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Study of Optical Properties of a Glutathione Capped Gold Nanoparticles using Linker (MHDA) by Fourier Transform Infra Red Spectroscopy and Surface Enhanced Raman Scattering

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***Abstract*—**16-Mercaptohexadecanoic acid (MHDA) and tripeptide glutathione conjugated with gold nanoparticles (Au-NPs) are characterized by Fourier Transform InfaRared (FTIR) spectroscopy combined with Surface-enhanced Raman scattering (SERS) spectroscopy. Surface Plasmon Resonance (SPR) technique based on FTIR spectroscopy has become an important tool in biophysics, which is perspective for the study of organic compounds. FTIR-spectra of MHDA shows the line at 2500 cm-1 attributed to thiol group which is modified by presence of Au-NPs, suggesting the formation of bond between thiol group and gold. We also can observe the peaks originate from characteristic chemical group. A Raman spectrum of the same sample is also promising. Our preliminary experiments confirm that SERS-effect takes place for MHDA connected with Au-NPs and enable us to detected small number (less than 106 cm-2) of MHDA molecules. Combination of spectroscopy methods: FTIR and SERS – enable to study optical properties of Au-NPs and immobilized bio-molecules in context of a bio-nano-sensors.

***Keywords*—**Glutathione; gold nanoparticles, Fourier transform infrared spectroscopy, MHDA, surface-enhanced Raman scattering.

I. Introduction

IT is well known fact that metal nanoparticles attract an extensive attention in various field of physics, chemistry, biology, due to their unique properties [1]. Among colloids of metallic, gold nanoparticles, which are one of the most stable colloid, does not form a stable oxide layer under ambient conditions. Further, the properties of gold nano-scale, which are dominated by electromagnetic resonances of plasmons and high surface to volume ratio are important in the context of direct electron transfer between bio-molecules and electrode surface and is particularly advantageous for biosensing technology [2]. Moreover, gold have strong binding affinity toward thiols, amines and disulfides [3].

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The infrared (IR) optical properties of gold nanoparticles are determinate by the plasmons - collective oscillations of electrons within a metal nanoparticles, activated by an incident electromagnetic field. Surface plasmon resonance (SPR) consists on an electromagnetic wave propagating along the surface of a thin metal layer stimulated by incident light [4]. The IR spectra are formed as a consequence of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of specific sets of chemical bonds from within a molecule. It is assumed that the intensity of the band is proportional to the concentration of the functional chemical groups in a molecule.

There are two types of vibrational motions, which are defined by stretching and bending modes. The stretching vibrations are characterized by rhythmic movement along the axis of tying, so the distance between atoms increases and decreases. The second one is bending mode, where an angle between atoms bond is changed [5].

Surface-enhanced Raman scattering (SERS) is a special phenomenon within the field of Raman spectroscopy and involves generation of enhanced Raman signal (by as much as 10^8) from analyzed molecules adsorbed on roughened metal surfaces (usually nanoparticles of silver and gold) [6]. SERS technique, attributed to large electromagnetic fields near nanostructured metallic surfaces and short range chemical effect, has the capability to detect a single molecule and provide information on molecular structure. Although some enhancement in SERS is due to chemical contribution (electronic interaction between metal and adsorbed molecules), the main reason why SERS produces extraordinary enhancements are surface plasmons - the electromagnetic property of nanostructures.

In a brief preliminary account of the present work, we have studied the immobilization on a surface of gold nanoparticles (Au-NPs) using a surfactant- 16-Mercaptohexadecanoic acid (MHDA), as a space arm. MHDA is a linker for covalent binding of proteins by condensation of carboxylic group and amino group of the protein. The surfactant bind to the gold surface through their thiols. As a protein we used a reduced glutathione, which is a tripeptide (Glu-Cys-Gly), as a model for proteinaceous ligand. Their physical properties were studied using FTIR and SERS.

