

Gold-Silver Hybrid Nanoparticles as a Novel Carrier for Electrochemical Study of Redox Protein

Khadijeh Eskandari^{1*}, Naimeh Mah-Heidari¹, Mahdi Fasihi-Ramandi², Mohammad Heiat³,

Fariba Dashtestani¹, Mehdi Kamali¹, Danial Ashiani¹

Abstract

Noble metal nanoparticles have a great potential for biological study, especially the use of gold nanoparticles is popular. In this work gold nanoparticles (GNPs), silver nanoparticles (SNPs) and gold-silver hybrid nanoparticles (HNPs) synthesized and used as a carrier for electrochemical investigation of redox protein. Optical characterization of these nanoparticles was performed by UV-Vis spectroscopy. The optical absorption spectra of HNPs solution shows only one plasmon absorption, it is concluded that mixing of gold and silver leads to a homogeneous formation of alloy nanoparticles. LCR meter study shows the HNPs is best conductance in compare of GNPs and SNPs. Therefore, the electron transfer of the homogenous glucose oxidase (GOx), horse radish peroxides (HRP) and hemoglobin (Hb) was investigated by electrochemical method in presence of HNPs. They demonstrated quasi-reversible cyclic voltammograms with a formal potential of -479, -178 and, 168 mV in 50 mM phosphate buffer solution at pH 7.4 respectively.

1. Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences Tehran, Iran
2. Molecular Biology Research Center, Systems Biology and Poisoning Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

* Corresponding Author

Khadijeh Eskandari
Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences Tehran, Iran
E-mail: kheskandari@alumni.ut.ac.ir

Submission Date: 8/12/2017

Accepted Date: 12/13/2017

Keywords: Gold Nanoparticles, Silver Nanoparticles, Gold-Silver Hybrid Nanoparticles

Introduction

The intense research in the field of nanoparticles by chemists, physicists, and materials scientists is motivated by the search for new materials in order to further miniaturize electronic devices as well as by the fundamental question of how molecular electronic properties evolve with increasing size in this intermediate region between molecular and solid-state physics. Also metal nanostructures attract much interest because of their unique properties, including large optical field enhancements resulting in the strong scattering and absorption of light. Alloy nanoparticles, on the other hand, have mainly been studied because of their catalytic effects [1]. Nanoparticle colloidal solutions of the noble metals copper, silver, and gold show a very intense color, which is absent in the bulk material as well as for the individual atoms. Their origin is attributed to the collective oscillation of the free conduction electrons induced by an interacting electromagnetic field. These resonances are also denoted as surface plasmon. The electric field intensity and the scattering and absorption cross-sections are all strongly enhanced at the LSPR frequency, which for gold, silver, and copper lies in the visible region. Since copper is easily oxidized, gold and silver nanostructures are most attractive for optical applications [2]. Also noteworthy is that Silver is the most nearly perfect conductor and silver mirrors are the best reflectors of light. Gold, however, is more chemically inert as it does not tarnish in air, and still exhibits excellent electrical conduction and light scatter properties [3].

Due to their large specific surface area and high surface free energy, nanoparticles can adsorb biomolecules strongly and play an important role in the immobilization of biomolecules in biosensor construction. Generally, the adsorption of biomolecules directly onto naked surfaces of bulk materials may frequently result in their denaturation and loss of bioactivity. However, the adsorption of such biomolecules onto the surfaces of nanoparticles can retain their bioactivity because of the biocompatibility of nanoparticles. Since most of the nanoparticles carry charges, they can electrostatically adsorb biomolecules with different charges. Besides the common electrostatic interaction, some nanoparticles can also immobilize biomolecules by other interactions. For example, it is reported that gold nanoparticles can immobilize proteins through the covalent bonds formed between the gold atoms and the amine groups and cysteine residues of proteins [4]. In this study the noble metal and their hybrid were compared to revealing of conductivity and electron transferring for redox proteins (GOx, HRP and Hb). Our experiments show that these Ag-Au nanoparticles can significantly enhance the current sensitivity of protein electrodes.

