An electrochemical approach for biomolecules detection involving electrocatalytic gold nanoparticles

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Abstract: In the last years the gold nanoparticles (AuNPs) have been used in various applications, due to their ability to provide a stable immobilization of biomolecules retaining their bioactivity, which is a major advantage for the preparation of biosensors. They are characterized by a good biological compatibility and excellent conductivity, too. Various synthesis procedures have been proposed in the recent years to better control their size, morphology and surface chemistry as the main parameters that may further influence the detection process.

The present work presents some preliminary experimental results regarding the use of electrocatalytic AuNPs prepared by a microemulsion assisted photoreduction procedure (MAPR) for bioanalytical detection involving chronoamperometry, a relatively easy electrochemical technique. Screen printed carbon electrodes (SPCE) were used as electrotransducers. The capture protein has been first immobilized on the carbon working electrode through passive adsorption to bind with corresponding antigen and AuNPs labelled antibody to build a sandwich assay. The electrochemical detection is based on the catalytic ability of AuNPs towards hydrogen evolution reaction in acidic media (1M HCl solution). A very good linearity of the cathodic current at a certain applied cathodic potential (of -1V vs. Ag ref.) against gold nanoparticles concentration has been determined. This methodology was applied to detect AuNPs as labels in an immunosandwich assay to the determination of a normal model antigen, human IgG. A linear relationship between the cathodic current at -1V/Ag ref. and human IgG concentration was obtained, respectively:

$$I(\mu A) = 28.22 + 0.161 \times [HIgG]_{ng/mL} \quad (1)$$

in the range of 0-500 ng/mL, with a correlation coefficient of 0.991.

1. Introduction

In the last years the gold nanoparticles (AuNPs) have been used in various applications, due to their ability to provide a stable immobilization of biomolecules retaining their bioactivity, which is a major advantage for the preparation of biosensors. Moreover, they are characterized by a good biological compatibility and excellent conductivity. Various synthesis procedures are usually proposed [1-4] to better control their size, morphology and surface chemistry as the main parameters that may further influence the detection process.

Recently it has been shown that the AuNPs prepared by a microemulsion assisted photoreduction procedure (MAPR) [5] showed a good electrocatalytic response towards hydrogen evolution reaction in acidic medium. A very good linearity of the cathodic current at

a certain applied cathodic potential (of -1V vs. Ag ref.) against gold nanoparticles concentration has been determined for thiol functionalized AuNPs using sodium 3-mercaptopropane sulfonate (symbolized as AuNP-MS) [6]. The obtained results were a good premise to develop further an easy-to-use biosensor for the identification and quantification of targeted metallic NPs toward specific cells (e.g. blood circulating inflammatory cells or tumor metastatic cells).

In the present work some preliminary experimental results are presented, regarding the use of electrocatalytic AuNPs-MS prepared by MAPR for bioanalytical detection involving chronoamperometry technique. The proposed methodology was applied for the determination of a normal model antigen, human IgG. Screen printed carbon electrodes (SPCE) were used as electrotransducers. The capture protein has been first immobilized on the carbon working electrode through passive adsorption to bind with corresponding antigen and AuNPs labelled antibody to build a sandwich assay. The electrochemical detection was based on the catalytic ability of AuNPs towards hydrogen evolution reaction in acidic media (1M HCl solution).

2. Experimental

2.1 Chemicals and instrumentation

All chemical reagents: goat anti-human IgG antibody (1 mg/mL), human IgG (0.1 mg/mL), bovine serum albumin (BSA) (1 mg/mL) and sodium 3-mercapto-1-propanesulfonate (MS) were purchased from Sigma-Aldrich. Chloroauric acid ($HAuCl_4$) was obtained from Fluka. 0.05M Sodium phosphate-buffered saline (PBS, pH 7.4) has been used as incubating and washing buffer. All other reagents were of analytical purity, and doubly distilled water was used throughout all the experiments. Carbon screen printed electrodes (SPCE) with carbon working electrode (4 mm diameter), carbon counter electrode and a silver pseudo-reference (purchased from Dropsens-Spain) have been used. The electrochemical experiments have been carried out involving a PGSTAT 12 (Metrohm Autolab) electrochemical system. Chronoamperograms were obtained in 1M HCl after holding the working electrode at a potential of +1.3V for 60 s and then applying a cathodic potential of -1.0V for 300 s. The generated cathodic current against time was recorded.

