

Synopsis

There is considerable current research interest in the use of biocompatible dielectric and metallic nanoparticles (NPs) for applications like biomedical imaging, diagnosis and therapy. NPs can be loaded with the dyes/drugs or genes and also functionalized for targeting specific cells/ cellular sites thus improving the diagnostic and therapeutic efficiency. Organically modified silica nanoparticles (SiNP) have been used as carrier of hydrophobic dyes/drugs and genetic materials in cells and animal models [1, 2]. These NPs are amphiphilic in nature having a hydrophobic core, and their surface is charged depending upon the silica precursors, used to synthesize them. Therefore, there is a possibility of electrostatic binding with oppositely charged molecules at the NP surface. While SiNP (size~30 nm) are transparent in the visible region metal NPs show localized surface plasmon resonance (LSPR) ranging from UV to NIR region (depending on the metal type, size, shape, structure, composition and also the dielectric constant of the environment). The strong SP fields enhance their extinction coefficients by 5-6 orders of magnitude (per NP) as compared to strongly absorbing/fluorescing organic dye molecules [3]. These NPs can therefore be used as contrast agents with the added advantage that in contrast to fluorescent probes these do not get bleached under long exposure. Since LSPR is sensitive to the surrounding environment, these NPs have also been used for sensing applications. As gold is inert, its NPs are being actively explored for imaging and therapeutic applications. Among the different shapes of gold NPs, gold nanorods show highest extinction coefficient. Their longitudinal surface plasmon (L-SP) band can be tuned from visible to NIR region, by a change in their aspect ratio. These are therefore

also being studied for photothermal effects. However, rods are commonly synthesized in the template of a surfactant, cetyltrimethylammonium bromide (CTAB), which has been shown to be potentially toxic. Therefore, these need to be coated with biocompatible polymers to minimize this toxicity. These polymers are generally charged and therefore offer the possibility of electrostatic interaction with the oppositely charged dyes/drugs. It should be noted that binding of a dye/drug to NPs may lead to some changes in its photophysical and photochemical properties and thus may affect its functionality. We have therefore, investigated the changes in the photophysical properties of dyes as a consequence of binding to the NP surface. The results of our investigations on several dyes of biomedical significance are presented in this thesis.

Interaction of SiNP with two sets of dyes was investigated. The first set included dyes, such as 8-anilino-1-naphthalenesulfonate (ANS), 6-p-toluidino-2-naphthalenesulphonate (TNS) and Merocyanine 540 (MC540), which are negatively charged polarity sensitive dyes. These dyes undergo structural changes in their excited states in polar medium due to electron re-distribution and dominantly decay non-radiatively. As a consequence, these are weakly fluorescent in aqueous medium. Studies on the interaction of these dyes with SiNPs, having positive charge on their surface, form the first part of the thesis. Significant shifts in the absorption and emission peak positions and enhancement in their fluorescence intensity was observed on binding of these dyes with SiNPs. This was attributed to the suppression of the excited state charge transfer process in case of ANS and TNS. In case of MC540, which is also a photodynamically active dye, the excited state photoisomerism process is suppressed due to binding to SiNP. This resulted in enhanced phototoxicity of the dye-NP complex against cancer cells in-vitro.

Another set of dyes studied were porphyrin type photosensitizers Chlorin p_6 (Cp_6) and purpurin 18 (PP18). Both are interesting photosensitizers because these have significant absorption in the longer wavelength (650-900 nm) where tissue absorb and

scatter weakly and provide high singlet oxygen yield. Cp_6 is amphiphilic and negatively charged at physiological pH due to the presence of three carboxylic acid groups. Electrostatic binding of this drug to SiNP leads to an increase in its photostability and is the probable cause for higher phototoxicity against cancer cells. Although PP18 absorbs strongly than Cp_6 in the therapeutic window, its use as a drug for photodynamic therapy (PDT) is limited by the fact that it is hydrophobic and therefore needs carrier to get solubilized in aqueous medium. However, in aqueous medium this gets converted to Cp_6 due to hydrolysis of its anhydride ring. Suitability of SiNP as carriers of PP18 in aqueous media was investigated by studying its time dependent conversion to Cp_6 . This forms the second part of the thesis.

In the last part of the thesis, results on studies on the effect of large localized fields generated due to longitudinal surface plasmon resonance (L-SP) of polymer coated gold nanorods on the absorption and emission properties of molecules attached on their surface are presented. For these studies the L-SP peak of the gold nanorods was tuned to resonance or off-resonance with respect to the absorption maximum of Methylene Blue (MB) and Nile Blue (NB). L-SP peak tuning showed significant change in the spectroscopic properties of MB and NB due to coupling between L-SP and molecular absorption. On the other hand, the spectroscopic properties of Cp_6 , which does not have an absorption maximum near the L-SP were observed to depend upon the chemical nature and thickness of the polymeric coatings over the gold nanorod.

The organization of the thesis is as follows:

Chapter 1: In this chapter a brief introduction on importance of nanoparticles in biomedical applications is given. The interest in silica NPs, especially ORMOSIL NPs and their use for drug encapsulation and delivery is reviewed. Possible effect of electrostatic interaction with NP on the photophysical properties of fluorophores/photosensitizers is discussed. Work carried out by different groups on the

size, shape and composition dependent surface plasmon resonance properties of gold nanostructures is reviewed. Biomedical applications of gold nanorods and the possible effect of their LSPR on the photophysical properties of fluorophores/photosensitizers attached to them are discussed.