Materials and Methods

Materials

GOx, HRP, Hb and $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and multi-wall carbon nanotubes were obtained from Sigma. Hydrogen tetrachloroaurate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), silver nitrate (AgNO_3), trisodium citrate Potassium, dihydrogen phosphate (KH_2PO_4) and

dipotassium hydrogen phosphate (K_2HPO_4) solution were purchased from Merck and were used without further purification.

Apparatus and measurements

All electrochemical experiments were carried out using a computerized Potentiostat/Galvanostat (model 263-A, EG&G, USA) equipped with power suite software package. Electrochemical studies were performed using a single-compartment conventional three-electrode cell at room temperature. A working modified glassy carbon (GC) electrode with a diameter of 3 mm, a saturated silver/silver chloride (Ag/AgCl) reference electrode, containing 3 M KCl and a platinum rod auxiliary electrode (from Azar electrode Co, Iran) were used. All potentials were measured and reported versus the Ag/AgCl reference electrode. Circular dichroism spectroscopy was analyzed by an Aviv model 215 Spectropolarimeter (Lakewood, NJ, USA). For Zeta sizer measurement, Zeta potential analyzer was used (Zeta Plus, Brookhaven Instruments Corporation, USA) and the UV-Vis spectroscopy made by Cary spectrophotometer, 100 Bio-model, LCR meter (from HAMEG Instruments, model HM8118).

Nanoparticles preparation

For preparation of gold colloidal nanoparticles (GNPs), 20 ml (36 μ M) $HAuCl_4 \cdot 3H_2O$ were heated up to 60°C, then 2 ml of 5% (w/v) sodium citrate added to it. The final red color was stored in dark glass bottles at 4°C [5]. Also the silver colloidal nanoparticles (SNPs) were prepared in same procedure by $AgNO_3$. Gold-Silver hybrid nanoparticle (HNPs) was synthesis in of ratio 1:1, 1:2 and 2:1 gold to silver. Thus for the predetermined mix ratio of $HAuCl_4 \cdot 3H_2O$ (36 μ M) and $AgNO_3$ (36 μ M) solutions were heated up to 60°C and similar above method the sodium citrate was used as a reduction.

Conductivity measurements of nanoparticles

A LCR meter was used for nanoparticles conductivity measurements. Two gold electrodes were placed in pure water and after, the LCR meter was set on zero, nanoparticles solution was added to it, and the conductivity of nanoparticles was measured.

Preparation of protein electrode for electrochemical method

For investigation of proteincyclic voltammograms, 2 μ l carbon nanotubes (CNTs) dropped on GC electrode and after drying, it immersed in 100 μ l nanoparticles and 30 μ l (3 mg/ml) proteins in 50 mM PBS, pH 7.4. All potentials were measured and reported versus the Ag/AgCl reference electrode.

Results and Discussion

Nanoparticles characterization

Gold and silver nanoparticles have strong surface plasmon giving an absorption peak in the visible region of the electromagnetic spectrum [6]. The absorption peak of GNPs is in red area, 500-600 nm and according to the calculated absorption spectrum to nano-sized metallic silver particles a surface plasmon peak characterized by a maximum centered at 400 nm is observed [7]. As shown in Fig.1 the maximum absorption peak of GNPs and SNPs are 524 and 392 nm. Also HNPs with the ratio of at 1:1, 1:2 and 2:1 gold to silver exhibit maximum absorption band at 465,

431, and 496 nm respectively. The optical absorption spectra HNPs show only one plasmon absorption it is demonstrate that mixing of gold and silver leads to a homogeneous formation of alloy nanoparticles. So the absorption peak became broad and shifted to longer wavelength, with increasing molar ratio of $HAuCl_4$.

