

Use of Silica-Gold Core Shell Structured Nanoparticles for Targeted Drug Delivery System

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Abstract

Drug targeting, using Silica-Gold core carrier particles loaded with antibiotic drugs is an emerging and significant method of disease treatment. Silica core particles were synthesized by Stober's method and it was functionalized with amine groups. The monodispersed Gold particles were prepared by conventional citrate reduction method. Both functionalized Silica and monodispersed Gold nanoparticles were combined and Silica-Gold core nanoshells were prepared in the range of 60-110nm. The morphology, elemental composition of the core nanoshell was characterized by UV-Visible spectroscopy, FT-IR spectroscopy, Scanning Electron Microscope (SEM), Energy Dispersive Spectroscopy (EDX) and X-ray Diffraction analysis. The antibiotic Gentamycin was loaded onto these Silica-Gold core nanoshell carriers and the drug release profile was observed.

Keywords: Drug loading; Gentamycin; Silica-Gold core nanoshell; Citrate reduction method

Introduction

During the last decade, owing to the emergence of a new generation of high technology materials, the number of research groups involved in nanomaterials has increased exponentially [1]. Core-shell particles are very important because of their applications in areas such as catalysis, cancer diagnosis and sensors [12]. Nanoshells are obtained by removing the cores of these particles or by over coating the inert core with a thin coat [2]. Core shell nanoparticles consist of concentric particles, in which particles of one material are coated with a thin layer of another material using specialized procedures [3]. The existing drug delivery systems can be categorized into two groups, namely the polymer- and lipid-based systems, in which the drugs were incorporated into or attached to the polymer or lipid [4].

In the recent past there has been considerable interest in nanosystems such as Gold nanoparticles, carbon nanotubes, etc. for drug delivery applications. To date, Gold nanoshells offer the added features of high biocompatibility and bioconjugation to antibodies via long-standing protocols adapted from Gold colloid bioconjugate chemistry, their near-infrared optical properties make them an ideal, if not unique, vehicle for a whole blood immunoassay [5]. Nanoshells made of oxides such as Silica and Titania find application in the field of drug delivery. The outer surface of these shells can be used for attaching antibodies so that the Silica shell-antibody complex can be used for targeted drug delivery in biological systems [6].

Silica-Gold core nanoshell in drug delivery system may offer plenty of advantages over conventional dosage forms, which improves efficacy, reduced toxicity, enhanced biodistribution and reduces patient's compliance. The particles have the ability to conjugate the molecules without affecting the core and also can be used to encapsulate the drugs [7]. The present study Silica-Gold core nanoshells were synthesized and characterized for use of targeted drug delivery.

Materials and Methods

All reagents (except water) were purchased from the Himedia and used without modification. Deionized water was purified by milli-Q

water system (Millipore Corporation) and filtered through various filter to remove any impurities.

Silica and Gold nanoparticles were separately synthesized by Sol-gel method and Silica particles were coated with respective shell materials (Au) in multistep process.

Silica nanoparticles were synthesized by well known Stober's method [8] Different size of monodispersed Silica nanoparticles of various sizes was synthesized by changing the concentration of ethanol and ammonium hydroxide. The Silica nanospheres were synthesized by hydrolysis of 1.2ml of tetraethoxysilane in a 10 ml of ethanol, 3 ml of water and 0.75 ml of ammonium hydroxide and solutions was stirred at room temperature for 3 hours. After completion of reaction, the Silica nanoparticles were collected by ultrafiltration. Since Gold had very little affinity towards Silica, so silane coupling agent was used to functionalize the Silica nanoparticles. The Silica nanoparticles subsequently functionalized by particles dissolved in ethanol followed by the addition of 0.5 ml of 3-aminopropyltriethoxysilane (APS). The mixture was vigorously stirred at 60°C for 15 hrs to promote covalent binding of APS moieties onto the Silica particles [9]. The amine functionalized Silica particles were centrifuged on R-24 refrigerated centrifuge (REMI) at 2000 rpm for 1 hr. The particles were thrice redispersed in 100 ml of ethanol to remove unattached APS and other contaminants.