2.2 Thiol functionalized AuNPs synthesis

Thiol functionalized AuNPs have been prepared through MAPR procedure [5]. Briefly, 0.02M sodium 3-mercaptopropane sulfonate (MS) in $HAuCl_4$ solutions (1g/L Au in 1M HCl) have been prepared at 25^{o}C, stirred for 30 min. and then mixed with n-heptane (82.5 % (w/w)) and Brij30 (15 %(w/w)), to form the microemulsion. UV irradiation was carried out for 1h using a photo reactor containing a medium-pressure UV lamp at 254 nm. The resulted NPs were 5 times alternatively washed with acetone and ethanol in order to remove the oil, surfactant and excess of thiol compounds. The resulting AuNP-MS nanoparticles were characterized by transmission electron microscopy (TEM) that indicated the particle size of colloidal gold was about 13 nm.

2.3 Preparation of the antibody-AuNP-MS conjugate

The antibody-AuNP-MS conjugate was prepared by adding 200 μL of goat anti-human IgG antibody (1 mg/mL) to 1 mL of AuNP-MS solution (0.1 g/L), followed by incubation at room temperature for 2 h. Subsequently, a blocking step with 750 μL of 1 mg/mL BSA incubating at 25^{o}C for 20 min. was undertaken, to minimize the non-specific adsorption and then kept overnight in refrigerator at 4^{o}C. Finally, centrifugation was carried out at 15000 rpm for 20 min. and the antibody-AuNP-MS conjugate was reconstituted in PBS solution.

2.4 Immunoassay procedure

A sandwich immunoassay procedure has been adopted to determine the human IgG, as shown in Figure 1.

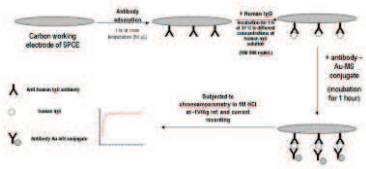


Figure 1. The immunoassay procedure

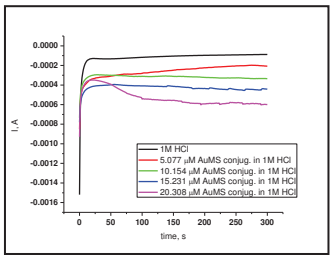
The carbon working electrode was firstly modified through antibody adsorption, by coating the electrode surface with 50 µL of goat anti-human IgG antibody solution (pH 7.4). After 1h the antibody-modified electrode was washed with PBS. Then 50 µL of BSA 1% has been applied to block the residue-free site, for 1 h at room temperature. The immunosensor has been then incubated for 1h at 37°C in various concentrations of human IgG solution (100-900 ng/mL), followed by the addition of antibody-AuNP-MS conjugate solution for another 1h. Finally the modified SPCE was rinsed with PBS solution to remove any unbound tracer and subjected to chronoamperometry in 1M HCl at -1V/Ag ref. and current recording.

3. Results and disscusion

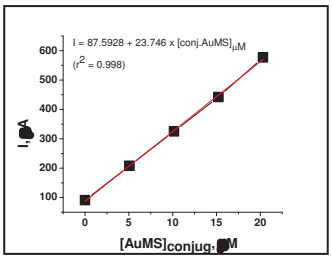
3.1 Evaluation of electrocatalytic effect of conjugated AuNP-MS NPs

The behavior of AuNP-MS NPs modified with antibodies has been evaluated involving cyclic voltammetry (not shown here) and chronoamperometry in 1M HCl.

As illustrated in Figures 2(a) and 2(b), a very good linearity between the absolute value of cathodic current (at 250 s) recorded at -1V/Ag ref. and the concentration of conjugated AuNP-MS nanoparticles (concentration range: 0-20µM).



(a)



(b)

Figure 2. (a) Chronoamperograms recorded at E = -1.0V/ Ag/AgCl for solutions having various conjugated AuNP-MS concentrations onto SPCE (after a pretreatment consisting in a 30 s polarization at +1.35V /Ag ref.); (b) Cathodic current at E = -1.0 V /Ag/AgCl vs. conjugated AuNP-MS concentration

3.2 Optimization of the amount of conjugating antibody on AuNP-MS

The interaction between the goat anti-human IgG antibody and AuNP-MS nanoparticles represents the key step of this electrochemical detection and the amount of coating antibody on AuNP-MS should be optimized.

