the charge transfer resistance (Rtc) is used (see scheme in Figure 1). The performed fitting using Randles model is fairly good and the calculated parameters obtained at different potentials (Table 1 in supporting

information) show that the Rct values decrease when the polarization potential increases. These measurements show the impedance response dependency from the applied DC potential.

This fact is due to a large dependence of the HER kinetics on AuNPs' surfaces in HCl solution and at negative over potentials. The total two-electron process of hydrogen evolution proceeds at least in two steps: first, the proton donors discharge with formation of adsorbed H atoms, and, second, the desorption of these atoms. In this case the hydrogen adsorption by the AuNPs is not observed, for these reason the experimental data was fitted in a common Randless circuit and used to evaluate HER. This phenomenon has been reported for metal electrodes such as gold that poorly adsorbs hydrogen (Khanova and Krishtalik 2011).

From the SEM images shown in Figure 2 it is possible to notice the physical deformation of the circular shape of the AuNPs, which after hydrogen evolution becomes irregular, furthermore the level of irregularities in the shape increases with the increase of the negative potential applied on the AuNPs (Figure 2 B,C,D). This indicates that high negative potential applied changes the nanostructure of AuNPs. For later measurements -1 V was fixed as a polarization potential due to the stable impedance response and less observed deformation of the circular shape of the AuNPs compared to the case where higher potentials are used.

Different AC potentials (10, 20, 50 y 100 mV) also were tested. Figure S1 in suportting information shows the obtained Nyquist plot without a significant difference in terms of Rct. Nevertheless, at low potentials (10, 20 mV) the impedance response is not stable at low frequency. On the other hand being the 100 mV AC a relatively large potencial an AC of 50 mV was used.

Finally, the +1.35 V was applied to release Au^{+3} ions from the surface of the gold nanoparticles into the measuring solution. This pretreatment condition enhances the catalytic

effect on the HER and was previously optimized (Ambrosi et al. 2007; de la Escosura-Muñiz et al. 2010; Maltez-da Costa et al. 2010)

After optimizing the experimental procedure, a series of EIS measurements for different amounts of AuNP are registered under the optimal conditions. Figure 3A shows the impedance measurements in a Nyquist plot. The smallest semicircle corresponds to the 1500 pM AuNPs, while the biggest semicircle corresponds to the blank (HCl without AuNP). The resulting charge transfer resistances are directly represented in a histogram (Fig. 3B) and the inverse of these resistances are plotted vs. the logarithm of the concentration to adjust the calibration curve (inset Fig. 3B). The LOD of 0.67 pM is calculated by the value obtained by interpolation of the blank plus three times its standard deviation. The method shows high reproducibility being its RSD of around 13 %, obtained for 3 repetitive assays.

Once the impedimetric detection of AuNP catalysis was performed and its effectiveness as a transduction method demonstrated, it is afterwards applied in a standard immunoassay for protein detection. In figure 4A a schematic of the performed magneto inmunosandwich for human IgG (HIgG) detection is displayed. The capturing antibody, immobilized onto the magnetic bead, is an antihuman IgG, polyclonal, developed in goat. The detection antibody, conjugated with gold nanoparticles, is another antihuman IgG developed in rabbit. Figure 4B shows the obtained Rct values with the corresponding error bars achieved using different amounts of HIgG. The signal of AuNP is directly related to the amount of the formed immunoconjugates. A linear biosensing response within the range from 2 – 500 ng mL^{-1} IgG with 0.31 ng mL^{-1} as limit of detection (LOD) was obtained from the inverse of the obtained Rct.

Figure 5 shows TEM image of magnetic beads with (Figure 5A) and without (Figure 5B) gold nanoparticles anchored through the inmunosandwich (Magnetic bead -Anti- human IgG

/ humanIgG/ Anti- human IgG-Au NPs). The small black points corresponding to AuNPs covering the surface of the MBs (big spheres) can be observed in Figure 5A demonstrating the magneto inmunosandwich formation.

