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Study of photodynamic activity of $Au@SiO_2$ core-shell nanoparticles *in vitro*

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ABSTRACT

Metal-semiconductor core-shell type $Au@SiO_2$ nanoparticles were prepared by Stober's method. They were characterized by absorption, XRD, HR-TEM and EDAX techniques. The resulting modified core-shell nanoparticles shows that the formation of singlet oxygen, which was confirmed by ESR technique. The photohemolysis studies were carried out under two different experimental conditions. It is observed that the photohemolysis increases with concentration as well as light dose. Cell viability of the core-shell nanoparticles against HeLa cell lines were studied by MTT assay method. The outcomes of the present study indicate that, the $Au@SiO_2$ core-shell nanoparticles are extremely stable with a very high photodynamic efficiency under visible light illumination.

1. Introduction

Cancer is one of the dreaded diseases which affect the human population. Cancer claims twice as many lives worldwide as AIDS. In fact, more than 12% of all deaths every year caused by cancer [1]. Surgery, radiation, chemotherapy, immunotherapy and gene therapy have their own limitations. When dealing with large tumors, there are some risk that the central cells are not affected, which leads to recurrences. Under this circumstance, people are looking for an alternative or a complementary treatment modality for the selective treatment of tumors without altering the normal surrounding tissues or organs. In this context, photodynamic therapy has emerged as a new treatment modality due to its selective interaction with the tumour tissues. Photodynamic therapy (PDT) involves administration of a photosensitizing agent followed by irradiation at a wavelength corresponding to an absorbance band of the sensitizer [2]. The photosensitizer is taken up more by the cancer cells than the healthy cells [3]. Hence, during PDT, only tissues that are simultaneously exposed to the photosensitizer and light in the presence of oxygen are the ones subjected to the cytotoxic reactions. The conventional photosensitizers used in PDT are the Porphyrin derivatives such as Dihaematoporphyrin ester, Photoporphyrin-IX, ALA induced endogenous porphyrin and the second generation photosensitizers such as Phthalocyanines and Naphthalocyanines [4,5]. However, these classical photosensitizers have certain limitations. To overcome the drawbacks of the conventional photosensitizers, in recent years, many researchers have considered the possibility of using the non-toxic nanoparticles in PDT [6].

Among the nanoparticles, plasmonic (noble metal) nanoparticles distinguish themselves from other nanoplatforms such as semiconductor quantum dots, magnetic and polymeric nanoparticle by their unique surface plasmon resonance (SPR). This SPR, resulting from photon confinement to a small particle size, enhances all the radiative and nonradiative properties of the nanoparticles [7–9] and thus offering multiple modalities for biological and medical applications [10–15]. In this respect, gold seems very useful due to its superior physic- chemical properties, *e.g.*: corrosion resistant, low toxicity, conformational flexibility which all make this noble metal very attractive for biomedical applications [16,17]. But Au nanoparticles with diameters less than 200 nm tend to aggregate spontaneously, and their stability in the air, water or sunlight is not good enough for long term applications, which will decrease their performance. To solve this problem, a wide range of materials TiO_2 [18], SiO_2 [19], Al_2O_3 [20], zeolites [21] and activated carbon fibers [22] have been employed to support Au nanoparticles, so that the ultra-fine Ag nanoparticles can be homogeneously form without aggregation [23]. Among the various protecting layers, silica nanoparticles have attracted widespread interest for biomedical use due to their easily accessible and uniformly sized pores, stable structures, easy modification of the internal and external surface, extremely high surface area and pore volume, and biocompatibility. The Au core-shell nanoparticles would provide a new possibility

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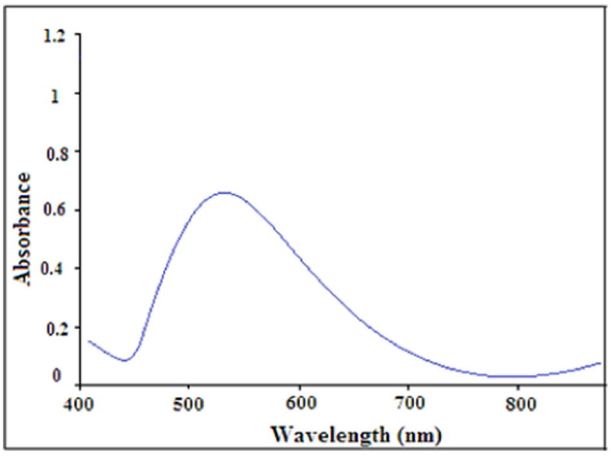

Fig. 1. UV–Vis spectra of $Au@SiO_2$ core-shell NPs.

due to their unique structures and at the same time the shell to protect Au nanoparticles and stabilize them against chemical corrosion.

In biological applications core-shell nanoparticles have major advantages over simple nanoparticles leading to the improvement of properties such as (i) less cytotoxicity, (ii) increase in dispersibility, bio- and cytocompatibility, (iii) better conjugation with other bioactive molecules, (iv) increased thermal and chemical stability and so on [24,25].