The Gold nanoparticles were synthesized through the common techniques of citrate reduction, which has been described in detail. The preparation of Gold nanoparticles by dissolve tetrachloroaurate

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(180 mg) and trisodium citrate (600 mg) in water (590 ml), then the mixture was kept in water bath and the temperature maintained at 80°C for 30 minutes. After that Gold colloid solution was cooled to room temperature and store at 5°C [10].

The synthesis of Silica-Gold core nanoshell by attachment of clusters to amine-functionalized Silica particles to the Gold nanoparticles by Osterloh method [11]. 10 ml of the SiO₂-APS particles in ethanol were taken and washed with 20 ml of water, then after redispersed in 20 ml of water and pH maintained at 5.0. To that 1.0 ml of Silica-APS solution was added into 20 ml of Gold-citrate solution by drop wise, with stirring. After 5 minutes, the solution was centrifuged at 3000 rpm and the deep red solid was collected, then it first washed with 20 ml of water and then washed with 20 ml of methanol.

Characterization methods

Different types of nanoparticles were characterized through plasmon spectrum analysis (UV-Visible Spectroscopy), Scanning Electron Microscopy (SEM) analysis, FT-IR (Fourier Transform Infrared) spectrum studies and EDX (Energy Diffusive X-ray analysis).

UV-Visible spectra were obtained using Hitachi UV-Visible spectrometer over the wavelength range of 200-800nm. This is used to find out the plasmon peak to detect the presence of specific molecule in the sample.

The FT-IR spectrum was recorded on Perkin Elmer FTIR 1650 spectrophotometer at ambient temperature using KBr pellet method. The pellet was scanned at 16 scans at wave number range of 400-4000cm⁻¹

For SEM studies dried Silica nanoparticles, Gold nanoparticles as well as Silica-Gold core nanoshell particles were coated with gold to improve the electrical conductivity. The samples were examined at a magnification of 20000 to 150000X to find out the shape and size of the particles.

Elemental analysis of Silica-Gold core nanoshell was done by Energy Diffusive X-ray (EDX) analysis for the presence of core material in the sample. The strong signal from the sample is observed and value is noted.

The crystallographic study was performed on the synthesized Silica-Gold core nanoshell by X-ray diffractometer. The sizes of the nanoparticles were calculated using Bragg's law.

Drug loading and releasing pattern

Loading of drug was carried out by the addition of 4 ml of Gentamycin to 20 ml of nanoshell and the mixture was stirred for 24 hrs [12]. At fixed time intervals, the nanoshells were separated from the liquid by ultrafiltration and optical density of residual Gentamycin in the supernatant was measured at 285nm by UV-Visible spectrophotometer. Drug loading was determined as the difference between the initial Gentamycin concentration and the concentration of Gentamycin in the supernatant.

Drug loaded nanoshell carriers were then separated through ultrafiltration and air dried. Drug release profile was obtained by dissolving the dried drug loaded nanoshells in 5ml PBS buffer at 37°C. Concentration of Gentamycin released in the aqueous solution was observed at fixed time interval at 285nm by UV - Visible spectrophotometer.

Results

Core shell type Silica-Gold nanoparticles clusters form by reaction of 3-aminopropyltriethoxysilane (APS) - modified Silica spheres with a solution of citrate-coated Gold nanoparticles at pH 5.0. The Silica-Gold nano coreshells were isolated by ultrafiltration and stored as dispersed in methanol.

The characterization of all type of nanoparticles was studied by UV-Visible spectroscopy. The absorbance spectrum of both Stobers Silica nanoparticles absorbed at 210 nm and functionalized Silica nanoparticles revealed the presence of peak at 220nm, a specific peak for Si-O-Si bond and it confirms the presence of Silica nanoparticles. The optical absorption of pure Gold nanoparticles solutions was found at 525nm as shown in Figure 1. The UV-Visible spectra of Silica-Gold core nanoshell revealed a peak at 510nm because it shifts the wavelength slightly from red to blue due to binding of Gold with Silica and form the coreshell.