LOD was calculated by the interpolation of the blank plus three times the standard deviation of the blank. With this new AuNP based strategy using EIS the LOD is improved in comparison to the previously one obtained when the chronoamperometry was used for AuNPs detection (Maltez-da Costa et al. 2010). Moreover, high reproducibility with a RSD of around 13% was observed for HIgG detection. The selectivity of the assay is demonstrated performing two control assays using a non-specific protein (BSA instead of HIgG) as 'control 1' and another one without AuNPs as 'control 2' (see Figure 4C).

On the other hand, the Au NP size effect in the electrochemical signal amplification was evaluated before (Bonanni et al. 2011). For this reason, this new method represents a quite fast and cost efficient method to determine the size of AuNPs. Figure 6 shows the results of EIS-HER characterization of AuNPs (from BritishBiocell) of different sizes (2, 5, 10, 15, 20, 50 nm). In Figure 6A the EIS-HER data is presented in a Nyquist plot, showing the semicircles resulting from the measurement of the nanoparticles of different diameters. The biggest semicircles correspond to the smallest gold nanoparticles (2 nm diameter) with a clear tendency of EIS decrease when AuNPs size is increased. Although EIS is applied before for AuNP size evaluation (Bonanni et al. 2011) this EIS-HER method clearly shows an improvement of the sensitivity and a better discrimination of AuNPs in the size range of 2-50 nm. The histograms for different AuNPs sizes as a function of Rct were represented in Figure 6B and the corresponding TEM images as insets are also shown. High reproducibility of the EIS-HER measurements with a RSD of 12 % is observed.

4. CONCLUSION

In summary we have demonstrated that the Electrochemical Impedance Spectroscopy (EIS) combined with hydrogen evolution reaction (HER) constitutes a high reproducible and efficient method for the detection of different amounts of AuNPs in acidic medium. This EIS-HER method combines the advantages of EIS with HER induced by AuNPs. Furthermore, the proposed method displays dramatic improvements of the analytical performance in terms of a wide linear range (2 – 500 ng mL^{-1}) of response along with a good LOD (0.31 ng mL^{-1}) and sensitivity for a model protein (Human IgG) detection in a magnetoimmunoassay and the gold nanoparticle size characterization. In addition, it is also important to emphasize that with this new strategy for the magneto immunosandwich a reduction of one order of magnitude in the LOD is obtained, compared with chroamperometric technique normally used for this detection reported before. On the other hand, the use of this EIS-HER as a sensitive technique capable of differentiating the signal generated by the presence of AuNPs of different sizes can be extended to other nanoparticles and related catalytic reactions. We envisage the use of such sensing technology to be with interest for various sensing and biosensing applications where nanoparticles or other nanomaterials may be involved.

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FIGURE CAPTIONS

Figure 1. Schematic of EIS sensing principle through hydrogen evolution reaction induced by gold nanoparticles. Inset: Transmission Electron Microscopy (TEM) images of AuNPs including a magnified image.

Figure 2. Scanning electron micrograph (SEM) and Nyquist plot for AuNPs after potentiostatic cathodic polarization: (A) -0.6 V, (B) -0.8 V, (C) -1 V and (D) -1.2 V. The scale bare of all the SEM images is 250 nm and continuous line correspond to fit data.

F**igure 3**. (A) Argand diagram recorded for increasing concentrations of AuNPs in 1 M HCl ranging from 1.5 to 1500pM (top to bottom); (B) Histogram representing Rct as a function of different AuNPs concentration. Inset: calibration curve obtained by plotting the Rct inverse value with logarithm of AuNPs concentration.

