

Review: Photodynamic cancer therapy of noble metal core-shell nanoparticles in vitroK.L.DHANALEKSHMI¹, K.S.MEENA²¹Associate Professor, ²PG& Research, Department of Chemistry,¹BIST, BIHER, Bharath University, Chennai-73,²Queen Mary's College, Chennai - 04ghanamveni88@gmail.com**Abstract**

The World Health Organization states "Cancer is the leading cause of death in the economically developed countries and the second leading cause of death in developing countries". Typically surgery, radiation and chemotherapy are the most common cancer treatment modalities being used effectively in the field of cancer therapy. However, these conventional modalities have their own limitations in particular in the selective treatment of cancer. Hence, a potential and effective treatment of cancer without affecting the surrounding normal cells and tissues has long been a top priority among the clinicians. In the past few decades, photodynamic therapy (PDT) has gained the attention of the medical community and is considered as an effective treatment modality in the selective treatment of cancer, by virtue of its overcoming the drawbacks of the conventional treatment techniques. In biosciences, nanoparticles are replacing organic dyes since they have photostability and nontoxicity. Due to their adjustable surface chemistry, they can be modified to become water soluble, biocompatible and target specific. Among the metal nanoparticles, noble metal nanoparticles such as gold and silver are particularly interesting due to their size and shape dependent unique optoelectronic properties. The coating of semiconductor nanoparticles on top of the noble metals makes the nanoparticles much less toxic and bio-compatible. Hence the present study is aimed to study the core-shell nanoparticles and study their photodynamic efficiency in vitro.

1. Introduction

Cancer has become one of the most devastating diseases worldwide. Cancer claims twice as many lives worldwide as AIDS. In fact, more than 12% of all deaths every year is caused by cancer [1-6]. The word cancer is derived from the Latin word for crab because cancers are often very irregularly shaped, and because, like a crab, they "grab on and don't let go." The term cancer specifically refers to a new growth which has the ability to invade surrounding tissues,

metastasize (spread to other organs) and which may eventually lead to the patient's death if untreated [7-9]. Photodynamic therapy is the one of the emerging cancer treatment modality that takes the advantage of interaction between light and photosensitizing agent to initiate the cell death. All the cancer treatments such as surgery, radiation, chemotherapy, immunotherapy and gene therapy have their own limitations. When dealing with large tumours [10-15], 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 tumours 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 [16-21].

The organic photosensitizers used for PDT have some disadvantages, including toxicity to the human body, suboptimal tumour selectivity, low absorption of light, poor light penetration into the tumour due to the relatively short wavelength absorption and prolonged skin photosensitivity, therefore the interest in non-toxic inorganic photosensitizers such as metal oxide nanoparticles is increasing nowadays. They also have high emission efficiency, large light absorption cross section, good photoluminescence quantum yields and long emission lifetime. The core-shell composite structures, as a kind of new nanostructures, have received intense attention due to their improved physical and chemical properties over their single components [22-26].

2. Photodynamic Therapy

Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic procedure that can exert a selective cytotoxic activity toward malignant cells [27-29]. The procedure involves administration of a photosensitizing agent followed by irradiation at a wavelength corresponding to an absorbance band of the sensitizer. Clinical studies revealed that PDT can be curative, particularly in early stage of tumours. It can prolong survival in patients with inoperable cancers and significantly improve quality of life. Minimal normal tissue toxicity, negligible systemic effects, greatly reduced long-term morbidity [30-34], lack of intrinsic or acquired resistance mechanisms and excellent cosmetic as well as organ function-sparing effects of this treatment make it a valuable therapeutic option for combination treatments. With a number of

recent technological improvements, PDT has the potential to become integrated into the mainstream of cancer treatment [35-40].

PDT has the potential to meet many currently unmet medical needs. Although still emerging, it is already a successful and clinically approved therapeutic modality used for the management of neoplastic and non-malignant diseases. PDT was the first drug device combination approved by the US Food and Drug Administration (FDA) almost two decades ago, but even so remains underutilized clinically[41-45].

Compared to current treatments such as surgery, radiation therapy and chemotherapy, PDT offers advantages of an effective and selective method of destroying diseased tissues without damaging surrounding healthy tissues, a type of treatment where repeated doses can be given without the total-dose limitations associated with radiotherapy and where the healing process results in little or no scarring [4-6]. In addition, in contrast to most other cancer therapies, PDT can induce immunity, even against less immunogenic tumours, and thus contribute to long term tumour control [7]. PDT can also be used when surgery is not possible. If a patient has cancer in an organ or part of an organ that cannot be removed surgically (e.g., the upper bronchi of the lung), PDT can still treat the site. Moreover, it is a procedure that is low in cost. Finally, PDT is a repeatable therapy. Unlike radiation therapy, PDT can be used again and again. Hence, it offers a means of long-term management of cancer even if complete cure is not possible [8].

Photodynamic therapy (PDT) may be considered to be coming of age. There have been a number of regulatory approvals worldwide (10 countries in Europe, Japan, Canada and U.S.A.) for the treatment of specific cancers with Photofrin (PF). In addition, at the end of 1999 Verteporfin (Benzoporphyrin derivative monoacid A (BPD-MA)) was approved in Switzerland for the treatment of the wet form of age-related macular degeneration (AMD), the leading cause of blindness amongst the elderly people in the Western world. A panel for the U.S.A. Food and Drug Administration (FDA) has also approved it in the U.S.A. Similarly, 6-aminolevulinic acid (ALA)-induced protoporphyrin based PDT has been approved by the FDA for the treatment of actinic keratoses, a pre-malignant dermatological disorder.

In the last decade, three major events in the field of PDT have possibly contributed to this transition into the clinic: (1) appropriate industrial involvement to sponsor controlled clinical trials [9], (2) recognition of the need for a larger variety of photosensitizers with improved optical and target localizing properties [10] and (3) a better, and a more sophisticated understanding of biological mechanisms involved in PDT [11]. The consequence of all this, and in particular of the mechanistic understanding, has been a dramatic increase in the potential applications of PDT such that it may now be considered a platform technology with a broad reach into many disease areas. In fact, while the initial focus of PDT was the treatment of cancer, there has been a rapid increase in studies investigating the use of PDT for the treatment of non-cancer diseases.