FTIR is used to identify types of chemical bonds (functional groups) between the atoms or molecules. The wavelength of light absorbed is characteristic of the chemical bond present in the chemicals. By interpreting the infrared absorption spectrum; the chemical bonds in a molecule can be determined. FTIR spectra of pure compounds are generally so unique that they are like a molecular fingerprint.

FTIR results of the present study in Figure 2 shows the spectrum of Silica, Functionalized Silica; Gold and Silica-Gold core nanoshell particles. Silica and Silica-Gold core nanoshell particles showed a peak at 1193.85cm⁻¹ which can be assigned to Si-O-Si bond and the peak at 945.05cm⁻¹ can be attributed to Si-OH bond while for functionalized silica particles peak at 3269.12 cm⁻¹ can be assigned to NH bond. In the spectrum of the functionalized silica particles coated with gold the intensity of Si-O-Si and Si-OH peaks has been reduced significantly. This indicates the presence of gold shell in silica particles.

Figure 3 represents a Scanning electron micrograph (SEM) of Silica nanoparticles, Gold nanoparticles and Silica-Gold nanoshells. It can be seen that Silica particles have well defined shapes. The sizes of the Silica particles have a diameter of 30-70 nm in Figure 3a. The sizes of the Gold nanoparticles were in the range of 10- 40 nm in Figure 3b and Silica-Gold nanoshell sizes in the range of 60-110 nm in Figure 3c. Figure 3d shows the Energy Diffusive X-ray (EDX) spectrum recorded in the particle mode from one of the densely populated nanoshell.

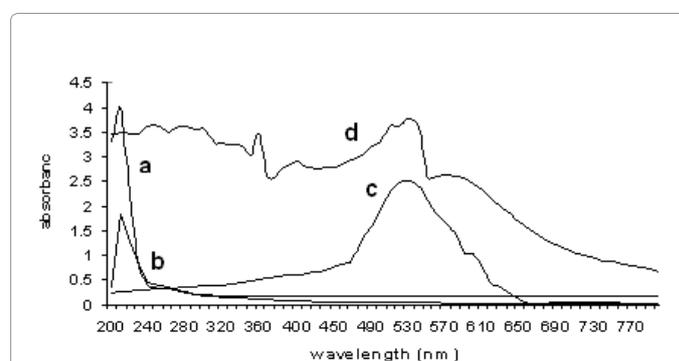


Figure 1: Comparative analysis of UV-Visible spectrum of nanoparticles (a) Absorption spectrum of Silica nanoparticles (b) Absorption spectrum of Functionalized silica nanoparticles (c) Absorption spectrum of Gold nanoparticles (d) Absorption spectrum of Silica-Gold core nanoshell.