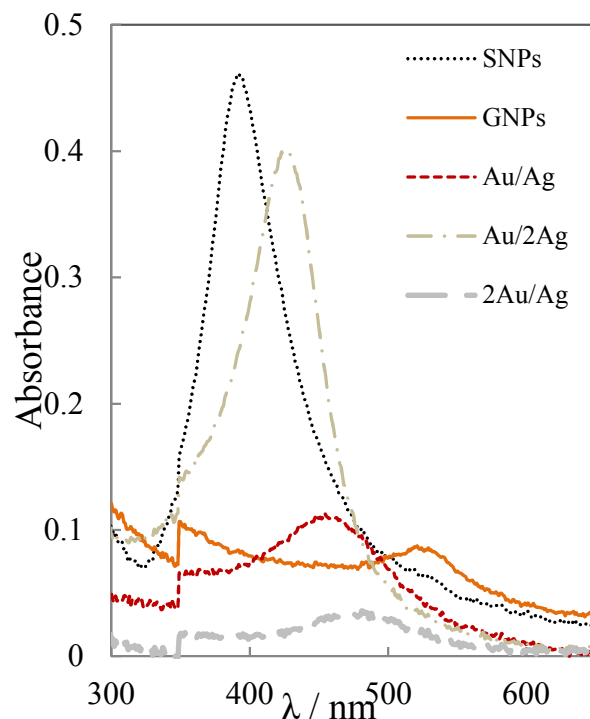


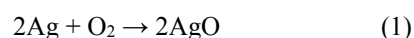
Figure 1. UV-Vis absorption spectra of GNPs, SNPs and HNPs (with ratio of 1:1, 1:2 and 2:1 gold to silver).

Size and surface charge analysis

The hydrodynamic size and the surface charge (zeta potential) was investigated by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively [8]. The average hydrodynamic size of GNPs, SNPs, and HNPs are 42, 66, and 59 nm and their surface charges are -15.1, -15.8 and -16.1 mV respectively.

Conductivity measurements of nanoparticles

The conductivity change of GNPs, SNPs and HNPs in different days was shown in Fig. 2. These results demonstrated that the SNPs have a significant conductivity change, because silver is prone to oxidation. In fact the equilibrium electrode potential of silver is +0.799 V. This value is only 0.01 V more negative than the equilibrium potential of an oxygen electrode in natural environments, so silver is susceptible to giving electrons to oxygen. Silver can still be thermodynamically oxidized by atmospheric oxygen at normal temperatures. At standard temperatures and pressures (STP), this thermodynamically equilibrium was occurred [9]:



Moreover, although the conductivity of GNPs is 1.8 time less than of SNPs, but it is almost constant with time. This results show the HNPs with the ratio of 1:1 has a less con-

ductivity change and in finally has a highest conductivity, so it selected for next test.

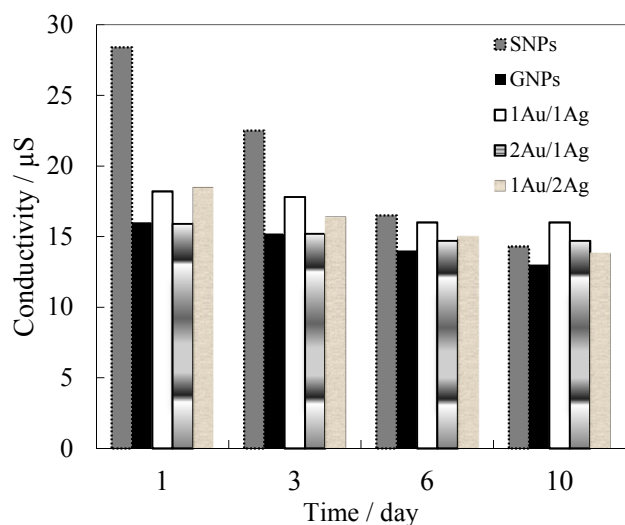


Figure 2. The conductivity of GNPs, SNPs and HNPs (with ratio of 1:1, 1:2 and 2:1 gold to silver) in different days.

Electrochemical investigation

The cyclic voltammograms (CVs) of homogenous, HNPs, SPNs+GOx, GPNs+GOx and HPNs+GOx on CNT/GC electrode in 50 mM pH 7.4 PBS were shown in Fig. 3. It's obviously that the only GOx without nanoparticle and nanoparticle without GOx have no response and SPNs+GOx is also.