3. Basic Components of PDT

PDT is a two stage procedure. After the administration of a light-sensitive photosensitizer in the tumour sites, which are irradiated with a light of appropriate wavelength. The latter can be delivered to virtually any organ in the body by means of flexible fiber-optic devices. Selectivity is derived from both the ability of useful photosensitizers to localize in neoplastic lesions and precise delivery of light to the treated sites.

PDT consists of three essential components: Photosensitizer (PS), light and oxygen (Fig.1). None of these is individually toxic, but together they initiate a photochemical reaction that culminates in the generation of a highly reactive product termed singlet oxygen ($^1\text{O}_2$). The latter can rapidly cause significant toxicity leading to cell death via apoptosis or necrosis. Antitumor effects of PDT derive inter-related mechanisms: direct cytotoxic effects on tumour cells, damage to the tumour vasculature, and induction of a robust inflammatory reaction that can lead to the development of systemic immunity. The relative contribution of these mechanisms depends to a large extent on the type and dose of PS used, the time between photosensitizer administration and light exposure, total light dose and its fluence rate, tumour oxygen concentration and perhaps other still poorly recognized variables. Therefore, determination of optimal conditions for using PDT requires a coordinated interdisciplinary effort [12,13].

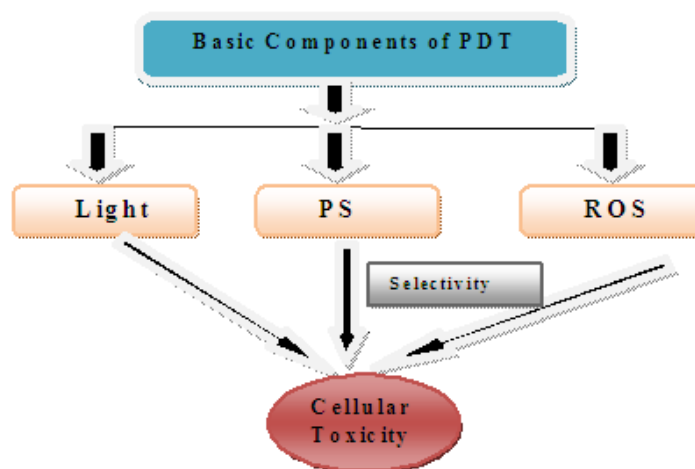


Fig.1. Factors for successful PDT

3.1. Light

The successful PDT required the photoactivation of the photosensitizer by light. Light is a form of wavelengths (λ). The energy content (E) of light is related to the wavelength of absorption by $E=h\nu = hc/\lambda$, where h is the Planck's constant, ν is the frequency, c is the speed of light in vacuum (3.0×10^8 m/s) and λ is the wavelength [14, 15]. Each photosensitizer has their particular wavelength of light needed to maximize penetration and excitation. The light penetration is limited by optical scattering within the tissue, the absorption by endogenous chromophores, and the absorption of light by the sensitizing drug (self-shielding).

The sensitizer activated by any wavelength above 800 nm is not effective to yield singlet oxygen, because the triplet state of the sensitizer is below the energy level of singlet oxygen.

Photosensitization had been performed initially with conventional gas discharge lamps. It is also possible to use metal halogen lamp, emitting 600-800 nm radiation at high power density and short-arc xenon lamp which is tunable over a bandwidth between 400-1200 nm. The broad light beam produced by incoherent lamps is useful for the treatment of large lesions. Endoscopes and

several optical fibers are developed further for interstitial therapy of larger tumours. The light delivery system has been greatly improved in the last 20 years.

3.1.1. Laser

Lasers have become the primary light source for activation because laser light is monochromatic (provide the exact selection of wavelengths), coherent (light waves are parallel permitting precise focusing), precise and intense (allowing for shorter treatment times). This property makes the laser beam to have monochromaticity and directionality which in turn make the lasers to produce a high optical energy density a few thousand times better than any non-laser light source. So we can deliver a high dose of optical energies on the surface or interior of the body. The high intensity laser radiation ensures a high rate of photochemical processes. Pulsed lasers, like the gold vapor laser (GVL) and the copper vapor laser-pumped dye laser (GVDL), produce brief pulses in duration of millisecond to nanosecond. There is no difference between the result of continuous wave and pulsed lasers. Tunable solid-state lasers, such as the neodymium: YAG laser, are particularly useful for PDT, while tunable dye lasers are frequently used in investigative studies because they allow maximum flexibility. Traditionally the normal light source for PDT has been a laser pumped dye laser. The tuneability of this device allows for maximum excitation of a variety of photosensitizers as well as facilitating endoscopic treatments to be performed via optical fiber light delivery. These large and expensive lasers are research devices requiring regular maintenance and not widely available in the clinical setting. This has restricted PDT to a few specialist centers, limiting progress due to a lack of controlled multi-centre trials.

3.1.2 Light Emitting Diode (LED)

Light Emitting Diodes (LEDs) are now being used as alternative light sources as more convenient than lasers and have longer treatment times. Preliminary evaluation of a new LED light source has shown its potential for photodynamic therapy. The light source has demonstrated a performance comparable with that of a copper vapour pumped dye laser in normal

rat liver. The results were sufficiently encouraging for us to believe the LED light source is a potentially useful tool for the treatment of superficial lesions with PDT. Many attempts have been made to improve the efficacy of PDT. Today the light-emitting diode (LED) is considered an emerging light source for PDT. An LED source can generate a high-energy beam of a desired wavelength, and can be assembled in a range of sizes and configurations, generating light by relaxation of excited electrons. They are less expensive and smaller than other types of lasers and can provide wavelengths in the range of 350 to 1100 nm. Besides light source improvement, several different classes of photosensitizers have been developed and clinical and/or preclinical trials have been performed. LED can generate high energy light of desired wavelengths and can be assembled in a range of geometries and sizes. The advance here is the highly portable energy source, for example, battery power, which is self-contained. Now the patient can be mobile during illumination. In addition various sizes of LED array are available. This prototype is currently undergoing clinical trials. This allows for the realization of prolonged outpatient illumination as well as metronomic repeated illumination (16 - 18).

