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ELECTROCATALYTICAL MAGNETOBIOSENSING BASED ON GOLD NANOPARTICLES

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ABSTRACT

In this work, electrocatalytic methods are developed in order to improve the sensitivity of model biosensors based on a labelling method using AuNPs. Microparamagnetic beads (MB) are used as primary biological molecules immobilisation platforms and AuNPs modified with secondary biological molecules as high sensitive electrochemical labels. The carbon electrodes, used as transducer, incorporate a magnet that allows the collection / immobilization on its surface of the biological sandwich attached to the MB. The final detection is based on the catalytic effect of AuNPs on the electroreduction of different species, like silver ions. The low levels of AuNPs detected with these methods allow the obtaining of biosensors with low protein and ss-DNA target detection limits, with special interest for further applications in clinical analysis, food quality and safety as well as other industrial applications.

1. INTRODUCTION

Catalysis is considered as the central field of nanoscience and nanotechnology. Interest in catalysis induced by metal nanoparticles (NPs) is increasing dramatically in the last years. The use of NPs in catalysis appeared in the 19th century with photography (use of gold NPs - AgNPs) and the decomposition of hydrogen peroxide (use of PtNPs) [1]. Usually, these NP catalysts are prepared from a metal salt, a reducing agent and a stabilizer. NPs have been widely used for their catalytic properties in organic synthesis, for example, in hydrogenation and C-C coupling reactions [2], and the heterogeneous oxidation of CO [3] on AuNPs.

On the other hand, immunoassays are currently the predominant analytical technique for the quantitative determination of a broad variety of analytes in clinical,

medical, biotechnological, and environmental significance. In most of the existing immunoassays, the immunological interaction is visualized via an auxiliary reaction, using a label. Recently, the use of metal and semiconductor nanoparticles as labels for different biorecognition and biosensing processes has received wide attention, due to the unique electronic, optical, and catalytic properties of these nanoparticles [4,5]. AuNPs stand out from the variety of nanoparticles used in electrochemical bioassays because of their biocompatibility [6], rapid and simple chemical synthesis, narrow size distribution and efficient coating by thiols or other bioligands.

A substantial sensitivity enhancement can be achieved, for example, by using the AuNPs as catalytic labels for further amplification steps. The most common strategy uses the catalytic deposition of gold [7] and especially of silver onto AuNPs to improve the sensitivity, and other catalytic strategies have been studied.

In most of the reported works, the silver enhancement relies on the chemical reduction of silver ions [8-11] to silver metal onto the surface of the AuNPs followed by anodic-stripping electrochemical measurement. However, the sensitivity of this procedure is compromised by nonspecific silver depositions onto the transducing surface.

In this work, an alternative method is developed, based in the silver electrodeposition. This process shows a very interesting advantage over the chemical deposition protocol, since silver only deposits on the AuNPs. This fact results in a high signal-to-background ratio by reducing the nonspecific silver depositions of the chemical procedure.

An electrocatalytic silver-enhanced metalloimmunoassay using AuNPs as labels and microparamagnetic beads (MB) as platforms for the

immunological interaction is performed. After the immunochemical reactions, the modified magnetic beads are easily captured on the electrode surface by using a graphite-epoxy composite electrode with a small magnet incorporated.

2. SYNTHESIS OF AuNPs AND CONJUGATION WITH PROTEINS

AuNPs were synthesized by reducing tetrachloroauric acid with trisodium citrate, a method pioneered by Turkevich et al. [12]. Briefly, 200 mL of 0.01% HAuCl₄ solution were boiled with vigorous stirring. 5 mL of a 1% trisodium citrate solution were added quickly to the boiling solution. When the solution turned deep red, indicating the formation of gold nanoparticles, the solution was left stirring and cooling down. Transmission electron micrographs (TEM) of the synthesized gold nanoparticles are shown in figure 1.

The AuNPs loading with the antibody was performed according to the following procedure: AuNP suspension was mixed with human-IgG and incubated. After that, a blocking step with BSA was performed. Finally, a centrifugation at 14000 rpm for 20 min was carried out, and then the AuNPs/ α -human-IgG was reconstituted in H₂O milli-Q.

Before their conjugation with proteins, a gold aggregation test was performed to detect salt-induced colloidal gold aggregation and find by this way the antibody concentration to be used for conjugation with gold nanoparticles. The antibody concentration which prevents gold aggregation was determined by measuring the difference between the absorbance at 520 and at 580 nm and plotting it against the concentration used. The minimum antibody concentration giving the highest absorbance difference resulted to be 7 μ g for 1 mL of gold nanoparticles and that corresponded to a number of protein molecules of 10 for each gold nanoparticle. This result was verified by theoretical calculations. We used the covering ratio calculations so as to define the configuration called a spherical code (or spherical packing). Anti-human-HRP was approximated to a sphere of a radius of 5.6 nm and using the geometrical model of sphere packing around a single central sphere, resulted that 13 spheres of radius 5.6 nm can be arranged around a single central sphere of radius 6.5 nm (gold nanoparticle). The good correspondence between theoretical and experimental results confirms that gold aggregation test is a simple and valid method to control protein conjugation to gold nanoparticles. A scheme of this conjugation process is shown in figure 2A.