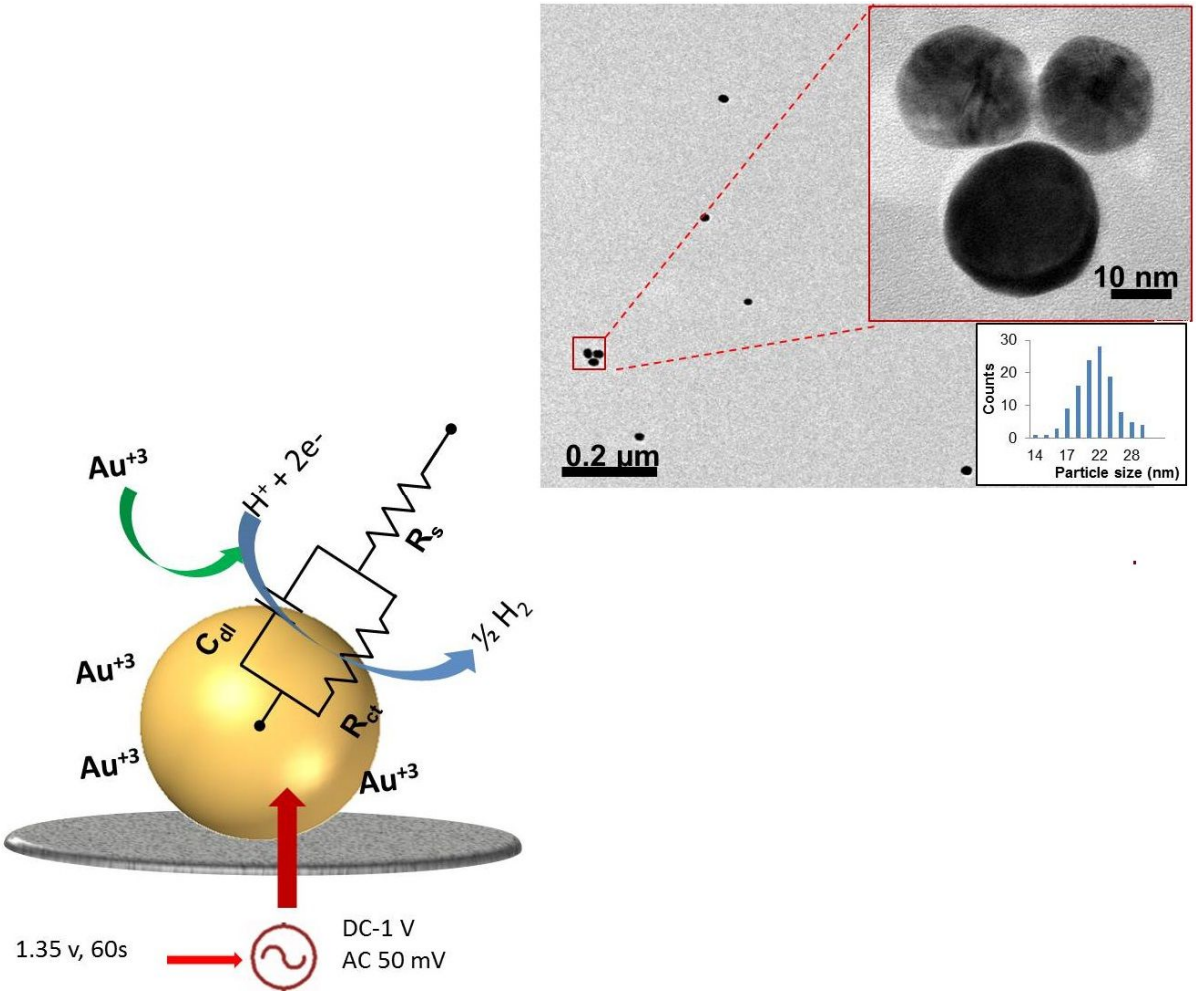
Figure 4. (A) Schematic of the magneto sandwich immunoassay for HIgG detection using AuNPs as label. (B) Rct values vs different concentrations of HIgG. Inset: Calibration curve obtained by plotting 1/Rct vs. the logarithm of different concentrations of HIgG.(C)Selectivity of the immunosandwich assay: Nyquist plots obtained for the control 1 (BSA), no antibody as a control two and 500ng/ml as a positive.

Figure 5. TEM images of magnetic beads modified with (A) and without (B) AuNP anchored through the inmunosandwich (Magnetic bead -Anti- human IgG / humanIgG/ Anti- human IgG-Au NPs).