Chapter 2: In this chapter details of the preparation procedure of the ORMOSIL NPs and gold nanorods and subsequently their characterization are given. NPs were prepared in the micellar core of surfactants following the wet chemical methods available in the literature. These were characterized for their size and polydispersity by transmission electron microscopy imaging as well as light scattering methods. The surface charge and coatings were characterized by Zeta potential measurements and Fourier transform infra-red spectroscopy respectively. Dye-NP interaction was studied by both steady state absorption and fluorescence spectroscopy and time resolved emission spectroscopy. Fluorescence correlation spectroscopy was also done to study the binding of dye to SiNP in serum media. Brief principles of these techniques along with the instrument details are discussed in this chapter.

Chapter 3: Results of the studies on the interaction of ANS and TNS and MC540 with SiNP-V (NP, prepared with silica precursor vinyl-triethoxysilane (VTES)) and SiNP-VA (NP, prepared with silica precursor VTES and 3-aminopropyl-triethoxysilane (APTS)) in aqueous media are presented in this chapter. ANS and TNS are very weakly fluorescent in aqueous medium at physiological pH. In the presence of SiNP-VA, a significant enhancement of the fluorescence yield of the dyes was observed. This has been attributed to the suppression of the charge transfer process from aniline group (for ANS) and p-toluidino group (for TNS) to the sulphonate group after binding to the positively charged amino groups at the SiNP surface due to electrostatic interaction. In comparison to protein environment, however, the fluorescence of the two dyes was observed to be significantly decreased in the NP environment. A comparison of radiative

and non-radiative decay rates indicated that this quenching was primarily static in nature however, time resolved fluorescence anisotropy decay measurements showed that the quenching mechanism was probe dependent in the nanoparticle environment. Our absorption/emission studies on MC540 showed that the fluorescence and photostability of the dye significantly increases when bound to SiNP-VA. This is attributed to suppression in the excited state photoisomerism process (around its central double bond in aqueous medium) due to binding. The efficacy of this dye-nanoparticle complex was also tested on cancer cells where it showed higher light induced toxicity as compared to the free dye. Details of the study are described in this chapter.

Chapter 4: In this chapter we describe studies on the pH dependent aggregation behavior of Cp_6 in the presence of SiNP-VA suspended in aqueous medium. The absorption and fluorescence properties of the dye in the presence of SiNP-VA indicated that the electrostatic binding of dye with SiNP results in significant change in the acid-base equilibrium of Cp_6 . Subsequently, phototoxicity, on cancer cell lines, due to Cp_6 -SiNP complex and free Cp_6 was compared which showed a significant increase in the phototoxicity due to the Cp_6 -SiNP complex. Details of the study are described in this chapter.

Chapter 5: In this chapter four different NPs have been studied as delivery vehicle for hydrophobic PP18. Since PP18 has high absorption coefficient at longer wavelengths (650-720 nm) it may be more suitable for PDT applications as compared to its hydrolytic product Cp_6 provided a suitable delivery vehicle is established. We have spectroscopically studied and compared the conversion of PP18 to Cp_6 as a function of time over the period of 24 hrs in NPs: SiNP-V, SiNP-VA, polymeric NP (prepared using polymer PLGA) and liposomes (prepared using phosphatidylcholine). Our results indicate that the conversion is least in SiNP-V and highest in liposome. The details of the study are described in the chapter.

Chapter 6: In this chapter, the effect of L-SP electric field of gold nanorods on the absorption and emission properties of two positively charged dyes MB and NB has been studied as a function of detuning the L-SP band from the absorption maxima of the dyes. Binding between dyes and nanorods were established by electrostatic interaction by making the nanorods negatively charged after coating them with polymer polystyrene sulphonate (PSS). Both the absorption and emission properties of the dyes conjugated to the rods were found to get modified due to the SP field coupling as well as the aggregation of the dyes on the NP surface. The details of the study are described in the chapter.

Chapter 7: In this chapter, the results of the effect of different coating materials (surfactant CTAB and polymers) and L-SP of gold nanorods on the photophysical properties of Cp_6 electrostatically bound to these coated rods are presented. For this the CTAB coated rods were first coated with PSS to make their surface negatively charged and then coated with positively charged polymers PDDAC & PAH. While the spectroscopic properties of the Cp_6 were observed to depend upon the nature of the coatings, no significant effect due to LSPR peak position, with respect to the Cp_6 'Q band' absorption peak, was observed. The radiative decay rate was found to be different and decreased in all the three coated rods and was attributed to the difference in the chemical nature of coatings. However, the nonradiative decay rate was found to increase in the case of CTAB coated rods while no change was observed in the case of PAH and PDDAC coated rods. This was attributed to distance dependent energy transfer between the drug and nanorod, which decreased as the coating thickness increased. In addition the photobleaching rate of Cp_6 conjugated to these rods was also found to be significantly decreased and coating dependent. These results suggest that the coatings on the rods should be appropriately done depending on the applications. Details of these studies will be described in the chapter.

Chapter 8 presents the conclusions with a summary of the results and a brief discussion on possible future work.

References:

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