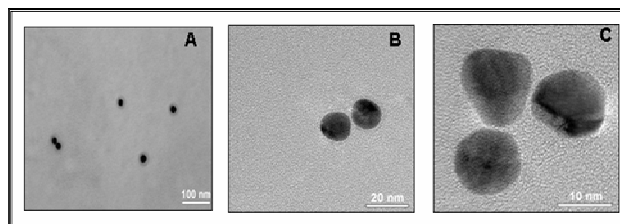


Figure 1. Transmission electron micrographs (TEM) of the self-synthesized gold nanoparticles.

3. SANDWICH TYPE MAGNETOASSAY

Briefly, MBs were incubated with biotinylated α -human IgG with gentle mixing in a TS-100 ThermoShaker. The preparation process was followed by resuspending the MB/ α -human IgG in the blocking buffer (PBS-BSA) to block any remaining active surface of MBs. After washing steps, the MB/ α -human IgG were incubated with human IgG antigen at different concentrations, forming by this way the immunocomplex MB/ α -human IgG/Human IgG. Finally, after the washing steps, the MB/ α -human IgG/Human IgG immunocomplex was incubated with the previously synthesized AuNPs/ α -human-IgG complex. A scheme of this procedure is showed in figure 2B.

A similar procedure can be followed in the case of DNA hybridization sensors [13-14] (not shown).

Clear evidences of the successful immunological adsorptions in a condition of the absence of unspecific adsorptions are the transmission electron micrographs (TEM) images showed in figure 3.

When the assay is carried out without the presence of the antigen (Figure 3A) only magnetic beads are observed with a very low amount of AuNPs non specifically bonded. However, if the assay is performed with antigen (Figure 3B), a high quantity of AuNPs is observed around the magnetic beads, which indicates that the immunological reaction has taken place.

4. ELECTROCATALYTICAL DETECTION

Once the magnetoassay has been performed, the AuNPs can be detected through their catalytic effect on the reduction of different species, like silver ions or protons.

The silver enhancement method, based on the catalytic effect of AuNPs on the chemical reduction of silver ions, has been widely used to improve the detection

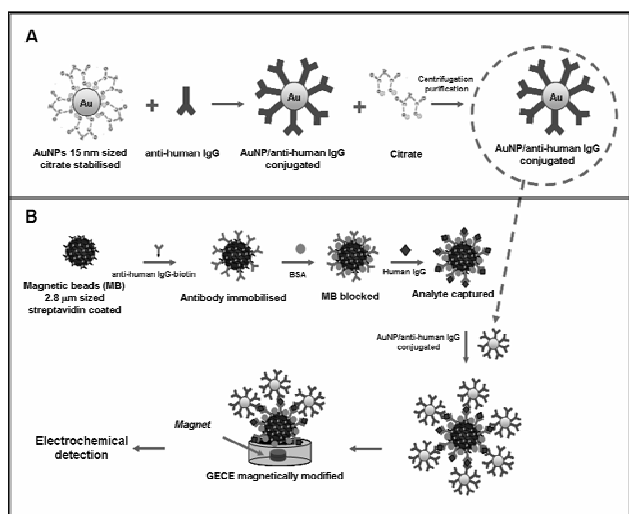


Figure 2. Scheme of the gold nanoparticles conjugation process (A) and the sandwich type magnetoassay protocol (B).

limits of several metalloimmunoassays. In these assays the

silver ions are chemically reduced onto the electrode surface in the presence of AuNPs connected to the studied bioconjugates without the possibility to discriminate between AuNP or electrode surface.

However, an alternative is to achieve the selective electro-catalytic reduction of silver ions on AuNPs and its advantages can be used to design novel sensing devices.

Other catalytic properties of AuNPs are currently studied.

4. CONCLUSIONS

In this work, electrocatalytic methods are studied in order to improve the sensitivity of previously developed magnetobioassays.

In the sensing device, AuNPs as label are used to detect human IgG as a model protein and the excess/non-linked reagents of the immunological reactions are separated using a permanent magnet, allowing the electrochemical signal coming from AuNP to be measured, and thus the presence or absence of protein be determined. The magnetic separation step significantly reduces background signal and gives the system distinct advantages for alternative detection modes of antigens. Finally, the sensible electrochemical detection of the AuNPs is achieved, based on their catalytic effect on the electro-reduction of silver ions.