Figure 6. Impedimetric measurement of AuNPs of various diameters and in the same concentration. Nyquist plot (A) and histograms of Rct value vs. AuNPs diameter (B). The scale bare of all the TEM images is 100 nm.

Figures

Figure 1:



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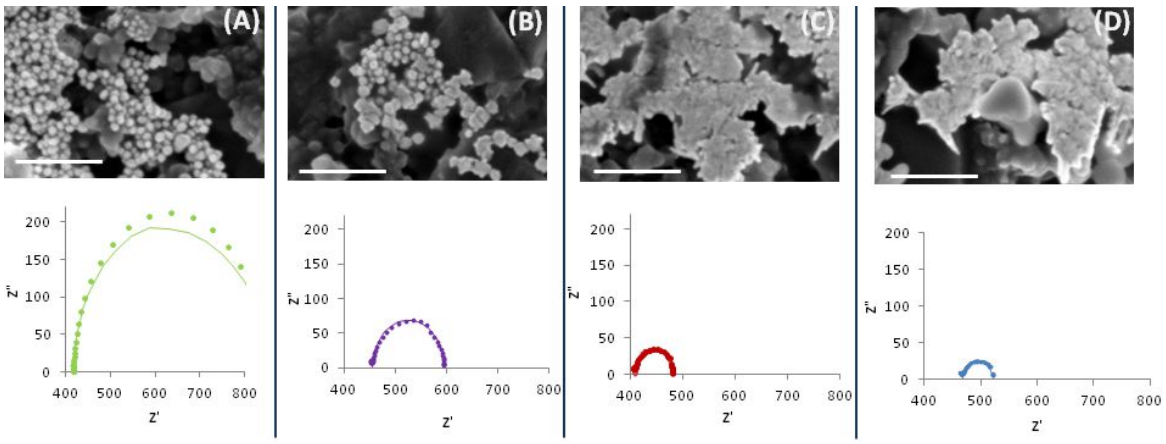