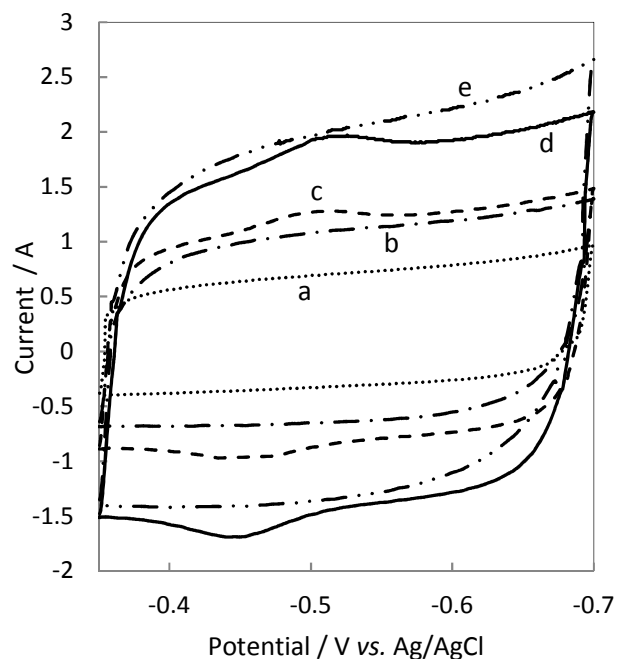


Figure 3. Cyclic voltammograms of homogenous GOx (a), SNPs+GOx (b), GNPs+GOx (c), HNPs+GOx (d) and HNPs (e) on CNT/GC electrode in 50 mM/L PBS (pH 7.4) at 50 mVs⁻¹.

In fact a nanoparticle as an electron-transporting shuttle was caused to facilitate of electron passing between redox active site and electrode. Thus the GOx have a direct electron transfer in presence of GNPs and HNPs. but as can be seen in Fig. 3, the redox response of HPNs+GOx is stronger than of GPNs+GOx, due to of high conductivity shown by LCR meter result. On the other hand Although silver is best conductor among the metals [10], but the ESR study of Ag nanoparticles indicates the existence of free radicals from Ag nanoparticles [11], thus it can be said that the oxidation pathway is associated with the production of radicals which are causing to protein oxidation.

It can lead to hydroxylation of aromatic groups and aliphatic amino acid sidechains, nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and to conversion of some amino acid residues to carbonyl derivatives. Oxidation can lead also to cleavage of the polypeptide chain and to formation of cross-linked protein aggregates. Furthermore, functional groups of proteins can react with oxidation products of polyunsaturated fatty acids and with carbohydrate derivatives (glycation/glycooxidation) to produce inactive derivatives [12]. Therefore the GOx no have electrochemical response in presence of SPNs. However, it was reported that gold GNPs could adsorb redox proteins (proteins) without loss of their biological activities [13], but its weak conductive in compare of silver, that causing to low electrochemical response as shown in Fig. 2. The HNPs has a highest conductivity, so HNPs has been used for study of the direct electron transfer of proteins, because in HNPs, gold sector with having a good biocompatibility and capability of protein absorption was caused to safety of proteins structure and its concentrates in nanoparticles surface and moreover the silver sector facilitate electron transfer and in result amplification of electrochemical response.

The formal potential of GNPs+GOx and HNPs+GOx is -477 and -479 mV respectively, similar to the reported result [14]. The formal potential (E^0) has been calculated by average of the cathodic and anodic peak potential. It was obvious that GOx in presence of HNPs is exhibited excellent redox properties for high conductivity of HNPs. Therefore, the HNPs with having of highest conductivity and electrochemical response are best substrate for electron transferring of redox proteins. The current density (ΔI) of GOx is 3 μ A was larger than GOx immobilized on Nafion-CNTs-CdTe-GC electrode [15] and NH₂-TiO₂-CNT/GC electrode [16].

Fig. 4A shows the cyclic voltammograms of GOx/NH₂-Fe@Au/Au electrode in PBS (50 mM, pH 7.4) at different scan rates. Fig. 4B shows the plot of cathodic and anodic peaks current (I_p) against the scan rate (ν). Both the anodic and cathodic peak currents increased linearly with scan rate in the region of 10-500 mVs⁻¹ indicating surface-confined electrode reaction. The kinetic parameters of electron transfer coefficient (α) and apparent charge transfer rate constant (k_s) can be calculated using Laviron's model [17]. Laviron derived general expressions for the linear potential sweep voltammetric response for the case

of surface-confined electroactive at small concentration Eqs. (2)- (5):

$$E_{pc} = E^0 + \frac{RT}{(1-\alpha)nF} \ln\left[\frac{(1-\alpha)}{m}\right] \quad (2)$$