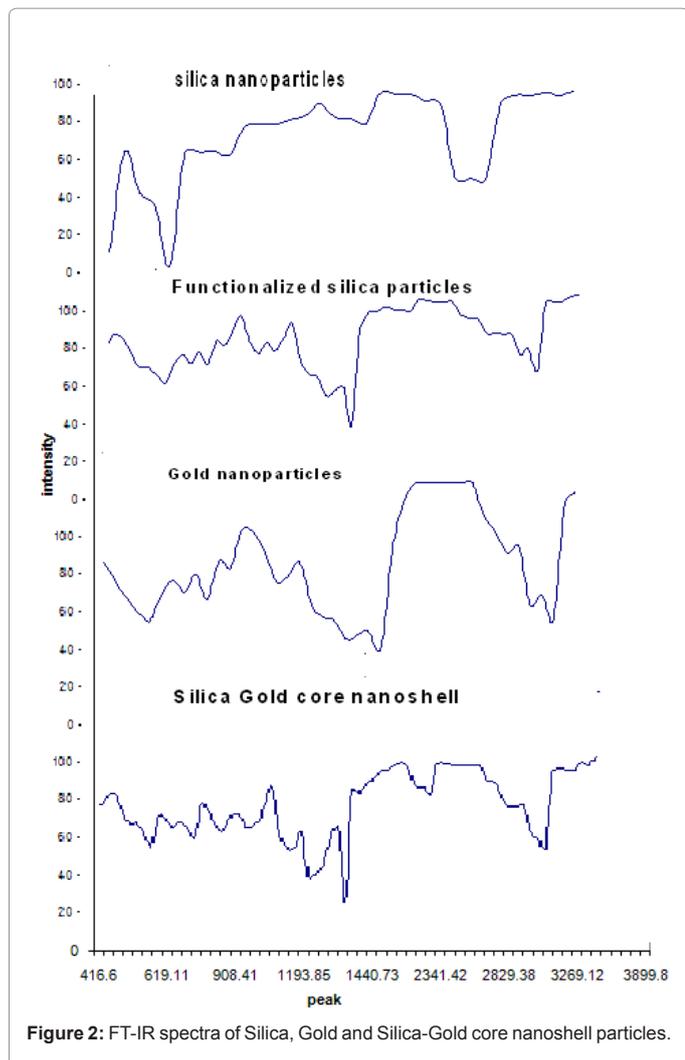


Figure 2: FT-IR spectra of Silica, Gold and Silica-Gold core nanoshell particles.

Strong signals from the Silica and Gold atoms in the nanoparticles were observed.

The elemental analysis measurements show that the Silica-Gold core nanoshell composed of metallic Au and Si and some other contaminants also present.

The X-ray diffraction pattern taken from Silica-Gold core nanoshell is shown in Figure 4. All the peaks were corresponding to the Silica-Gold diffraction. The mean values for Silica-Gold core nanoshell was calculated as 32nm based on Bragg's law. The X-ray Diffraction pattern of Silica is hidden under the pattern of Gold due to overlapping of their diffraction peaks at $2\theta=56.66; 64.82; 75; 77.8517$. The numbers of diffraction patterns of Si-OH were observed in the X-ray Diffraction spectrum indicating that the Silica nanoparticles were well protected by the Gold shell. The diffraction studies confirm a high degree of crystallinity and uniformity in the particles.

The drug loaded Silica-Gold core nanoshell has potential application in drug carrier and delivery system. The Silica-Gold core nanoshell mixed with Gentamycin solution and the amount of Gentamycin absorbed by core shell was calculated. The drug Gentamycin loading and releasing pattern to Silica-Gold core nanoshell as shown in Figure 5. Initially, the adsorption of Gentamycin was rapid by nanocarriers,

the adsorption rate slow down and reached saturation level. In 30 h of loading 87 μ g are loaded per mg of Silica - Gold core nanoshell.

The drug release pattern of nano carriers was observed in Figure 6. It is apparent from Figure 6 there is a continuous release of drug up to 30h beyond which slows down. A maximum of 25% of adsorbed drug was released in 90 h from nano carriers.

Discussion

For pure Gold nanoparticles, Plasmon shift of pure Gold nanoparticles has been observed at 525 nm and absorption spectrum of MTMOS sol-gel: Similar shift of Plasmon band for Gold nanoparticles solution has been observed at 524 nm [12]. The results of the present study were also referred to Pd-coated Silica nanoparticles, the plasmon peak observed at 520 nm and THPC Gold nanoparticles have plasmon absorption peak at 520 nm [13].