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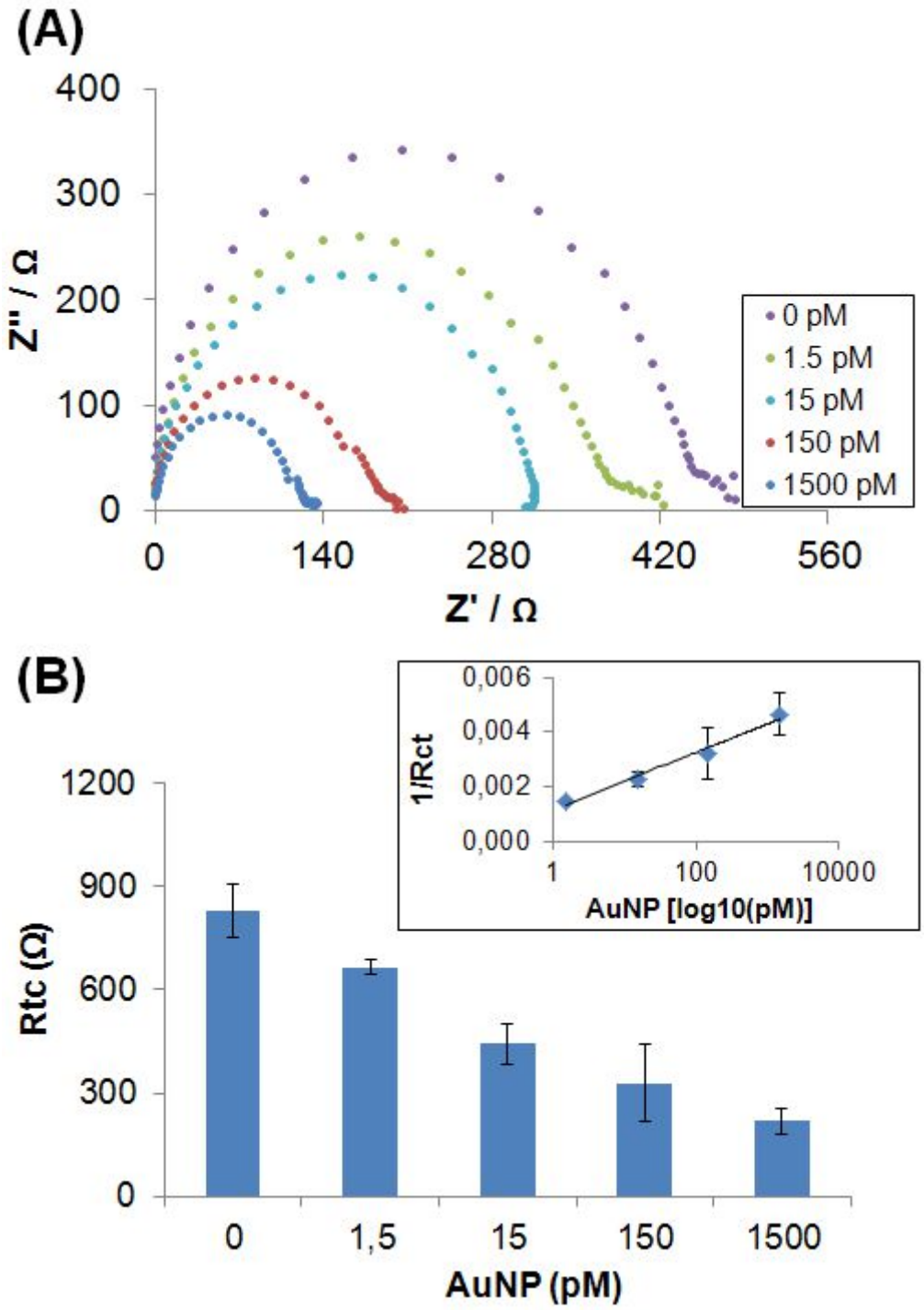
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15 pM
150 pM
1500 pM
(B)
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300