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF} \ln\left[\frac{\alpha}{m}\right] \quad (3)$$

$$E_{pa} - E_{pc} = \Delta E_p > \frac{200}{n} \text{ mV} \quad (4)$$

$$K_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log\left(\frac{RT}{nFv}\right) - \frac{\alpha(1-\alpha)nF\Delta E_p}{2.3RT} \quad (5)$$

where, $m = (RT/F) (k_s/nv)$, k_s is the apparent charge transfer rate constant, α is the charge transfer coefficient, n is the number of electron transferred in the rate-determining reaction, ΔE_p is the peak potential separation; R , T , and F have their usual meanings ($R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$, $T = 298 \text{ K}$, $F = 96485 \text{ C mol}^{-1}$) and v is the scan rate. It can be calculated using the plot of peak potentials (E_p) vs. logarithm of scan rate ($\log v$).

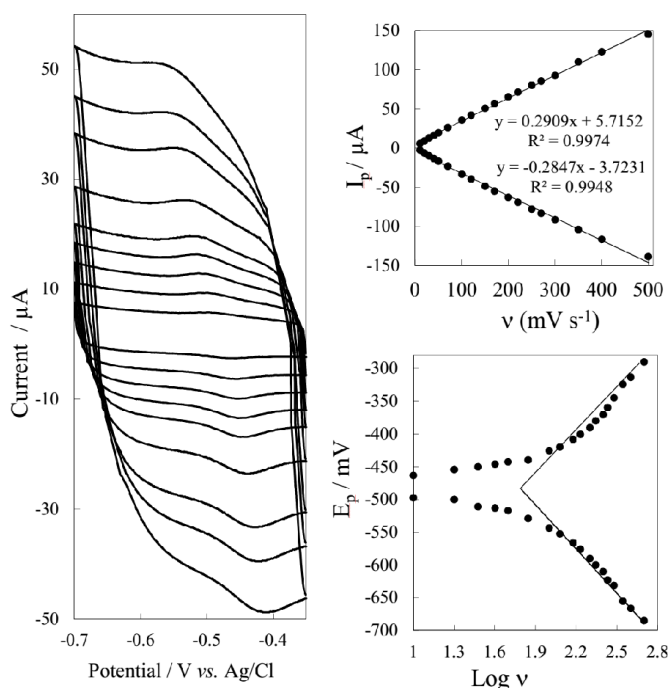


Figure 4. Cyclic voltammograms of HNPs/GOx electrode in 50 mM/L PBS (pH 7.4) at various scan rates (A) plot of I_p vs v (B) plot of E_p vs. $\log v$ (C).

The slope of the linear section in cathodic and anodic peaks in Fig. 5C is equal to $-2.303RT/\alpha_c F$ and $2.303RT/\alpha_a nF$, respectively (Linear regression equations: $y_a = 262.43x - 996.37$, $R^2 = 0.9932$ and $y_c = -249.25x - 15.84$, $R^2 = 0.9901$). As seen in Fig. 5C, it was found that

for scan rates above 200 mVs^{-1} , ΔE was proportional to the $\log v$, as indicated by Laviron. Therefore, we extracted the average value of 0.22 and 0.47 s^{-1} for α and k_s , respectively [18]. Also, α and k_s were extracted for HRP+HNPs and Hb+HNPs in same method, that was 0.28 and 0.48 s^{-1} and 0.25 and 0.36 s^{-1} , respectively (Fig. 5 and 6).