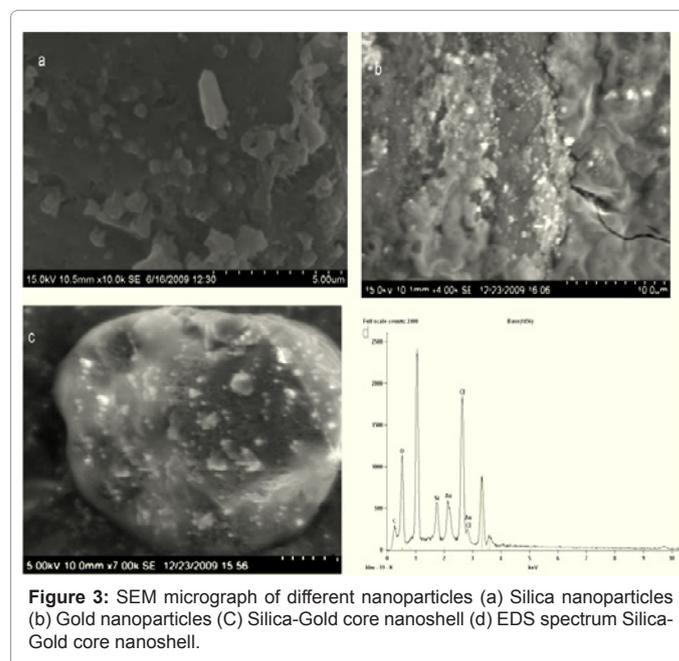


Figure 3: SEM micrograph of different nanoparticles (a) Silica nanoparticles (b) Gold nanoparticles (c) Silica-Gold core nanoshell (d) EDS spectrum Silica-Gold core nanoshell.

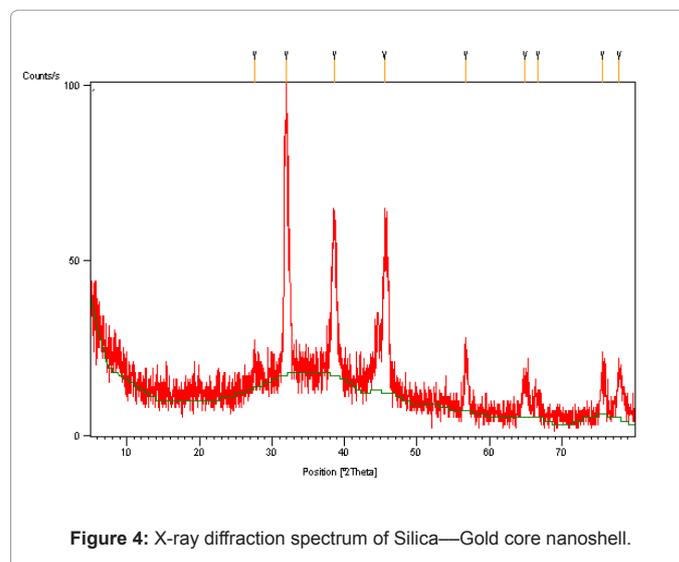


Figure 4: X-ray diffraction spectrum of Silica-Gold core nanoshell.

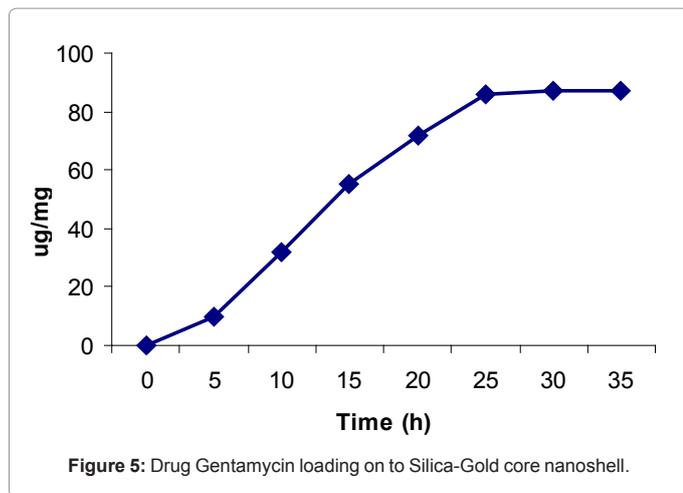


Figure 5: Drug Gentamycin loading on to Silica-Gold core nanoshell.

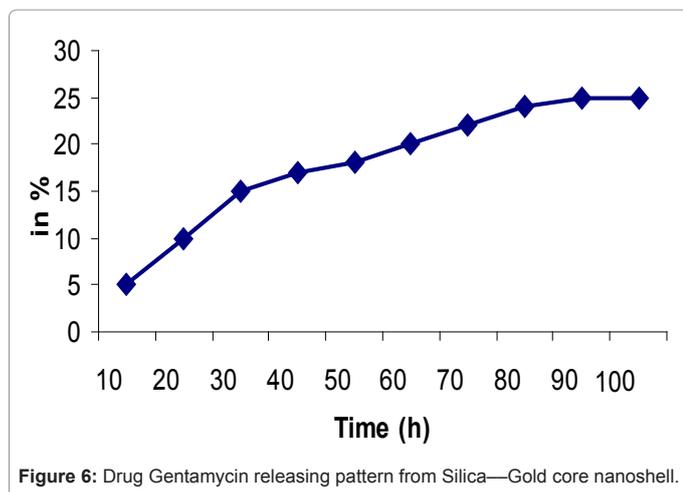


Figure 6: Drug Gentamycin releasing pattern from Silica—Gold core nanoshell.