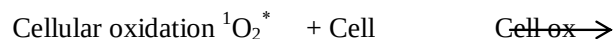
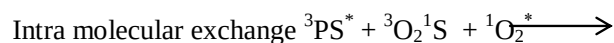
3.1.2.1. Advantages

- Due to large illumination fields, useful in the treatment of large skin lesions
- Low cost and easy availability
- Different photosensitizers with varying absorption maxima can be used.
- Shows similar efficacies as laser irradiation

3.2. The photochemical generation of oxidizing species

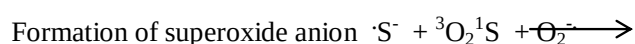
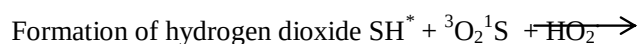
The ability of PDT to produce ROS needs the presence of molecular oxygen. The ground electronic state of oxygen is a triplet, whereby the two outer most orbitals are unpaired but spin parallel and this triplet can undergo energy transfer upon collision with the excited PS triplet. This **Type II** process involves “flipping the spin” of the outermost O_2 electron and shifting it in to the orbital containing the other electron, which in turn leaves one orbital entirely unoccupied (a violation of Hund's rule). Termed singlet oxygen ($^1O_2$), this form of oxygen (not considered a radical as its electrons are spin paired) is extremely short lived and reactive, owing to its electron configuration instability.

Type II Reactions



An alternative photochemical mechanism is termed the **Type I** when the PS triplet directly transfers an electron, sometimes in concert with proton donation to O_2 , yielding superoxide anion (O_2^-) which can then go on to form other ROS including the hydroxyl radical ($\bullet\text{OH}$), and hydrogen peroxide (H_2O_2).

Type I Reactions



The ROS formed through the Type I process have a range of different reactivities. $\bullet\text{OH}$, arguably the most reactive of the three ROS formed, is a strong electrophile that is able to chemically attack a very wide range of biomolecules. H_2O_2 is less reactive and O_2^- is least reactive.

3.2.1. Singlet oxygen

Singlet oxygen (${}^1\text{O}_2$) was first observed in 1924 [19] and then defined as a more reactive form of oxygen [20]. The main method of production of ${}^1\text{O}_2$ is by photosensitization reactions [21]. In 1931, Kautsky first proposed that ${}^1\text{O}_2$ might be a reaction intermediate in dye-sensitized photooxygenation [22]. Following this suggestion, many studies have showed that ${}^1\text{O}_2$ is an important intermediate species in the detrimental oxidation of biomolecules. It has been demonstrated now that ${}^1\text{O}_2$ can react with many kinds of biological molecules such as DNA, proteins and lipids [23]. Since oxygen is ubiquitous and efficiently quenches electronically excited states, ${}^1\text{O}_2$ is likely to be formed following irradiation in countless situations and involved in various chemical and biological processes as well as in several diseases.

processes. Singlet oxygen is used in several main areas; such as wastewater treatment, fine chemical synthesis and photodynamic applications [20].

3.2.1.1. Electronic structure of singlet oxygen

Molecular oxygen has two low-lying singlet excited states, $^1\Delta_g$ and $^1\Sigma_g^+$; 95 (22.5 kcal mol⁻¹) and 158 kJmol⁻¹ (31.5 kcal mol⁻¹) above the triplet state respectively. Electronic configurations of these states differ only by the structure of the p-antibonding orbitals. The configuration of the molecular orbitals of the first excited state, $^1\Delta_g$, is as follows: $O_2 KK (2\sigma_g)^2 (2\sigma_g)^2 (3\sigma_g)^2 (1\sigma_g)^4 (1\pi_g^+)(1\pi_g^+)$. In the second excited state, $^1\Sigma_g^+$ electronic configuration is identical to that of the ground state, except that the last two electrons have antiparallel spins [24].

The transition from the $^1\Delta_g$ state to the $^3\Sigma_g^-$ state is spin forbidden, thus the $^1\Delta_g O_2$ is a relatively long-lived species. The second excited state of oxygen, on the other hand, is short-lived due to a spin-allowed transition to the $^1\Delta_g$ state. This difference in stability is confirmed by the radiative lifetimes of $O_2(^1\Delta_g)$ and $O_2(^1\Sigma_g^+)$; which are 45 min and 7-12 s in the gas phase [25] and 10^{-6} – 10^{-3} s, and 10^{-11} - 10^{-9} s in solution [26], respectively. Due to the metastability of the $^1\Delta_g$ state, the $^1\Delta_g \rightarrow ^3\Sigma_g^-$ transitions at 1268.7 nm are observed in absorption and emission spectra despite being spin and symmetry forbidden [27].

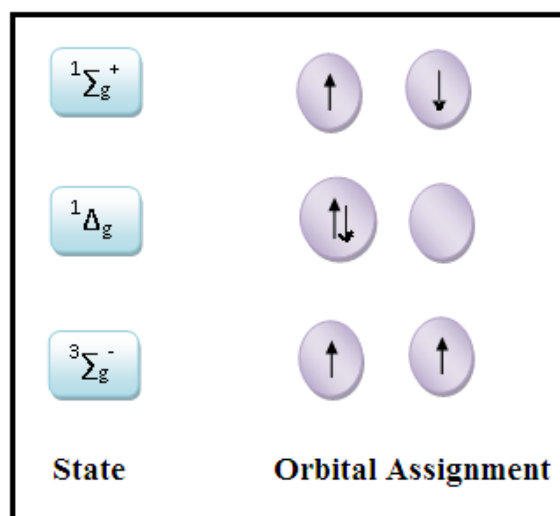


Fig.2. Primitive representations of molecular oxygen lowest singlet and triplet states.

