Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section A: Food Biotechnology

CODEN (USA): JCBPAT Research Article

Development of a Nano-immunosensor Composed of Proteins-Coated Gold Nanoparticles for Biosensing Applications

Leslie Susana Arcila-Lozano[1], Marlon Rojas-Lopez[1]*.

[1] Centro de Investigación en Biotecnología Aplicada del Instituto Politécnico Nacional. Km. 1.5 Carretera Tecuexcomac-Tepetitla. CP 90700 Tlaxcala. México.

Abstract: A colloidal immunosensor based on gold nanoparticles was developed. It was obtained by using gold nanoparticles conjugated with streptavidin and a biotinylated antibody in their surface. Gold nanoparticles (of nanometer size ~ 20 nm) were synthesized through the chemical reduction of chloroauric acid with sodium citrate. In order to determinate the critical protein concentration for covering the surface of the gold nanoparticles a titration experiment were carried out. The arrangement (gold nanoparticle-streptavidin-immunoglobulin) was characterized by UV/VIS spectroscopy and electron microscopy.

Key words: Gold nanoparticles, streptavidin, immunoglobulin, nano-immunosensor, biosensing.

INTRODUCTION

Gold nanoparticles (AuNPs) expose considerable applications in optics, catalysis, materials science and nanotechnology also including biology, nanomedicine and food microbiology[1]. A variety of biological molecules can be labeled with gold nanoparticles. Conjugation of inorganic nanoparticles to biomolecules generates hybrid materials that can be used to let the nanoparticles interact specifically with biological systems[2]. Nanoparticle-biomolecule conjugates bring together the unique properties and functionality of both materials, for example fluorescence or magnetic moment of the inorganic particles and the ability of biomolecules for highly specific binding by molecular recognition[3]. Affinity-based systems found in nature have attracted increasing attention during past years. Maybe the most well-known example is the streptavidin/biotin system. The strong bond and specificity of the streptavidin/biotin system has allowed researchers to employ it for a large number of applications in bio-nanotechnology, and a large variety of

biotinylation reagents and biomolecules like DNA oligomers, peptides, fluorescent dyes and antibodies readily modified with biotin or streptavidin are available[4].

The objective of this work was the development of a nano-immunosensor composed of streptavidin-coated nanoparticles united to specific biotinylated antibodies for biosensing applications.

METHODS

AuNps were synthesized through the chemical reduction of chloroauric acid ($HAuCl_4$) with sodium citrate ($C_6H_5Na_3O_7$)[5]. UV–VIS spectra were carried out using an Evolution 606 Spectrophotometer (Thermo Scientific™). It was used to measure the surface plasmon resonance of both single gold nanoparticles and conjugate as well the surface plasmon resonance of the arrangement (conjugated- immunoglobulin)[6].

RESULTS

An aqueous solution of chloroauric acid ($HAuCl_4$), which functioned as a precursor, and a solution of sodium citrate ($C_6H_5Na_3O_7$) which acted as a reducing agent were used in this synthesis method. These solutions were mixed, to start no coloration was observed after a few minutes the solution turned purple and then the ruby red color characteristic of gold nanoparticles of nanometer size (~20 nm). There is a range in which no coloration occurs during the reaction, this is due to the formation of precursor called dicarboxylic acid ketone such as oxidation product of citrate which functions as a reducing agent for gold ions atoms zerovalent. Monodisperse gold nanoparticles (AuNps) were synthesized and dissolved in deionised water exhibiting a pH of 6, Figure 1.

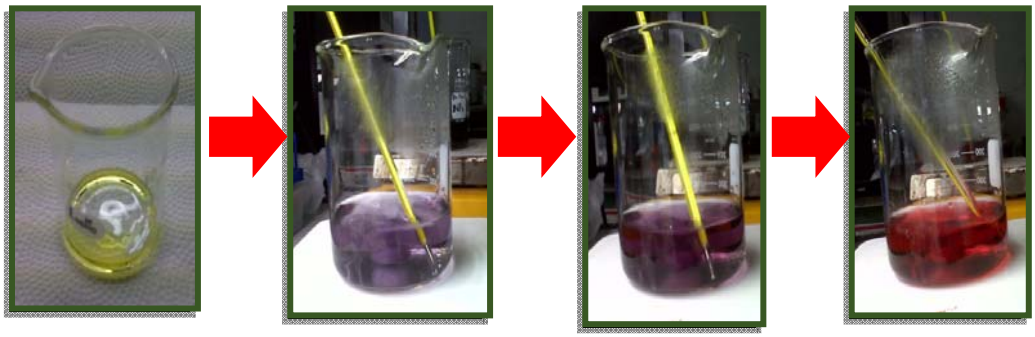

Figure 1: Synthesis of metallic gold nanoparticles.