Pos. [°2Th.]	FWHM [°2Th.]	d-spacing [Å]
27.5748	0.3149	3.23488
31.9434	0.2362	2.80175
38.5700	0.7085	2.33428
45.5367	0.3936	1.99205
56.6618	0.4723	1.62452
64.8822	0.7872	1.43716
66.5903	0.6298	1.40438
75.5772	0.7872	1.25816
77.8517	0.7680	1.22598

Table 1: Data for X-ray diffraction spectrum of Silica-Gold core nanoshell.

When an aqueous solution of Gold nanoparticles was mixed with an ethanolic solution of APS – Functionalized Silica nanoparticles, the Gold nanoparticles become immobilized on the surfaces of the Silica nanoparticles; the coverage of Gold nanoparticles is 30%, consistent with the reported coverage of charged nanoparticles on planar amine – functionalized surfaces [13]. The attachment was sufficiently strong, so that the Gold nanoparticles remain attached to the Silica nanoparticles.

The antimicrobial drugs loaded nano core shell advantage to act as vehicle for drug delivery, because it was biocompatible and protective surfaces. Also the Gold coated Silica has more hydrophobicity, which helps for the penetration of drugs into bacterial cells [14].

The pore size and environment can be tailored to selectively store

different molecules of interest [15], while the size and scope of the particles can be turned to maximized cellular uptake. Unlike polymer-based nanoparticles, these robust inorganic materials tolerated many organic solvents [16]. Silica based particles have been successfully used as drug delivery vectors [17], gene transfection agents [18] and carriers of molecules [19].

This report demonstrates the synthesis of Silica-Gold core nanoshell on the surface of Silica nanoparticles cores. The method described herein enables the synthesis of core-shell particles having dimensions substantially greater than 60-100nm. Monodispersed particles (Silica and Gold) with various core sizes 30-200nm were obtained by this reduction method. All the analyses collectively confirm the formation of Silica-Gold core nanoshell in sizes ranging from 10-100nm. The results of drug loading and releasing show that a significant amount of Gentamycin could be loaded onto the Silica-Gold core nanoshell. The amount of Gentamycin loaded in Silica-Gold core nanoshell is observed as 87µg/mg. The Gentamycin attached to the Silica-Gold core nanoshell through amine group of Gentamycin with the Gold particles. NH₂ group of doxorubicin drug was involved in binding of drug on to the gold nanoparticles surface [20]. NH group involved in finding 5-Flurouracil on to Gold nanoparticles surface area [21]. The drug release can be explained by the covalent conjugation model postulated by Ringsdorf [22], where break of Au–Gentamycin coordinate linkers results in the release of drug from core shell. In conclusion, a Silica-shell based drug delivery system can be achieved by incorporating Gentamycin in Silica-Gold core nanoshell. Future studies aiming to find out the antibacterial activity of Gentamycin drug loaded Silica-Gold core nanoshell.