The developed system establishes a general detection methodology that can be applied to a variety of immunosystems and DNA detection systems, including lab-on-a-chip technology.

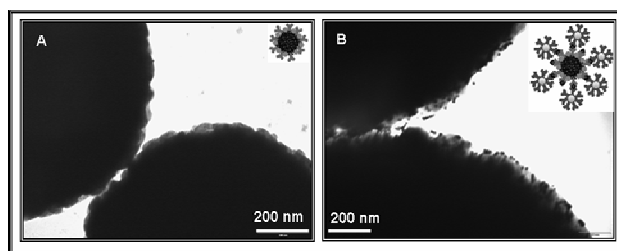


Figure 3. Transmission electron micrographs (TEM) of the micromagnetic beads after performing the sandwich assay without antigen (A) and with the human IgG (B).

5. REFERENCES

- [1] Bradley, J.S., *Clusters and Colloids*, **1994**, chapter 6, pp 459.
- [2] Reetz, M.T., Schulenburg, H., López, M., Spliethoff, B., Tesche, B. "Pt/C catalysts for the oxygen reduction reaction: correlation of carbon support morphology and catalytic activity". *Chimia* **2004**, 58, 896-899.
- [3] Lang, H-F., Maldonado, S., Stevenson, K.J., Chandler, B.D. "Synthesis and characterization of dendrimer templated supported bimetallic Pt-Au nanoparticles". *J. Am. Chem. Soc.* **2004**, 12949-12956.
- [4] Fritzsche, W.; Taton, T.A. „Metal nanoparticles as labels for heterogeneous, chip-based DNA detection". *Nanotech.* **2003**, 14 (12), R63-R73.
- [5] Seydack, M. "Nanoparticle labels in immunosensing using optical detection methods". *Biosens. and Bioelectron.* **2005**, 2454-2469.
- [6] Daniel M.C.; Astruc, D. "Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology". *Chem. Rev.* **2004**, 104, 293-346.
- [7] Liao, K.T.; Huang, H.J. "Femtomolar immunoassay based on coupling gold nanoparticle enlargement with square wave stripping voltammetry". *Anal. Chim. Acta* **2005**, 538, 159-164.
- [8] Karin Y.; Torres, C.; Dai, Z.; Rubinova, N.; Xiang, Y.; Pretsch, E.; Wang, J.; Bakker, Y. "Potentiometric biosensing of proteins with ultrasensitive ion-selective microelectrodes and nanoparticle labels". *J. Am. Chem. Soc.* **2006**, 128, 13676-13677.
- [9] Guo, H.; He, N.; Ge, S.; Yang, D.; Zhang, J. "MCM-41 mesoporous material modified carbon paste electrode for the determination of cardiac troponin I by anodic stripping voltammetry". *Talanta* **2005**, 68, 61-66.
- [10] Chua, X.; Fu, Xin; Chen, K.; Shen, G.L.; Yu, R.Q. "An electrochemical stripping metalloimmunoassay based on silver-

enhanced gold nanoparticle label". *Biosens. Bioelectron.* **2005**, 20, 1805–1812.

[11] Chua, X.; Xiang, Z.F.; Fua, X.; Wang, S.P.; Shen, T.G.; Yu, R.Q. "Silver-enhanced colloidal gold metalloimmunoassay for *Schistosoma japonicum* antibody detection". *J. Immunol. Meth.* **2005**, 301, 77–88.

[12] Turkevich, J.; Stevenson, P.; Hillier, J. "A study of the nucleation and growth processes in the synthesis of colloidal gold". *Discuss. Faraday Soc.* **1951**, 11, 55-75.

[13] Pumera, M.; Castañeda, M.T.; Pividori, M.I.; Eritja, R.; Merkoçi, A.; Alegret, S. "Magnetically triggered direct electrochemical detection of DNA hybridization using Au₆₇ Quantum Dot as electrical tracer". *Langmuir* **2005**, 21, 9625-9629.

[14] Castañeda, M.T.; Merkoçi, A.; Pumera, M.; Alegret, S.; "Electrochemical genosensors for biomedical applications based on gold nanoparticles". *Biosensors and Bioelectronics* **2007**, 22, 1961-1967.

[15] De la Escosura Muñiz, A.; González García, M.B.; Costa García, A. "Catalytic effect on silver electrodeposition of gold deposited on carbon electrodes". *Electroanal.* **2004**, 16, 1561-1568.

[16] Ambrosi, A.; Castañeda, M.T.; Killard, A.J.; Smith, M.R., Alegret, S.; Merkoçi A. "Double-codified gold nanolabels for enhanced immunoanalysis". *Anal. Chem.* **2007**, 79, 5232-5240.