II. Experimental Method

A. Samples

Au-NPs were prepared by the Turkevich method [8]. This can be done by using hydrogen tetrachloroaurate ($HAuCl_4$) as source of gold ions and trisodium citrate ($Na_3C_6H_5O_7$) as a reduction and stabilized agent. Tetrachlorauric acid is heated to its boiling on a magnetite stirrer, to which reducing agent sodium citrate is injected. Heating is continued till the color of the solution changed from colorless to pink/red. The colloidal gold is formed because the citrate ions act as both a reducing agent, and a capping agent. As a result of the citrate molecules settle on the particle surface, Au-NPs are stabilized trough electrostatic repulsion. The size of the produced particles can be controlled by varying the amount of available gold in the solution.

In order to attach the proteins to the citrate stabilized gold nanoparticles mercaptoalkane linkers are used. 16 – mercaptohexadecanoic acid. Au-structures were incubated in MHDA in ethanol for overnight at 4ºC. During incubation MHDA replace the citrate molecules and the thiol group of the mercaptoalkanes interact with the gold surface through an Au-S interaction. After rinsing with dimethylformamide (DMF), the MHDA-covered Au-structures were incubated in DMF solution of pentafluorophenyl (PFP) and *N*, *N*-diisopropylethylamine (DIPEA) and N-cyclohexyl-N′-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate (CMC), during 30 min at 25ºC. After repeated rinsing with DMF, condensation of the activated Au-linked carboxylic groups with first amine group of the reduced glutathione was carried out. On the surfaces of the electrode, solution with enzyme were put and incubated for 1 hour at 25ºC. After rinsing with phosphate buffer, blocking of un-reacted carboxylic groups with *2-(2*-aminoethoxy*)* ethanol (AEE) was performed, using solution of AEE in bicarbonate buffer. The bio-functionalized Au-structure was rinsed with phosphate buffer [7]-[8].

B. Measurements

The Fourier spectrometer Nicolet 6700 provided measurements of optical reflectivity in the range of far- as well as middle-infrared: from 500 cm^{-1} to 4000 cm^{-1} with resolution about 2 cm^{-1}. Reflectivity was measured using silver mirror as reference. The FTIR spectra of the samples were collected by this way at room temperature. The resolution of 1-4 cm^{-1} at 32 scans during one minute characterized these measurements.

The Raman-spectra were examined using Smart Raman DXR, which has a laser 780nm and maximal power of this laser is 14mW. This spectrometer enables us to measure the Raman shift spectra in the range from 50 to 3300 cm^{-1}.

III. Result and Discussion

A. X-ray Diffraction

The synthesized gold nanoparticles by Turkevich method were then checked for the structure and phase purity based on the X-ray diffraction (XRD) analysis. The XRD pattern (Fig. 1) shows five intense peaks in the whole spectrum of 2θ values ranging from 20 to 100 degree. The presence of intense peaks of nanoparticles (111), (200), (220), (222) and (311) confirms that the gold nanoparticles are crystalline in nature way and suggest that the Au-NPs are stable and do not aggregate [9]-[10].

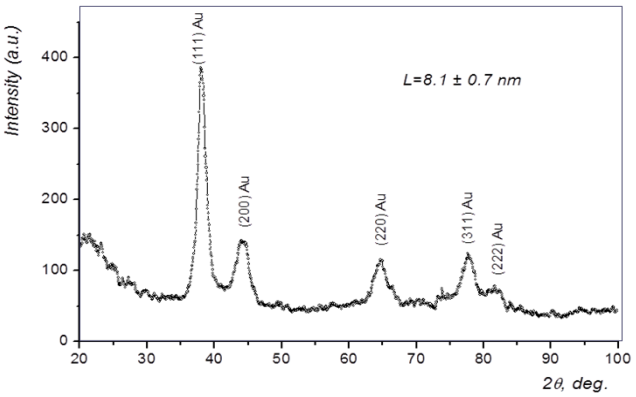

Fig. 1 XRD of Au-NPs

B. Surface Enhanced Raman Scattering

The effect of size of Au-NPs on signal SERS intensity is demonstrated in Fig. 2. It could be seen that the SERS signal became larger with the size of nanoparticles decreasing, so the size of Au-NPs have significant effect on signal SERS [11].