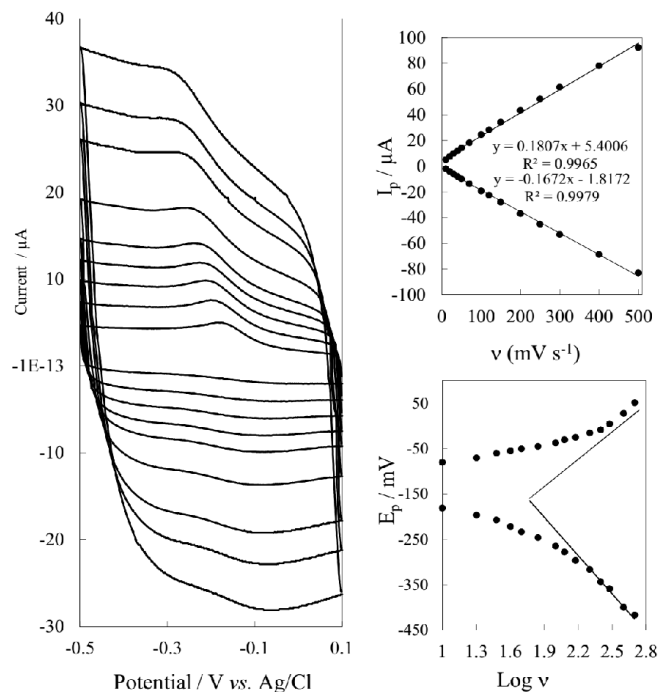


Figure 5. Cyclic voltammograms of HNPs/HRP electrode in 50 mM/L PBS (pH 7.4) at various scan rates (A) plot of I_p vs v (B) plot of E_p vs. $\log v$ (C).

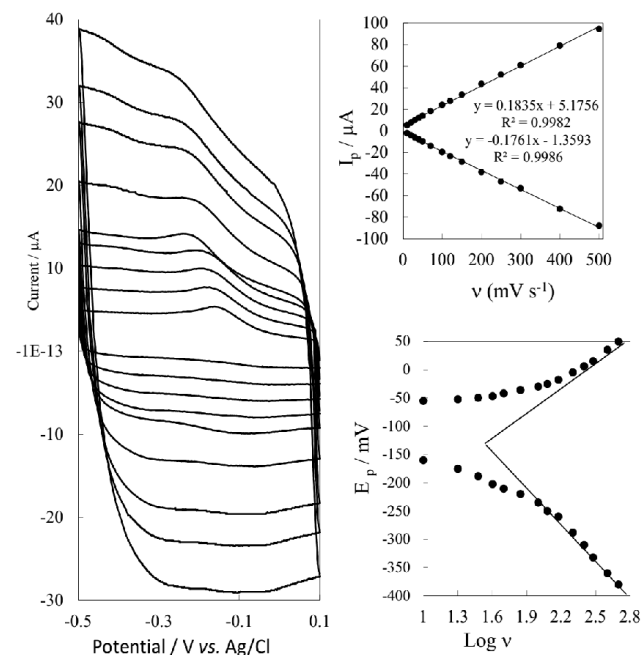


Figure 6. Cyclic voltammograms of HNPs/Hb electrode in 50 mM/L PBS (pH 7.4) at various scan rates (A) plot of I_p vs v (B) plot of E_p vs. $\log v$ (C).

Conclusion

In this paper different noble metal nanoparticles synthesized and investigated for redox protein immobilization. In first, conductivity change of GNPs, SNPs and HNPs was studied by LCR meter measurement. The LCR meter data shows the HNPs have highly conductivity and so in electrochemical study, its best electron transferring for redox proteins. In fact the silver sector was caused to high conductive property of HNPs and also the gold sector was led to good biocompatibility and high absorption of protein to concentrates of proteins in electrode surface. Thus these advantages caused to the HNPs have a highest current intensity, as shown in cyclic voltammograms. So it is clearly shows in hybrid nanoparticles we used advantage of multiple nanoparticles. Also it shows although the SNPs have a highest conductivity in first, but the process of decreasing of its conductivity is high too. Moreover unlike SNPs, the conductivity of GNPs is least but it has a minimal change in time. Finally HNPs was selected for direct electrochemistry investigation of GOx, HRP and Hb and to obtain of apparent charge transfer rate constant and the charge transfer coefficient.

Acknowledgments

Authors are grateful to Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences for providing financial support to undertake this work.