A series of reactions for the synthesis of gold nanoparticles were carried out by the method of reduction with sodium citrate, which were individually characterized by Spectrophotometry UV/ Vis in order to observe the surface plasmon resonance (SPR), which is the frequency at which the conduction electrons oscillate in response to an alternating electric field incident electromagnetic wave. The SPR is responsible for the attractive colors of colloidal solutions of some metal particles[5]. Gold nanoparticles absorb in the visible range (300-500 nm) with a small valley between 500-600 nm. Figure 2.

Gold nanoparticles, once they have been synthesized have a negatively charged surface mainly formed by citrates, obtained from the same synthesis. The negative surface charge repulsion occurs between each nanoparticle, constituting the colloidal state, remaining stable for some time. However, with the passage of

time this negative charge will weaken the adhesion of positive charges remaining compounds formed by the synthesis process. Once the surface charge of the nanoparticles is exhausted, there are no electrostatic repulsion forces which prevent the approach each other, thus giving raise to the aggregation process. In this process, large amounts of nanoparticles are attached to form micro particles which are unstable and tend to precipitate even.

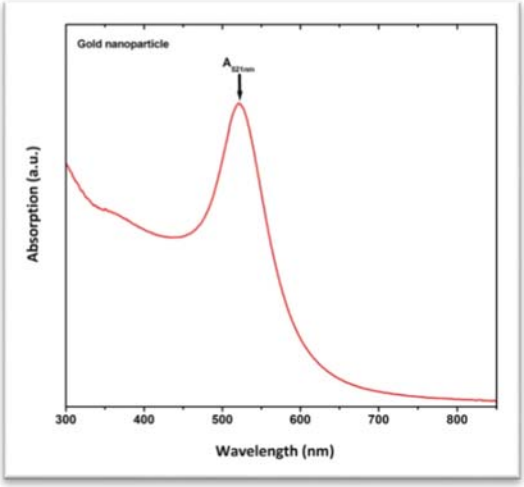

Figure 2: Surface plasmon resonance band characteristic of gold nanoparticles with ~ 20 nm of size.

It is therefore necessary to apply much larger molecules such as proteins to the surface of gold nanoparticles. The proteins may bind to the surface of the nanoparticle by type interactions: ionic, covalent or hydrophobic. Once the protein has attached to the nanoparticle, this becomes much more stable and less susceptible to the presence of ions in the medium in which they are suspended. Streptavidin protein is a viable molecule for this use because for one hand serves as a stabilizing agent of the gold nanoparticles, and on the other as receiver element option biotinylated antibodies[7]. We estimated at least 10 µl of streptavidin as sufficient for covering the surface of gold nanoparticles. Figure 3 shows transmission electronic microscopy (TEM) images of the gold nanoparticles and also of the conjugated. In the first case (Figure 3A), is shown the control with an increasing of 160,000X. In the second case (Figure 3B), is shown the conjugated gold nanoparticle-streptavidin, with an increasing of 210,000X. In the latter image, it is clearly seen the streptavidin layer surrounding the surface of the gold nanoparticle. TEM images and UV/VIS spectroscopy validation assays, confirmed that the process of functionalization of the gold nanoparticles with streptavidin protein was carried out successfully.

In order to determinate the critical immunoglobulin concentration for covering the surface of the conjugate (AuNp-Streptavidin) a titration experiment were carried out. The conjugates were aliquotted into a 1.5mL eppendorf tubes, then increased concentrations of immunoglobulin were added to each tube. Absorbance spectra were measured for AuNps, AuNP-Streptavidin conjugates and also for the array AuNp-Streptavidin-Immunoglobulin (nano-immunosensor).

UV/VIS measurements can be used to check the adsorption of proteins onto gold nanoparticles by the shift in wavelength of the absorbance band with respect to the SPR band of AuNPs. Upon addition of

immunoglobulin λmax shifted, indicating an interaction between the conjugate and the immunoglobulin and a modification of the refractive index due to the layers of proteins on the surface of AuNPs (Figure 4).