It can be seen in Fig. 4 that the group of peaks in the range of 1200-1600cm^{-1} is characteristic for glutathione (spectrum a). The band at 1296 cm^{-1} and a group of bands in the region from 1400 to 1500 cm^{-1} are observed due to (C–C) stretching vibrations. The other spectra represent glutathione capped Au-NPs of different size: 127nm (spectrum b) and 90nm (spectrum c). So, the 5-times enhancement of Raman-intensity is observed in case of 90 nm size Au-NPs.

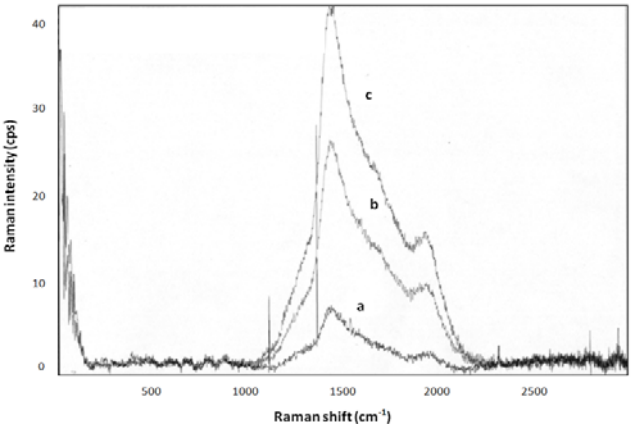

Fig. 2 Raman spectra of glutathione (a) , Au-NPs of 127 nm diameter + glutathione(b) and Au-NPs of 90 nm diameter r+ glutathione (c)

C. FTIR

IR reflectance spectra in Fig. 3 shows several groups of lines associated with the citrate molecules linked with surface of gold nanoparticles. The peak at the wavenumber 1077 cm^{-1} is assigned to the stretching vibrations of C-O whereas peak

at 1680 cm^{-1} is attributed to C=O [12]. The peak at the wavenumber at 1250 cm^{-1} is characteristic for the sample investigated. It can also be found that there are two bands at 2853 and 2914 cm^{-1} which are attributed to stretching vibrations of CH_2, and band at 1412 cm^{-1} which can be assigned to bending vibrations of CH as well as the line at 1507cm^{-1} – to the bending vibrations of OH.