Hence, the present study pronounces the synthesize of metal-semiconductor core-shell type $Au@SiO_2$ NPs, characterization of the NPs, *in vitro* photodynamic activity of them using human erythrocytes and the cell viability of the NPs against HeLa cell lines.

2. Material and methods

2.1. Reagents

Tetraethoxysilane (TEOS) was purchased from Sigma Aldrich. Tetrachloro auric acid was obtained from CDH chemicals and all the other chemicals used were of Analar grade. Milli–Q type water was used throughout the study.

2.2. Synthesis of $Au@SiO_2$ core-shell nanoparticles

Silica nanoparticles were prepared by the dropwise addition of a mixture of ethanol (18 ml) and TEOS (6 ml) to the mixture of ethanol (42 ml) and NH_4OH containing 25% NH_3 (9 ml) under constant stirring (1500 rpm) at room temperature. The titration speed was controlled to 20 s per droplet. After the titration, the stirring was performed continuously for 1 h at 1500 rpm. The obtained silica colloid was aged for 1 day and no purification treatment was carried out.

One day old silica colloid (7.5 ml) was mixed with a water-ethanol (1:9) solution containing 0.3 mmol $HAuCl_4 \cdot 3H_2O$ with stirring. A dilute ethanol solution having formaldehyde (10 ml) was added dropwise to the above mixture at a titration speed of 14 s per droplet under constant stirring. The resultant yellow solution was then aged for 1 day; centrifuged and washed with water for three cycles to remove the residual reactants.

2.3. Characterization

UV–Visible spectra of the NPs were recorded in a Perkin Elmer Lambda 35 spectrophotometer. X-ray diffraction (XRD) patterns were taken from X'pert PRO PANalytical diffractometer operated with CuK radiation (k = 1.5406 Å) source. High resolution transmission electron microscopy (HRTEM) photographs were taken using a JEOL JEM-3010 electron microscope operated at 300 keV. The magnifying power used was 600 and 800k times. The electron spin resonance (ESR) signals were recorded at ambient temperature on a JEOL JEM FA200 instrument.

2.4. Photohemolysis

Fresh human blood was obtained from healthy volunteers and mixed with anticoagulant EDTA in the ratio 3:1. The erythrocytes were allowed to settle for an hour and the plasma leukocytes and thrombocytes were separated by aspirating the supernatant. The sediment was washed 4–5 times with phosphate buffered saline (PBS) to remove any left out plasma. A stock solution of 0.5% erythrocyte suspension was prepared by diluting 2 ml of the solution with 38 ml of PBS.

Light from LED source at 540 nm was used for irradiating the sample. The microtitre plates having wells (2.5 cm dia) containing 1 ml of the sample were irradiated at different fluences using different concentrations of $Au@SiO_2$ nanoparticles. The irradiated cell suspension was centrifuged at 1500 rpm for 10 min and the supernatant was pipetted out and its O.D at 413 nm was measured using the spectrophotometer to quantify the percentage hemolysis. The same procedure was repeated to study the role of scavengers such as sodium azide and glutathione reduced (GSH) by adding 1 ml of each scavenger separately with 1 ml of $Au@SiO_2$ nanoparticles in PBS during hemolysis.

2.5. Cell culture

The human cervical cancer line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum. In this study a total of 2×10^4 HeLa cells/ml was taken in a 2.5 cm diameter wells containing medium. The cells were maintained at 37 °C in a humidified incubator with 5% CO_2 and 95% air. After 24 h, the cells were treated with the NPs at a concentration of 50, 100, 150 and 200 μg/ml. HeLa cell plate without any treatment was used as a control. HeLa cells were incubated with the NPs in 5% CO_2 atmosphere at 37 °C for 3 h. After that, the NPs containing media were removed, and the cells were washed with PBS and then illuminated for different time periods using LED light source. After illumination, PBS was removed and complete culture medium was added.

2.6. Cell viability

The viability of HeLa cells was assessed after 3, 8, 12 and 16 h of treatment with the core-shell NPs. Cell viability was assessed using MTT assay. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate dehydrogenase cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. MTT was dissolved in PBS (5 mg/ml) and 10 μl of it was added to each well after the treatment with NPs. The resultant formazan

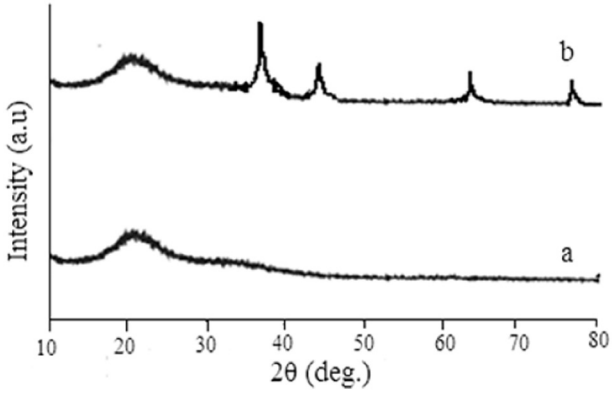

Fig. 2. XRD spectrum of $Au@SiO_2$ core-shell NPs a) air dried sample and b) sample annealed at 650 °C.