4. Nano – PDT

The first generation photosensitizers such as porphyrin derivatives have some disadvantages due to their planar structures, porphyrin derivatives tend to aggregate due to strong π - π stacking in concentrated solutions, which can reduce the quantum yield of singlet oxygen [28], porphyrin derivatives are synthetically inaccessible, and it takes great effort to produce large amount of such photosensitizers by conventional organic synthetic strategy. In addition, porphyrin derivatives usually exhibit small stoke shifts, thus limiting their applications in imaging. Although the second generation PS has been shown some disadvantages, including suboptimal tumor selectivity [29], low absorption of light and poor light penetration into the tumor due to the relatively short wavelength absorption [30], prolonged skin photosensitivity of patient [31] and the fact that it was a complex mixture of uncertain structure [32]. Some other chromophores have been reported for PDT study, such as phthalocyanine, squaraine dye, and perylene diimide however, these photosensitizers suffer from poor chemical stability and photostability and easily aggregate in a polar environment. To overcome the draw backs of conventional photosensitizers many researchers in recent year have consider the possibility of using non –toxic nanoparticles in PDT and it is often referred as Nano – PDT [33-35].

4.1. Advantages of Nanoparticles in PDT

Nanoparticles are having the following advantages over the conventional organic compounds

- They are photostable and nontoxic.
- They have also large absorption cross section, good photoluminescence quantum yields and long emission lifetimes.
- Due to their adjustable surface chemistry, they can be modified to become water soluble, biocompatible and target specific.

5. Noble Metals as Nanosensitizers

Noble metal nanoparticles such as gold and silver are particularly interesting due to their size and shape dependent unique optoelectronic properties. These noble metal nanoparticles, have elicited a lot of interest for important biomedical applications because of their ease of synthesis, characterization and surface functionalization. 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 [36-38] and thus offering multiple modalities for biological and medical applications [39-44]. Au and Ag show largely red-shifted properties boosting their values in photodynamic cancer therapy [45-47]. The strongly enhanced radiative properties such as absorption, scattering and plasmonic field for surface enhanced Raman of adjacent molecules make them extremely useful for molecular cancer imaging [48-51]

5.1. Noble metal nanoparticles in Cancer therapy

The size and shape of nanoparticles also play a large role in relation to cellular uptake in vitro. Spherical gold nanoparticles have higher cellular uptake than gold nanorods owing to variable biophysical properties such as receptor diffusion kinetics [52]. Noble metal nanoparticles might prove to be more effective since they have been shown to target multiple pathways.

AuNPs were passively taken up via diffusion through the cellular membrane. It may also be possible to tailor the endocytotic uptake of nanoparticles. The cellular uptake of metallic nanoparticles has also been reported to be size specific, with 40–50 nm having the greatest effect on internalization into cells [53-55]. Chan et al. has reported that 50 nm gold particles can enter into cell at a faster rate with higher amount relative to the other sizes. Silver nanoparticles have been found in the cytoplasm and mitochondria of primary liver cells as well as the mitochondria and the nucleus of fibroblasts [56,57]. AgNPs are directly toxic to the cancer cells through DNA damage and increased production of reactive oxygen species [58].

Surface charge is also an important factor that moderates cellular uptake of nanoparticles. The functionality of the nanoparticle surface further allows specific or nonspecific interactions within the cellular lipid bilayer. Since the cellular exterior is largely anionic, positively charged nanoparticles can easily transverse the cellular membrane via electrostatics. Nonetheless, negatively charged nanoparticles have also been observed in the cytosol [59,60]. This is most likely due to the nanoparticles passively targeting lipophilic domains. Cationic nanoparticles have also been shown to modulate membrane potential of cells and their subsequent downstream intracellular events. In addition, the Rotello group has also reported that zwitterionic nanoparticles can be highly efficient delivery system [61,62]. Pioneering work by Pitsillides illustrated that the surface plasmon resonance (SPR) of nanoparticles is easily exploited for PDT anti-cancer therapy [63]. Noble metal nanoparticles to thermally ablate cancer cells and tumor tissue in vitro and in vivo [64].

The uptake of nanoparticles in tumour site was decreased by 25% with aggregated nanoparticles. Ag and 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 [65] which will decrease their performance. To solve this problem, a wide range of materials such as TiO_2 [66], SiO_2 [67] and ZrO_2 have been employed to support Ag and Au nanoparticles, so that the ultra fine Ag and Au nanoparticles can be homogeneously formed without aggregation [68]. It could be reasonably envisioned that Ag and Au cores would release Ag and Au ions slowly through the outer porous coating layer, thereby producing and maintaining the excellent biological effects [69].

6. Core- Shell nanoparticles

Core-shell nanoparticles have a core made of a material and coated with another material on top of it. 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 cyto-compatibility, (iii) better conjugation with other bioactive molecules, (iv) increased thermal and chemical stability and so on. More elaborately, when the desired nanoparticles are toxic which may cause plenty of trouble to the host tissues and organs. The coating of a benign material on top of the core makes the nanoparticles much less toxic and bio-compatible. Sometimes shell layer not only acts as a nontoxic layer, but also improves the core material property. In case of semiconductor materials shell of other material improves the optical property, photo stability. The hydrophilicity of nanoparticles is very important to disperse them in biological systems (aqueous). The increase in bio-dispersivity, bio- and cyto- compatibility makes it a useful alternative to conventional drug delivery vehicle. The ease of synthesis also plays an important role in attracting the attention of researchers to this class of materials. When the core material is hydrophobic, coat of a hydrophilic material onto core surface in the form of core/shell nanoparticles can overcome the problem of dispersibility, bio and cytocompatibility. The presence of semiconductor shell enhances PDT by redshifting the original absorbance [70].

