FUNCTIONALIZATION OF GOLD NANOPARTICLES WITH STREPTAVIDIN FOR BIOSENSING APPLICATIONS

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Introduction. Due to their small dimensions metallic gold nanoparticles (AuNPs) are widely used for labeling specific biomolecules. For this application one critical issue is the coating of their surface with some molecules capable of preventing the flocculation of these AuNPs in liquid solutions as well as creating a robust link with targeted biomolecules (1). A variety of biological molecules can be labeled with gold nanoparticles. Proteins are the most common gold probes: toxins, antibodies, immunoglobulin binding proteins such as streptavidin have been labeled with colloidal gold to form highly sensitive reagents (2). The choice of streptavidin biomolecule is very versatile since the robust biotin/streptavidin interaction could be further used for assembling these AuNPs into more sophisticated molecular arrays (3).
The aim of this work was to obtain stable conjugates of gold nanoparticles with streptavidin for biosensing applications.

Methods. AuNps were synthesized through the chemical reduction of chloroauric acid ($HAuCl_4$) with sodium citrate. UV/Vis measurements were carried out using a spectrophotometer.

Results. AuNps dissolved in deionised water and having a pH of 6 were synthesized. UV/Vis spectrum of AuNPs suggests monodisperse solutions; and the particle size for each solution ranges from 15-25 nm. In order to determinate the critical streptavidine concentration for covering the surface of the AuNPs a tritation procedure of gold colloids varying the concentration of streptavidin was carried out in presence of NaCl. If most of the AuNPs are still covered with citrate groups (COO^-) then the negative charge of the AuNPs will be screened by Na^+ ions and aggregation of the colloid take place. This process is accompanied by a change of colour in the solution. On the other hand, if a sufficient quantity of streptavidin molecules is coating the surface of the AuNPs, then the adjunction of NaCl not affects the stabilization of the AuNPs in the solution and no colour change is observed. UV/Vis spectra of AuNps and their respective Streptavidin-AuNPs conjugates were measured. Spectral line shape indicates adsorption of proteins onto gold surface with a change in absorbance peak for the AuNPs. Due to the surface plasmon resonance of colloidal gold a strong absorbance peak between 500-560nm was observed. Upon addition of streptavidin λ_{max} shifted, indicating an interaction between the AuNPs and the protein and a modification of the refractive index due to the layer of protein on the surface of AuNPs (Figure 1).

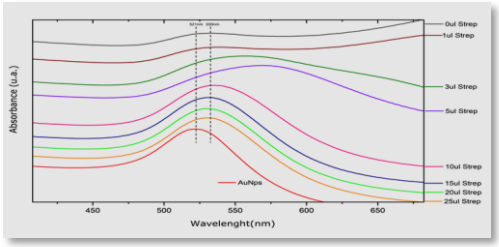

Fig.1 Uv/Vis spectra of colloidal gold and the Streptavidin- AuNp conjugates.

Conclusions. AuNP-streptavidin conjugates were obtained, and their UV/Vis spectra suggest the adsorption of the streptavidin protein on the metallic surface only under specific conditions. We have applied a titration procedure to determine the concentracion of streptavidin for covering in a full monolayer the surface of the gold nanoparticles. For further assembly purposes with biotinilated molecules, it is crucial to get the critical concentration of protein.

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References.

1.- Park S., Hamad-Schifferli K. (2010). Current Opinion in Chemical Biology.14:616-622.
2. Thanh N.T.K., Green L.A.W. (2010). Nano Today. (5):213-230.
3.- Thobhani S., Attree S., Boyd R., Kumarswami N., Noble J., Szymanski M., Porter R.A. (2010). J. of Immunological Methods.356:60-69.