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Rtc (Ω)
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1,5
15
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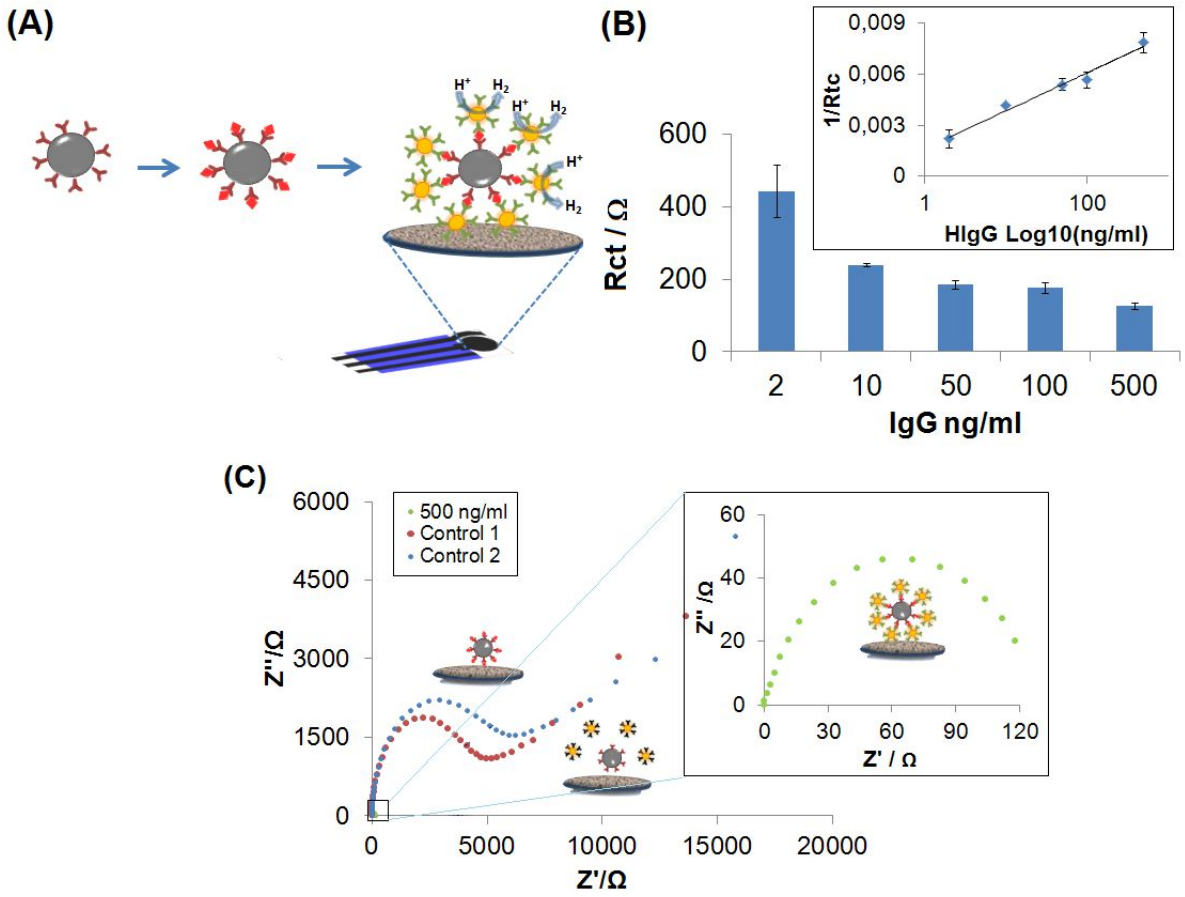
AuNP [log10(pM)]