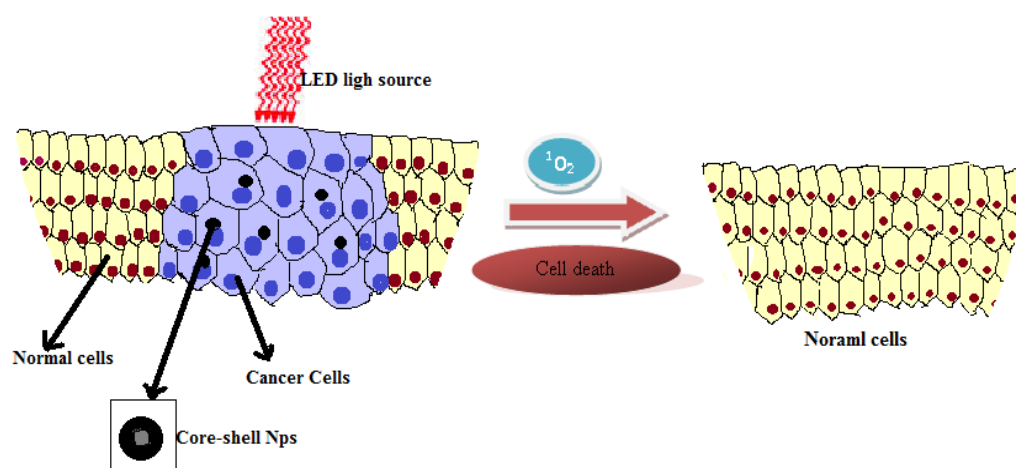


Fig.3. Photodynamic therapy illustration using core-shell NPs

7. Photohemolysis

Photohemolysis refers to the opening of red blood cell (RBC) membrane and release of hemoglobin induced by exposure to light. Photohemolysis of RBC in vitro is photosensitized by many other dyes and clinical drugs. This test is used as a predictor of agents that may photosensitize human skin. In the present work we used photohemolysis of human erythrocytes as a model system. Red blood cell (RBC) photohemolysis is a convenient system for assaying cell membrane disruption because the hemoglobin released is due exclusively to membrane damage and can be easily measured.

The "colloid-osmotic" model is generally accepted as the basic explanation for photohemolysis. According to this theory, photochemical damage to the RBC membrane initiates cation leakage, followed by swelling of the cells and hemolysis. These responses to light have been observed experimentally. Other effects which may be related to the photohemolysis mechanism include changes in RBC morphology and fragility induced by dark binding of some photosensitizers, photoperoxidation of membrane lipids, photolysis and photocrosslinking of membrane proteins, and photooxidation of encapsulated hemoglobin. The relationship between specific damage modalities in the photochemical stage of the process and hemolysis during dark incubation has not been established. Different primary photochemical reactions are feasible depending on the photosensitizer and reaction conditions. These include the well-known Type 1 and Type 2 photosensitization pathways mediated by the sensitizer [71].

6.1. Estimation of percent hemolysis

An ideal sensitizer should have negligible dark toxicity (i.e.) it should not produce any cytotoxic effect on the cells when added to the cellular suspension. The increase in the O.D at 413 nm is an indication of the lysis (damage) of the erythrocytes.

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 2ml of the solution with 38ml of PBS.

Light from the LED source was used for irradiating the sample. The microtitre plates having wells (2.5cm dia) containing 1ml of the sample were irradiated at different fluences using different concentrations of nanoparticles. The irradiated cell suspension was centrifuged at 1500 rpm for 10min and the supernatant was pipetted out and its O.D at 413nm was measured using a spectrophotometer to quantify the percentage hemolysis.

7. Cell viability

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. The measurement of cell viability plays a fundamental role in all forms of cell culture. Cell-based assays are often used to show direct cytotoxic effects that eventually lead to cell death. Among the cell viability assays, the MTT assay (3 – [4,5 – dimethylthiazol -2-yl] -2,5 – diphenyl tetrazolium bromide) is the best known method for determining mitochondrial dehydrogenase activities in the living cells, because it is easy-to-use, safe, has a high reproducibility, and is widely used in both cell viability and cytotoxicity tests. In this method, viable cells with an active metabolism convert MTT into a purple coloured formazan product with an absorbance maximum near 570 nm. When cells die, they lose the ability to convert MTT into formazan, thus color formation serves as a useful and convenient marker of only the viable cells. The exact cellular mechanism of MTT reduction into formazan is not well understood, but likely involves reaction with NADH or similar reducing molecules that transfer electrons to MTT [72].

7.1. 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% CO₂ and 95% air. After 24 h the cells were treated with nanoparticles at different

concentration. HeLa cell plate without any treatment was used as a control. HeLa cells were incubated with nanoparticles in 5% CO₂ atmosphere at 37°C for 3h. Afterward, compounds 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.

7.2. MTT assay

The viability of HeLa cells were assessed 16 h after treatment. 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 (5mg/ml) and 10 µl of it was added to each well following the 16h of incubation. The cells were then incubated for further 4h. The resultant formazan crystals were dissolved in dimethyl sulfoxide (200µl) and the absorbance intensity was measured at 570nm [73-75].

8. Ag@SiO₂ and Au@SiO₂ core-shell nanoparticles

Inorganic materials are superior to organic materials in terms of their durability, heat resistance, toxicity, selectivity and various other characteristics [76]. Among the inorganic nanoparticles, silver and gold that exhibit low toxicity in human, has diverse in vitro and in vivo applications [77]. The principal characteristic of Ag and Au nanoparticles is their high surface area to volume ratio, resulting in the appearance of new mechanical, chemical, electrical, optical, magnetic, electro-optical, and magneto-optical properties of the nanoparticles that are different from their bulk properties [78]. Coating of metal nanoparticles to semiconductor nanoparticles, makes it more susceptible to corrosion [79]. An efficient way to overcome these drawbacks is to exploit a core-shell type structure in which the noble metal is introduced as core and the semiconductor dioxide such as shell [80]. By changing the thickness of the shell and the particle radius the overlap of the wave functions and the bandgap can be changed [80]. The presence of a shell covering metal nanoparticles is important [81,82] because it will separate the metal nano-

spheres, avoiding touching with one another, and can also make metal particles more stable in air.

Among the various protecting layers, SiO_2 can be an ideal candidate for its facile synthesis, abundant available precursors and environmental compatibility. Silica-coated noble metal particles are important metal-based hybrid nanostructures [83]. In a metal@silica particle, the silica shell is inert and optically transparent. Silica-coated nanoparticles have been intensively studied due to their feasible manipulation and extensive application in biosensors and non-linear optical materials. Silica nanoparticles have attracted wide spread interest for biomedical use due to their easily accessible and uniformly sized pores, stable structures, easy accessible and uniformly sized pores, stable structure, easy modification of the internal and external surface, extremely high surface area and pore volume, and biocompatibility. On the basis of their properties, silica nanoparticles have emerged as an efficient drug delivery platform, and also stood out in their versatility as an ideal platform for building up of multimodeltheranostic nanoparticles [84-89].