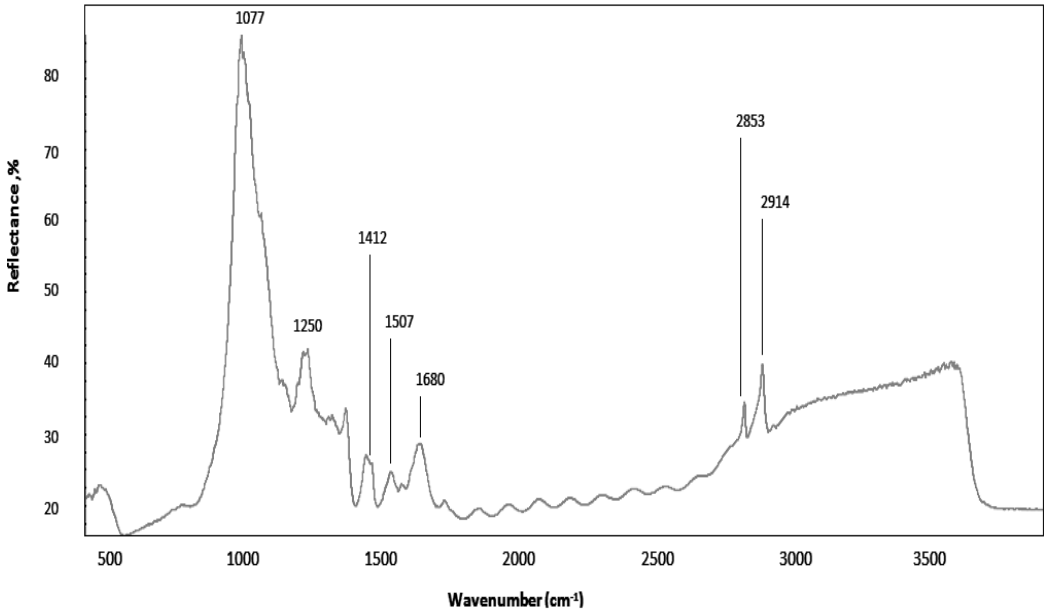

Fig. 3 IR reflectance spectra of citrate-stabilized Au-NPs

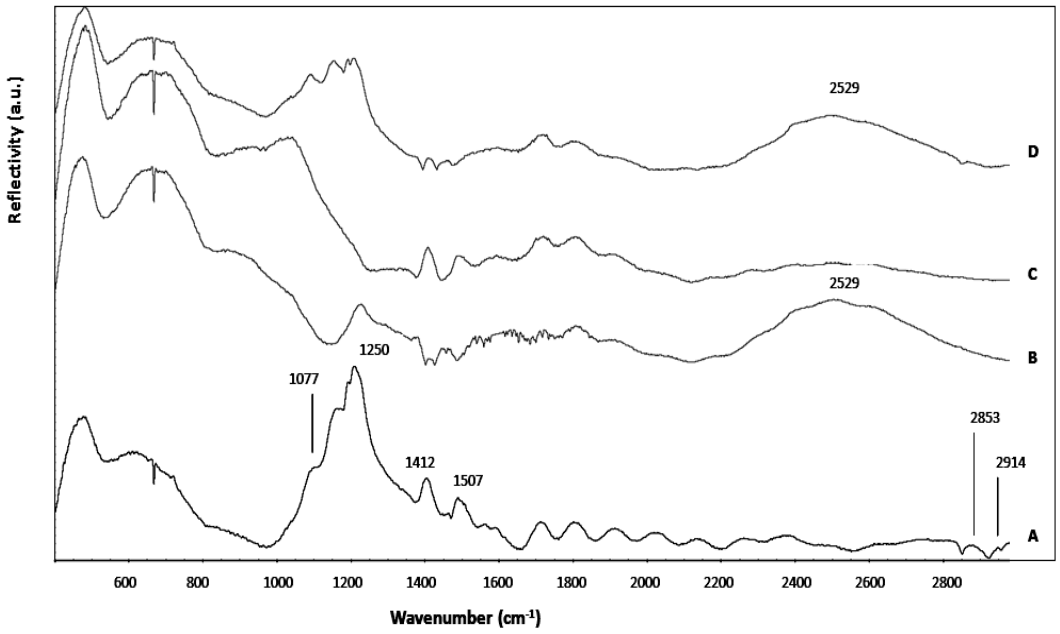

Fig. 4 Infrared spectra of different samples: the citrate stabilized gold nanoparticles (spectrum A), the pure MHDA (spectrum B), the Au-NPs + MHDA (spectrum C), Au-NPs + MHDA + glutathione (spectrum D)

Fig.4 shows the curves of the citrate stabilized Au-NPs (spectrum A), the pure MHDA (spectrum B), the MHDA liked with Au-NPs (spectrum C) and the glutathione capped gold nanoparticles (spectrum D). In the curve B is observed the group of specific broad bands at 2529 cm^{-1} caused by thiol vibrations which are appeared obviously in MHDA [12]. This group of lines is suppressed in MHDA linked with Au-NPs giving evidence, that MHDA anchors on the gold surface through the sulfur atom in the thiol group (see the C spectrum) and suggests that the bonding of the biofuncional ligands to the Au surface takes place through the S-H group. The Au-NPs-MHDA researched is a model system for the binding of biofuncional surfactants onto the Au-NPs surface. Thiol bonds are again shown in the AuNPs + MHDA + Glutathione

spectrum (at 2529cm^{-1}, see curve D) because glutathione is composed of glicyne, glutamic acid and cysteine, which also has a mercaptan group [13]-[14].

IV. Conclusion

FTIR enable us to monitor the interaction of MHDA and glutathione with the gold matrix. The technique in mid-IR can achieve sensitivity that is comparable and even higher that in the visible range and used to detect of biomolecules according to their "fingerprints".

FTIR and Raman spectra can be used to identify unknown molecules, determinate their concentrations, and to study bond strengths in molecules, so both of these spectroscopy techniques gives a lot of possibilities for study chemical and physics properties of thiol stabilized Au-NPs and proteineous ligand.

The colloidal-bound proteins enable to build bio-nano-sensors with great control over functionality and sensitivity.

Acknowledgment

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