Figure 4:



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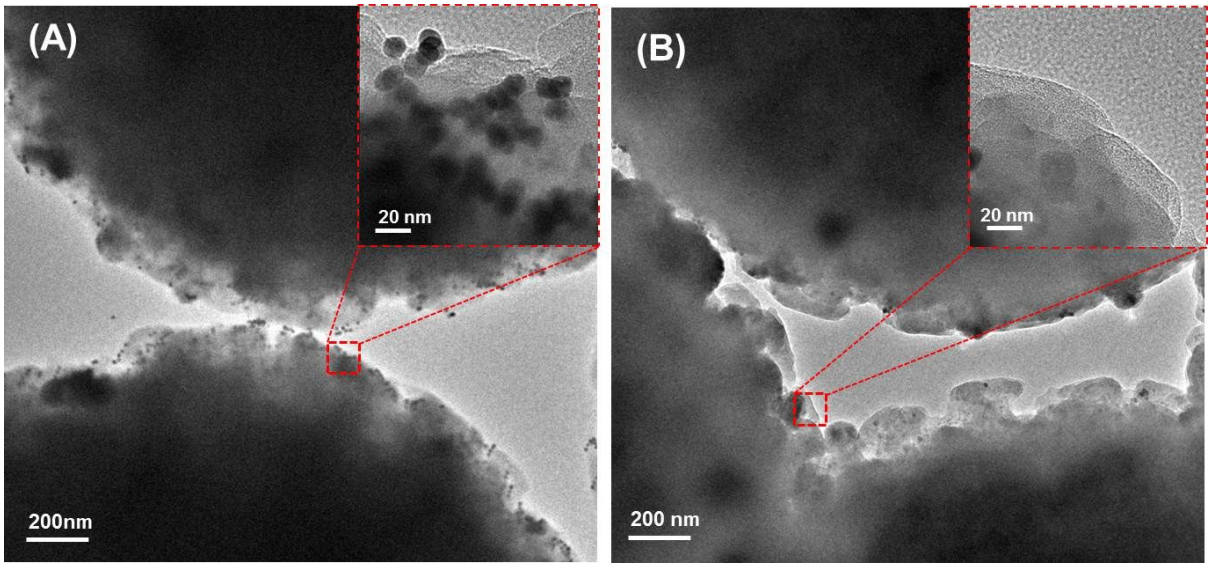
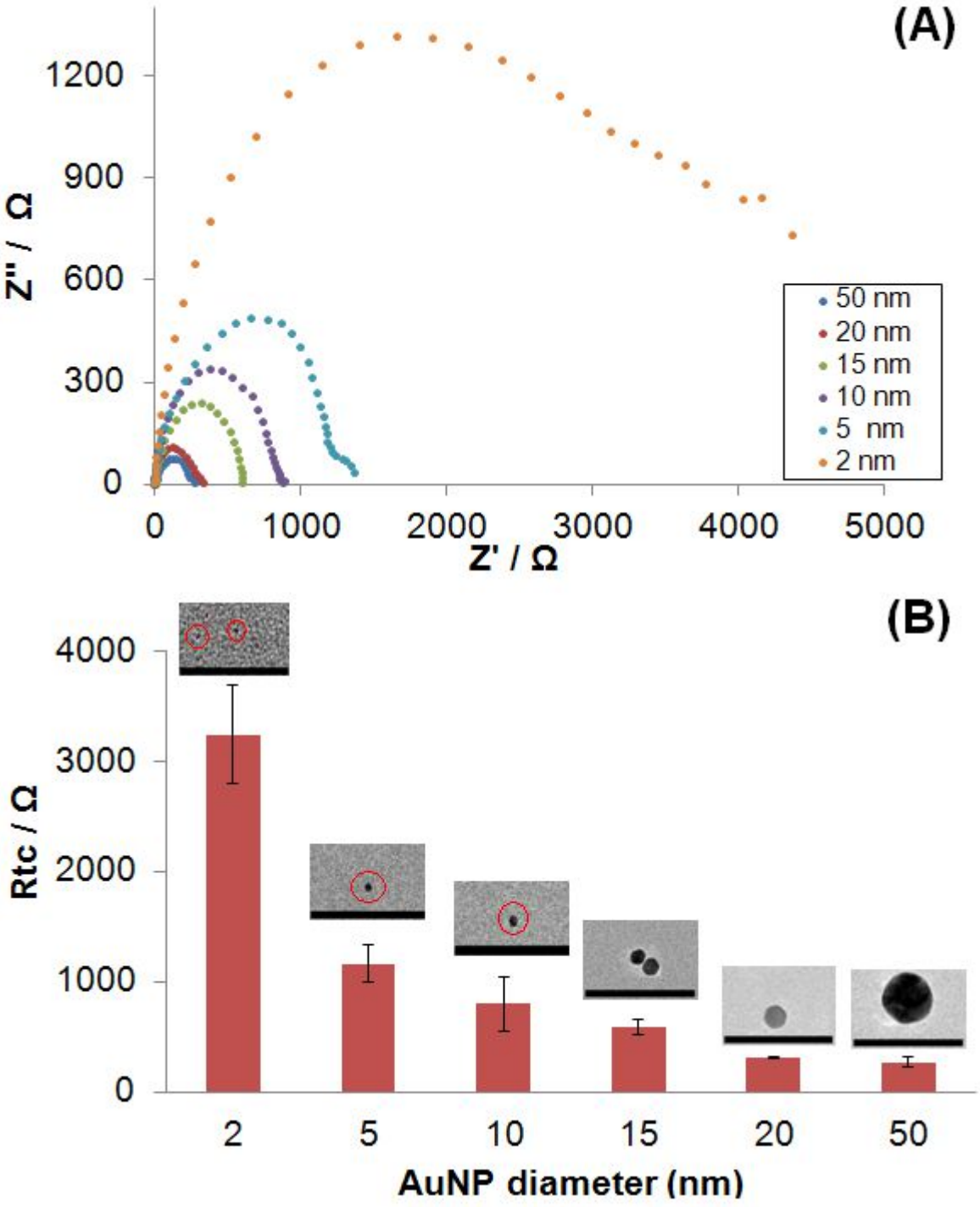


Figure 6: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AuNP diameter (nm)