Protected by an outer SiO_2 layer which has many polar hydroxyl groups at the surface, here the Ag@SiO_2 and Au@SiO_2 hybrid nanoparticles are highly stable. Silica is an inert, robust and optically transparent material. The silica shells not only enhance the colloidal stability, but also control the distance between core particles within assemblies by the thickness of the shell. Furthermore, this shell is hydrophilic, biocompatible and easy to functionalise with several groups using different silane coupling agents. These unique characteristics and the possibility of bio-conjugation made silica coating an attractive route for bio-analytical and medical applications of metal nanoparticles [90]. The concentric silica shell formation around metal NPs would provide a means to further tune the functional and interfacial properties of the NPs and thus make them more stable, processable and versatile [91-97]. While a thin dielectric silica coating has the potential to modify and stabilize the plasmonic properties [98-101]. Compare to the other nanoparticles, core-shell nanoparticles shows better photodynamic activity because the rate of production of reactive oxygen species is high. Fig 4 (a-d). shows the variation of photodynamic activities of different concentration of core-shell nanoparticles with varying light dose [102]. In Fig.4 (a-d), it is observed that the photodynamic activity increases with concentration as well as light dose.

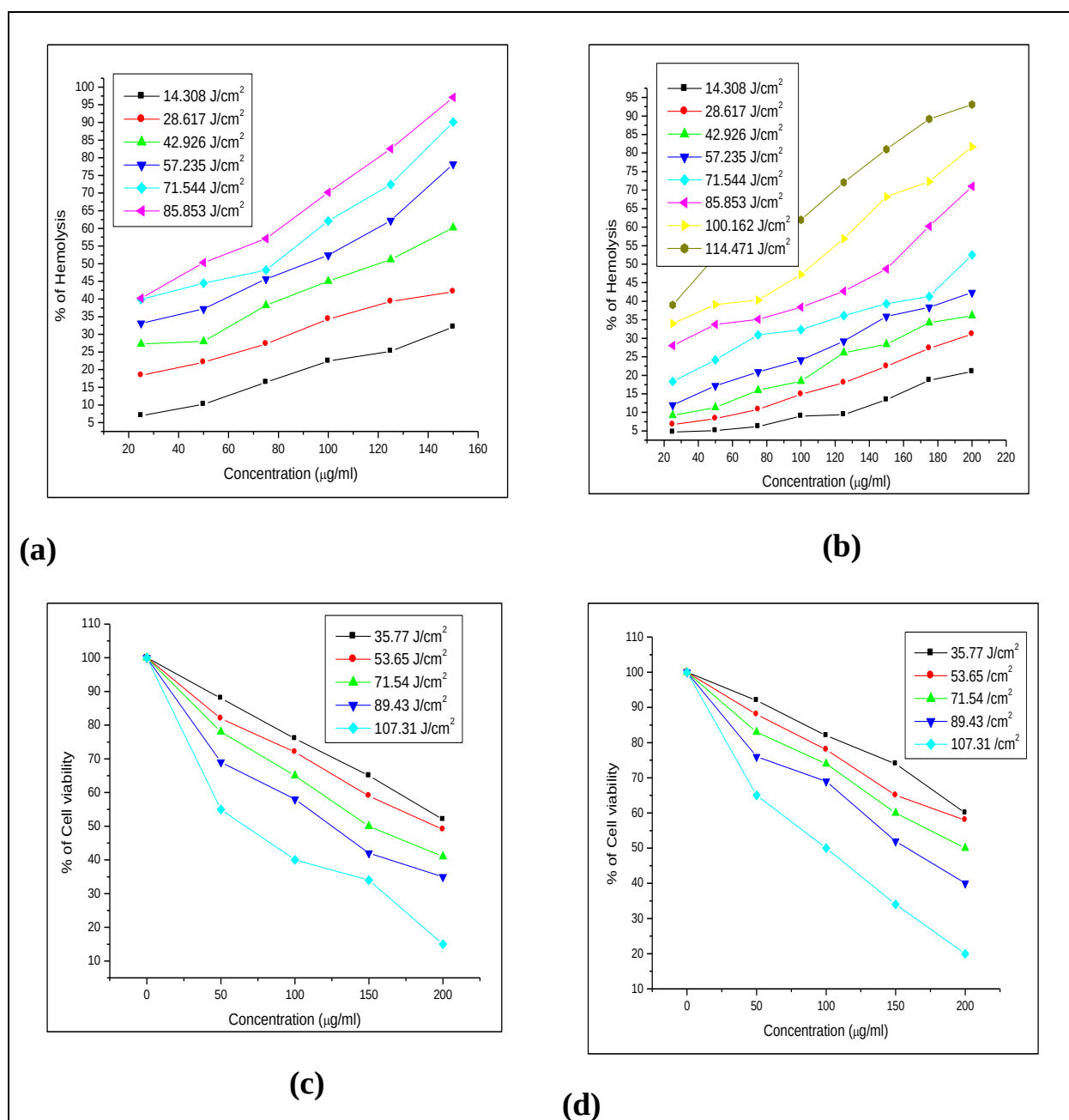


Fig. 4 – a& b) Photohemolysis of Ag@SiO₂ and Au@SiO₂ core-shell NPs, c & d) Cell viability of Ag@SiO₂ and Au@SiO₂ core-shell NPs

9. Ag@TiO₂ and Au@TiO₂ core-shell nanoparticles

In recent years gold and silver nanoparticles coated with TiO₂ semiconductor nanoparticles are increasing, because TiO₂ has proven to be the most suitable for widespread environment application for its high chemical stability, non-toxicity, low cost and excellent degradation of organic pollutants [103-106]. However, it lacks its efficiency due to high band gap and low quantum yield. As one of the application of TiO₂ nanoparticle photocatalysts, there is a report that suggested the photokilling property by photoexcited TiO₂, which can be used in the biology application, especially in the field of cancer treatment [107]. When TiO₂ absorbs the light with energy higher than its band-gap energy in the UV region, holes and electrons are generated. The photogenerated holes and electrons have the oxidation and reduction properties respectively, which can cause various photocatalytic reactions [108-112]. The reaction between the oxygen and electrons in the conduction band of TiO₂ can produce reactive oxygen species, which can destroy the structure of cancerous cells. The photokilling property of TiO₂ made it possible to utilize TiO₂ in the photodynamic therapy as a photosensitizer. TiO₂ however, has a disadvantage of being excited only in the UV light region, but UV light cannot penetrate into the biological tissues deeply.