Thus, chronoamperograms in 1M HCl for conjugated AuNP-MS with various antibody concentrations solutions on SPCE (after a pretreatment consisting in a 30 s polarization at +1.35V /Ag ref.) have been recorded and the dependence of the cathodic current at E = -1.0 V /Ag ref. (at t=200 s) against the anti-human IgG antibody concentration was constructed to determine the amount of antibody necessary to

coat the exterior of the nanoparticles, as illustrated in Figure 3. As shown in the figure, the cathodic current increases with the increase of antibody concentration. When the concentration is over 5 μg/mL, the current becomes quasi-stable. Therefore, 8μg/mL of anti-human IgG antibody has been selected for AuNP-MS conjugation.

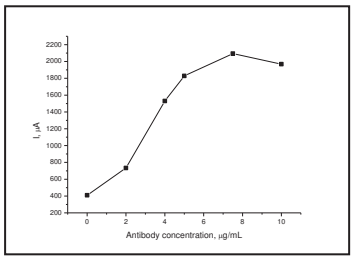


Figure 3. The dependence of the cathodic current at E = -1.0 V /Ag ref. (at t=200 s) in 1M HCl vs. anti-human IgG antibody concentration (AuNP-MS concentration: 5 μg/mL)

The conjugation of the AuNP-MS nanoparticles was confirmed using TEM images, as exemplified in Figure 4.

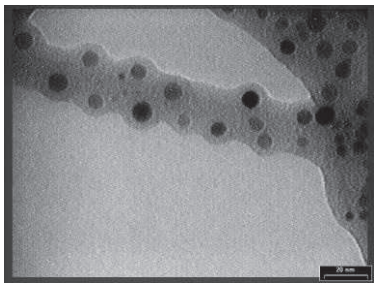


Figure 4. TEM images of the conjugated AuNP-MS nanoparticles

The conjugated AuNP-MS nanoparticles showed sizes of about 25-26 nm, doubled as compared to simple ones.

3.3 Detection of human IgG

After the immunosensor has been incubated with 50 μL of different concentrations of human IgG and then with antibody-AuMS-NP conjugate chronoamperograms were recorded, as presented in Figure 5.

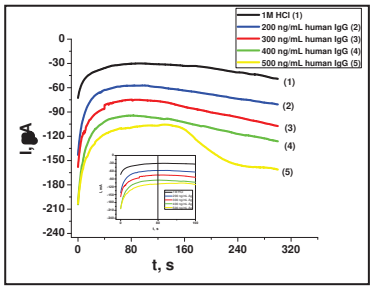


Figure 5. Chronoamperograms in 1M HCl for various antigen concentrations solutions (after a pretreatment consisting in a 60 s polarization at +1.3V /Ag ref.)

The values of cathodic current for t=60s against human IgG concentration followed a linear relationship in the range 0-500 ng/mL. The linear regression equation was:

$$I(\mu A) = 28.22 + 0.161 \times [HIgG] \quad (1)$$

with a correlation coefficient of 0.991, as shown in Figure 6.

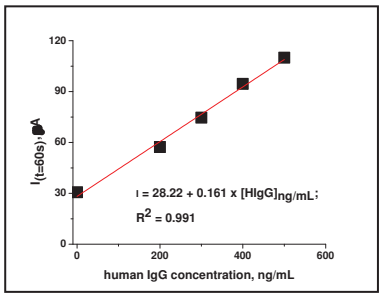


Figure 6. The dependence of cathodic current (t = 60s) at E = -1.0 V /Ag ref. vs. the concentration of human IgG

The detection limit was 142 ng/mL calculated by the 3σ-rule, relatively higher than expected. This behavior might be associated with the electrochemical pretreatment procedure. Further investigations will be performed to optimize the electrochemical response in order to improve the detection limit.

4. Conclusions

The use of AuNP-MS nanoparticles in bioanalytical detection associated with a relatively easy electrochemical technique (chronoamperometry) may represent a suitable strategy for a lower cost and faster methodology. Based on the obtained results, the sandwich procedure seemed to offer promising results based on the obtained correlation coefficient values. The established methodology also allowed the operation using microvolumes with an adequate accuracy. Further investigations will be performed to improve the detection limit, mainly by optimizing the working electrode pretreatment step.

Acknowledgement

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