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References

1. Deepika K, Suchita K, Kulkarni SK (2007) Synthesis and characterization of Silica- Gold core shell (SiO₂@Au) nanoparticles. *J Physics* 69: 277-283
2. Saran P, John DB, Son-Jong H, Alexander K (2005) Investigation of the Core-Shell Interface in Gold@Silica Nanoparticles: A Silica imprinting approach. *Langmuir* 21: 12348-12356
3. Jie Lu, Monty L, Sean S, Tian X, Michael K, et al. (2007) Mesoporous Silica Nanoparticles for Cancer Therapy: Energy-Dependent Cellular Uptake and Delivery of Paclitaxel to Cancer cells. *Nanobiotechnology* 3: 89-95
4. Nagarajan S, Yong Z (2008) Use of Core/Shell Structured Nanoparticles for Biomedical Applications. *Biomedical Engg* 1: 34-42
5. Hirsch LR, Jackson JB, Lee A, Halas NJ, West JL (2003) A Whole Blood Immunoassay Using Gold Nanoshells. *Anal Chem* 75: 2377-2381
6. Rosemary MJ, Ian ML, Pradeep T (2006). Investigation of the Antibacterial properties of Ciprofloxacin@SiO₂. *Langmuir* 22: 10125-10129
7. Zhou W, Peng GT, Lei S, Daniela C, Minghui Y, et al. (2005) Drug-Loaded, magnetic, hollow silica nanocomposites for Nanomedicine. *Nanomedicine* 1: 233-237
8. Stöber W, Fink A, Bohn E (1968) Controlled growth of mono-disperse silica spheres in the micron size range. *J Colloid Interface Sci* 26: 62–69
9. Kim JH, Chung HW, Lee TR (2006) Preparation and Characterization of Palladium Shell with Gold and Silica Cores. *Chem Mater* 18: 4115-4120
10. Kimling J, Maier M, Okenve B, Kotaidis V, Ballot H, et al (2006) Turkevitch method for Gold nanoparticles synthesis revisited. *J Phys Chem* 110: 15700-15707
11. Osterloh F, Hiramatsu H, Rhiannon P, Ting G (2004) Alkanethiol- Induced Structural Rearrangements in Silica-Gold Core shell type Nanoparticles Clusters: An opportunity for Chemical sensor Engineering. *Langmuir* 20: 5553-5558

12. Kefalides PT (1998) New methods for drug delivery. *Annals Internal Medicine* 128: 1053-1055
13. Westcott SL, Oldenburg SJ, Lee TR, Halas JH (1998) Formation and Adsorption of clusters of gold nanoparticles onto Functionalized Silica nanoparticles surfaces. *Langmuir* 14: 5396-5401
14. Darryl NW, Sheryl HE, Tracey RPH (2006) Evaluation of the microbial growth response to inorganic nanoparticles. *J Nanobiotechnology* 4: 1-8
15. Munoz B, Ramila A, Diaz I, Perez-Pariente J, Vallet-Regi M (2003) MCM-41 Organic modification as drug delivery rate regulator. *Chem Mater* 15: 500-503
16. Stein A, Melde BJ, Schroden RC (2000) Hybrid Inorganic-Organic Mesoporous Silicates-Nanoscopic Reactors Coming of Age. *Adv Mater* 12: 1403
17. Arruebo M, Fernandez PR, Ricardo IM, Jesus S (2007) Magnetic nanoparticles for drug delivery. *Nanotoday*. 2: 22-32
18. Radu DR, Lai CY, Jeftinija K, Rowe EW, Jeftinija S, et al. (2004) A polyamidoamine dendrimer-capped mesoporous Silica nanospheres based gene transfection reagent. *J Am Chem Soc* 126: 13216-13217
19. Lai CY, Jeftinija DM, Jeftinija K, Xu S, Jeftinija S, et al. (2003) A Mesoporous silica nanospheres-based carrier system with chemically removable CdS nanoparticle caps for stimuli-responsive controlled release of neurotransmitters and drug molecules. *J Am Chem Soc* 125: 4451-4459
20. Abdiche YN, Myszkowski DG (2004) Probing the mechanism of drug/lipid membrane interactions using Biacore. *Anal Biochem* 328: 233-243
21. Huang X, El-Sayed IH, Qian W, El-Sayed MA (2006) Cancer cell imaging and photothermal therapy in the near-infrared region by using Gold nanorods. *J Am Chem Soc* 128: 2115-2120
22. Ringsdorf H, Haussling L, Schmitt F-J, Knoll W (1991) Biotin-functionalized self-monolayers on gold: surface plasmon optical studies of specific recognition reactions. *Langmuir* 7: 1837.