As we know, silver and gold nanoparticles have very nice absorption ability in the visible light region because of the surface plasmon oscillation. Contact of silver and gold with TiO₂ can form the metal-semiconductor Schottky junction [113, 114], which can possibly make the charge separation lifetime long, if electron transfer occurs from silver to TiO₂. Electron collective motion in noble metal nanoparticles may enhance the electric field between metal nanoparticles and TiO₂, which may enlarge the electron density in TiO₂ making the electron transfer efficiency higher. The nice absorption property of silver and gold spherical nanoparticles will make bioapplication of silver/gold-TiO₂ systems possible and interesting utilization of visible light, because the visible light can easily penetrate into biological tissues. As a result, the electrons in the TiO₂ can react with oxygen to generate reactive oxygen species, which may kill cancer cells [115,116]. Fig. 5 (a-d) shows the photodynamic activity with different concentration of core-shell nanoparticles and light dose.

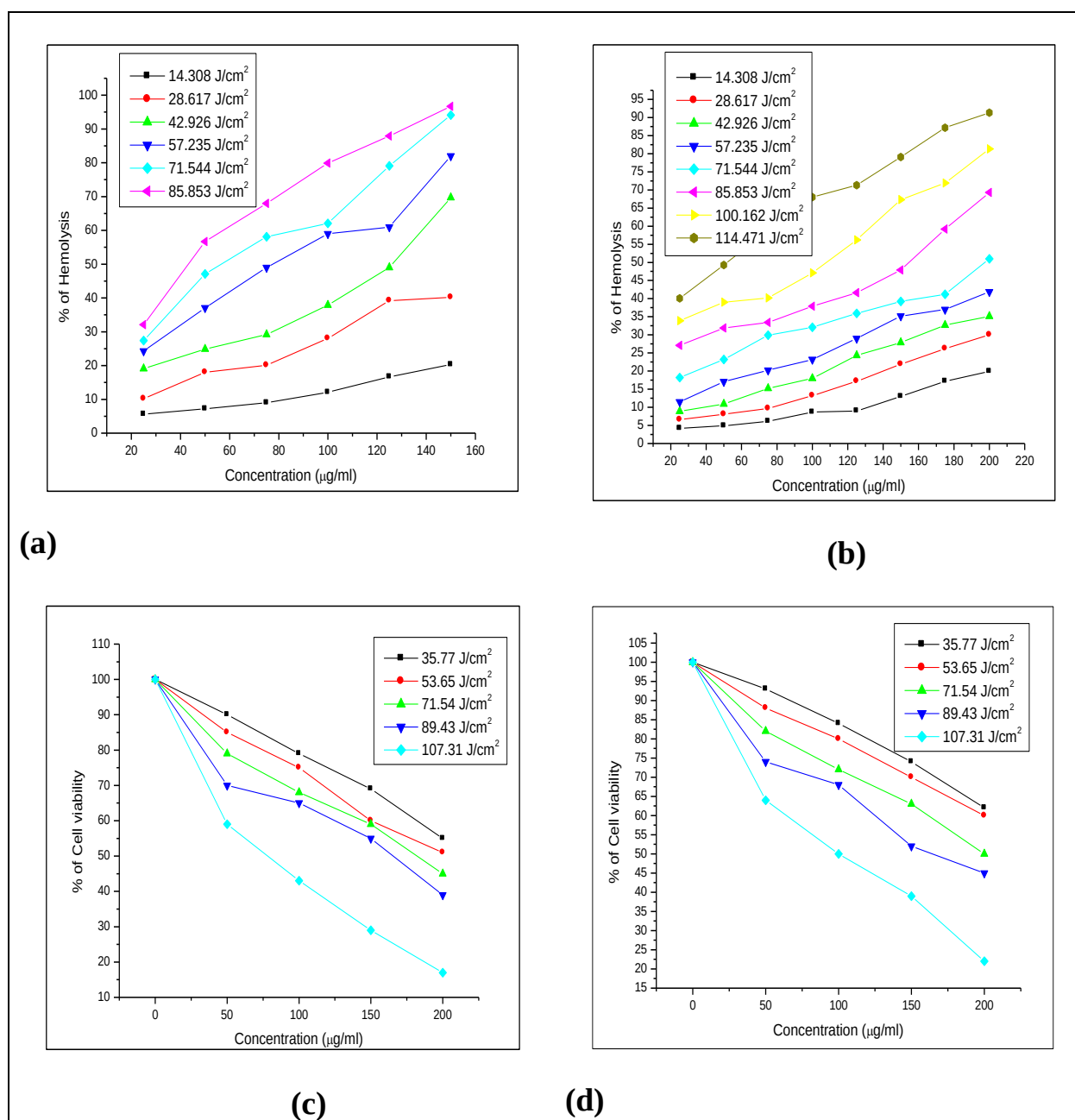


Fig. 5 – a & b) Photohemolysis of Ag@TiO₂ and Au@TiO₂ core-shell NPs, c & d) Cell viability of Ag@TiO₂ and Au@TiO₂ core-shell NPs

10. Ag@ZrO₂ and Au@ZrO₂ core-shell nanoparticles

Recently gold and silver samples supported on mesoporous zirconia have also been studied [117-119]. The opportunity of use ZrO₂ as support is due to its intrinsic characteristics: chemical and surface features of zirconia, as surface acidity, redox properties, porosity and surface area, can be adjusted by choosing different precursors and synthesis conditions and by dopants addition. Researchers have demonstrated that ZrO₂ had strong affinity for the phosphoric group and proved to be a fixed ideal material of biological molecules [120-121]. ZrO₂ which can attach with the phosphate group of DNA was used for the antibody loading, which can not only prevent the leakage of antibody, but also accelerate the electrons between the protein molecule and electrode [122].