References

1. Link, S., Wang, Z.L., El-Sayed, M.A., Alloy formation of gold-silver nanoparticles and the dependence of the plasmon absorption on their composition. *J Phys Chem B*, 1999, Vol. 103, pp. 3529-3533.
2. Jain, P.K., Huang, X., El-Sayed, I.H., El-Sayed, M.A., Noble metals on the nanoscale: Optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. *Account Chem Res*, 2008, Vol. 41, pp. 1578-1586.
3. Siiman, O., Burshteyn, A., Preparation, microscopy, and flow cytometry with excitation into surface plasmon resonance bands of gold or silver nanoparticles on aminodextran-coated polystyrene beads. *J Phys Chem B*, 2000, Vol. 104, pp. 9795-9810.
4. Luo, X., Morrin, A., Killard, A.J., Smyth, M.R., Application of nanoparticles in electrochemical sensors and biosensors. *Electroanalysis*, 2006, Vol. 18, pp. 319-326.
5. Mashhadizadeh, M.H., Eskandari, K., Foroumadi, A., Shafiee, A., Copper(II) modified carbon paste electrodes based on self-assembled mercapto compounds-gold-nanoparticle. *Talanta*, 2008, Vol. 76, pp. 497-502.
6. Yu, H., Chen, M., Rice, P.M., Wang, S.X., White, R.L., Sun, S., Dumbbell-like bifunctional Au-Fe₃O₄ nanoparticles. *Nano Lett*, 2005, Vol. 5, pp. 379-382.
7. Taleb, A., Petit, C., Pileni, M.P., Synthesis of highly monodisperse silver nanoparticles from AOT reverse micelles: a way to 2D and 3D self-organization. *Chem Mater*, 1997, Vol. 9, pp. 950-959.
8. Patila, S., Sandberg, A., Heckert, E., Self, W., Seal, S., Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. *Biomaterials*, 2007, Vol. 28, pp. 4600-4607.
9. Rooij, A., The oxidation of silver by atomic oxygen. *ESA J*, 1989, Vol. 13, pp. 363-382.
10. Ren, X., Meng, X., Tang, F., Preparation of Ag-Au nanoparticle and its application to glucose biosensor. *Sens Actuat B Chem*, 2005, Vol. 110, pp. 358-363.
11. Kim, J.S., Kuk, E., Yu, K.N., Kim, J., Park, S.J., Lee, H.J., Kim, S.H., Park, Y.K., Park, Y.H., Hwang, C., Kim, Y., Lee, Y., Jeong, D.H., Cho, M., Antimicrobial effects of silver nanoparticles. *Nanomed Nanotechnol*, 2007, Vol. 3, pp. 95-101.
12. Stadtman E.R., Levine, R.L., Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids*, 2003, Vol. 25, pp. 207-218.
13. Zhao, S., Zhang, K., Bai, Y., Yang, W., Sun, C., Glucose oxidase/colloidal gold nanoparticles immobilized in Nafion film on glassy carbon electrode: Direct electron transfer and electrocatalysis. *Bioelectrochemistry*, 2006, Vol. 69, pp. 158-163.
14. Liu, Y., Wang, M., Zhao, F., Xu, Z., Dong, S., The direct electron transfer of glucose oxidase and glucose biosensor based on carbon nanotubes/chitosan matrix. *Biosens. Bioelectron*, 2005, Vol. 21, pp. 984-988.
15. Liu, Q., Lu, X., Li, J., Yao, X., Li, J., Direct electrochemistry of glucose oxidase and electrochemical biosensing of glucose on quantum dots/carbon nanotubes electrodes. *Biosens Bioelectron*, 2007, Vol. 22, pp. 3203-3209.
16. Tasvir, M., Rafiee-Pour, H. A., Ghourchian, H., Gholami, M. R., Amine functionalized TiO₂-carbon nanotube composite: synthesis, characterization and application to glucose biosensing. *Appl Nanosci*, 2011, Vol. 1, pp. 189-195.
17. Laviron, E., General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *J Electroanal Chem*, 1979, Vol. 101, pp. 19-28.
18. Rahimi, P., Rafiee-Pour, H.A., Ghourchian, H., Norouzi, P., Ganjal, M.R., Ionic-liquid/NH₂-MWCNTs as a highly sensitive nano-composite for catalase direct electrochemistry. *Biosens Bioelectron*, 2010, Vol. 25, pp. 1301-1306.