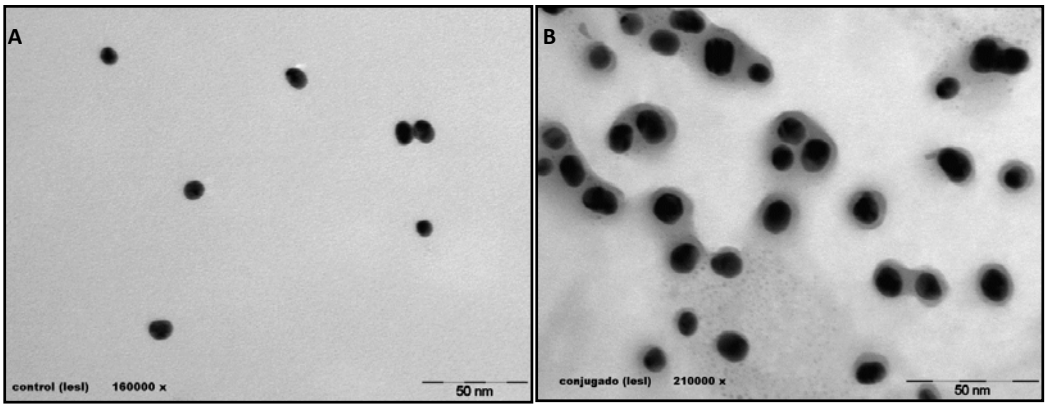

Figure 3: Transmission electronic microscopy images (TEM). Figure 3A, Gold Nanoparticles (control). Figure 3B, Nanoparticle-Streptavidin conjugate.

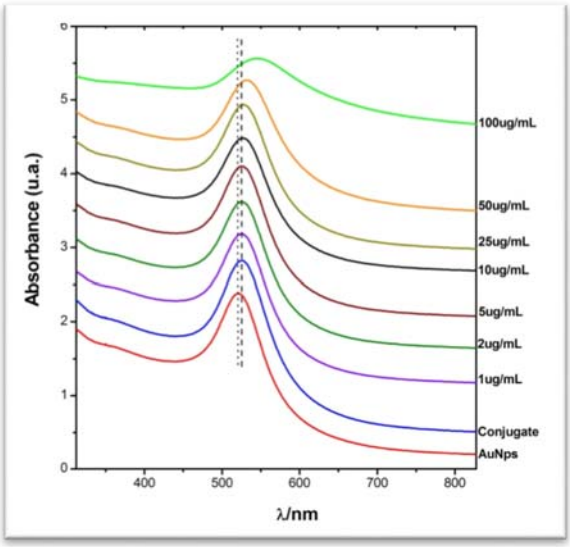

Figure 4: UV/VIS spectra of: colloidal gold (red), AuNp-Streptavidin conjugate (blue) and nano-immunosensor (AuNP-Streptavidin-Immunoglobulin).

CONCLUSIONS

We have obtained a colloidal immunosensor, which is based on gold nanoparticles (~20 nm) conjugated with streptavidin and biotinylated immunoglobulin covering the surface of the nanoparticles. Bioconjugation

experiments by titration procedures were applied to get the critical concentrations of streptavidin and biotinylated immunoglobulin. This kind of colloidal nano immunosensor could be used in diagnostics areas such as health and food.

ACKNOWLEDGEMENTS

This project has been supported by grants from PIFI and CONACyT.

REFERENCES

1. S.Park, K. Hamad-Schifferli, *Curr. Opin. Chem. Biol., 2010, 14:616*-622.

2. N.T.K.Thanh,L.A.W. Green, *Nano Today,2010,* (5):213-230.

3. K.E.Sapsford,W.R. Algar,L. Berti,K.B. Gemmill,B.J. Casey,E. Oh,I.L. Medintz, *Chem. Rev.*,2013, *113*(3), 1904–2074.

4.E.Boisselier,Astruc,Didier, *Chem. Soc. Rev.*,2009,38,1759-1782.

5. W.Haiss,N.T.K. Thanh,J. Aveyard, &D.G. Fernig, *Anal. Chem.*, 2007, *79* (11), 4215–21.

6.K.E.Sapsford,K.M. Tyner,B.J. Dair,J.R. Deschamps, &I.L. Medintz, *Anal. Chem.*,2011, *83*(12), 4453–88.

7.S.Thobhani, S. Attree,R. Boyd,N. Kumarswami,J. Noble,M. Szymanski,R.A. Porter, *J. of Immunol. Methods, 2010, 356:60*-69.

*** Corresponding author: Rojas-Lopez Marlon***
Centro de Investigación en Biotecnología Aplicada del Instituto Politécnico Nacional. Km. 1.5 Carretera Tecuexcomac-Tepetitla. CP 90700 Tlaxcala. México.
marlonrl1@hotmail.com.mx