In recent years, nano size zirconia (ZrO₂) has been generated with a lot of interest due to its specific optical, thermal, chemical and electrical properties. It can be used for many potential applications such as transparent optical devices and electrochemical capacitor electrodes, sensors, synthetic gemstone, fuel cells, catalysts [123], photo catalysts and advanced ceramics [124-126]. It is a well known industrial ceramic material for the present generation because of its high refractive index and high oxygen ion conduction. These properties have led to the use of ZrO₂ based components in many engineering applications such as automobile engine parts, and cutting tools. The relatively high coefficient of thermal expansion and low thermal conduction make nano ZrO₂ a suitable material for thermal barrier coating on metal components. The unique high temperature dielectric properties of nano zirconia (ZrO₂) make it useful in the preparation of a variety of functional ceramic components such as oxygen sensors [127,128] piezoelectric, pyroelectric ceramics, ferroelectric ceramics and a variety of transparent toughened structural ceramic products. Due to its interesting features ZrO₂ has drawn a great attention and studied extensively. Among the ceramic semiconductors, zirconia is a special type of material that has unique bifunctional characteristics of weak acid and weak base properties [129, 130]. The approach for designing and synthesizing such material in a core-shell model has attracted considerable attention. Fig.6 (a-d) shows the variation of photodynamic activities of different concentration of core-shell nanoparticles with varying light dose [131].

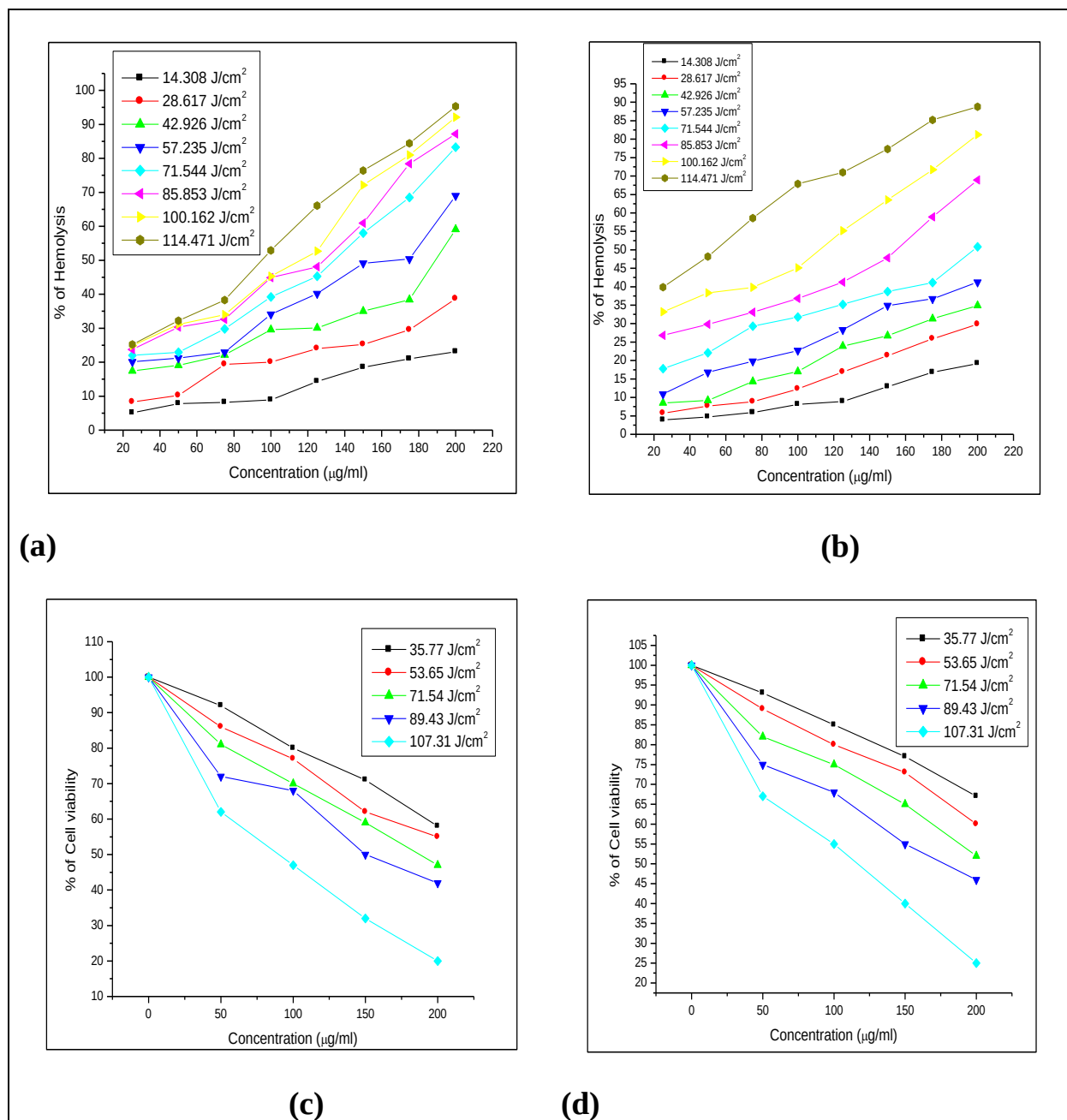


Fig. 6 – a & b) Photohemolysis of Ag@ZrO₂ and Au@ZrO₂ core-shell NPs, c & d) Cell viability of Ag@ZrO₂ and Au@ZrO₂ core-shell NPs

11. Conclusion

Photodynamic therapy has emerged as one of the important therapy for the treatment of cancer. In Nano-PDT the nanophotosensitizer that can be preferentially accumulated in the tumour site due to their small size. Although new sensitizers are required with absorption band at longer visible region to achieve deeper penetration in the tissues, core-shell nanoparticles is still considered for PDT, as it may be used for treating superficial tumors lesions. This study concludes that the core-shell nanoparticles such as Ag@SiO₂, Ag@TiO₂, Ag@ZrO₂, Au@SiO₂, Au@TiO₂ and Au@ZrO₂ can very well be explored as novel nano-photosensitizers in the place of conventional photosensitizers such as organic dyes. Among the above core-shell nanoparticles, Ag@SiO₂ core-shell nanoparticles shows higher photodynamic activity than the other core-shell nanoparticles under LED light source illumination because it produced higher reactive oxygen species than the other core-shell nanoparticles.

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