

# CHAPTER 7

## PHOTOPHYSICAL PROPERTIES OF CHLORIN $P_6$ BOUND TO COATED GOLD NANORODS

In this chapter we present the results on our study on the interaction of photosensitizer  $Cp_6$  with coated gold nanorods. The effect of different coating materials (surfactant and polymers) and surface plasmon resonance of gold nanorods (AuNRs), on the photophysical properties of  $Cp_6$  such as quantum yield, radiative and non-radiative decay, photobleaching etc is described.

### 7.1 Introduction

Since colloidal gold is stable, biocompatible and nontoxic [190] there exists a considerable interest in the development of multi-functional gold nanostructures for diagnostic and therapeutic applications [191, 192, 193, 194]. In particular, gold nanorods

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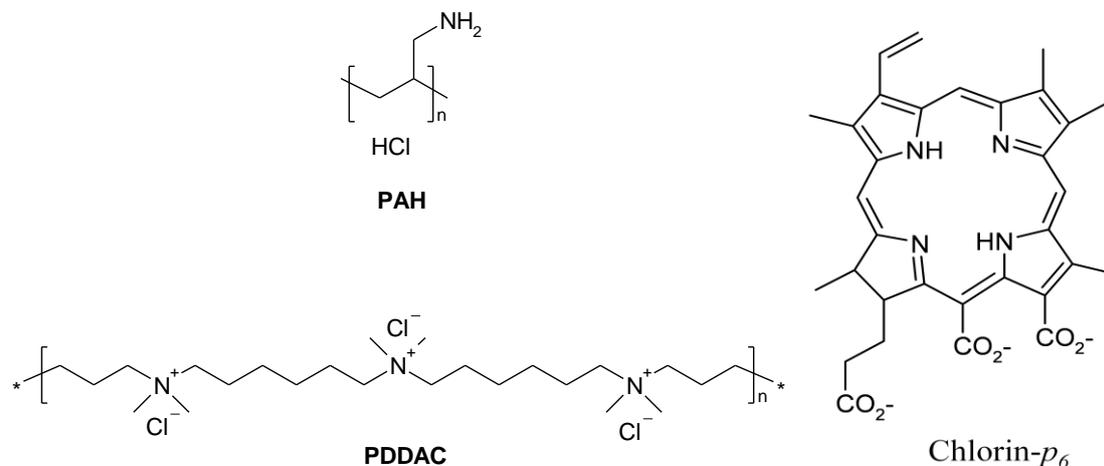
*Work discussed in this chapter has been published as follows:*

1. 'Photophysical Properties of Chlorin  $p_6$  Bound to Coated Gold Nanorods' B. Jain, A. Uppal, P. K. Gupta and K. Das, *Journal of Molecular Structure*, Vol. 1032, pp. 23-28 (2013).

(AuNR), with absorption in the near infra-red (NIR) spectral region (~650-900 nm), are being actively investigated for contrast based imaging and hyperthermia [195, 196]. This follows because the optical transparency of biological tissue is highest in this region and AuNR are particularly suited for these applications because of their large absorption cross-section and high photothermal conversion efficiencies [197]. A recent study suggests that a combination of photodynamic therapy (PDT) and hyperthermia can be more effective than either PDT or hyperthermia alone and this can be easily realised by conjugating NIR absorbing photosensitizers with NIR absorbing AuNRs [95]. One of the most popular methods for the preparation of AuNR is the use of rod-shaped micelles, formed from the surfactant, cetyl trimethylammonium bromide (CTAB), as templates [76, 77, 98]. However, the high concentration of CTAB employed in synthesis of AuNRs has raised some concerns regarding their toxicity [198, 199, 80, 200, 201]. Therefore, efforts have been made to remove the extra CTAB from the NP suspension and to overcoat the CTAB coating with other materials that lead to better biocompatibility and stability of the AuNRs [195-201].

AuNRs coated with positively charged polymers like poly allylamine hydrochloride (PAH) and poly diallyl dimethyl ammonium chloride (PDDAC) have been shown to have reduced cytotoxicity and improved cellular uptake [80, 200]. In this chapter we have used PAH and PDDAC coated AuNRs to study the binding between them and  $Cp_6$  a negatively charged photosensitizer having substantial absorption in the 660 nm region. The binding is investigated by monitoring the absorption and fluorescence properties of  $Cp_6$  in the presence of AuNRs with two different aspect ratios such that their L-SP was tuned to and away from the Q band absorption of  $Cp_6$ .

We show that the photophysics of  $Cp_6$  bound to AuNR depend significantly upon the nature of the coating material of the AuNRs. Chemical structures of  $Cp_6$  and polymers PAH and PDDAC are shown in Fig. 7.1.



**Figure 7.1** Chemical structure of  $Cp_6$  and repeating unit of the two polymers PAH and PDDAC used in this study.

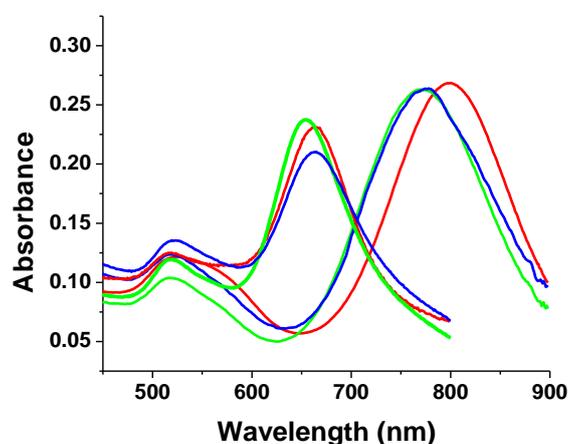
## 7.2 Experimental Details

CTAB-coated AuNRs having different L-SPR were synthesized described in chapter 2. These CTAB coated rods are positively charged. As these polymers are also positively charged, the rods were first coated with PSS which is negatively charged and then coated with PAH or PDDAC. The details of the procedure are given in chapter 2, section 2.1.2. These coated rods were finally suspended in Millipore water at pH 7.4 for further experiments. The molar extinction coefficient of AuNRs used in this study with aspect ratio of  $\sim 2.5$  was estimated to be  $\sim 3.17 \times 10^9 \text{ M}^{-1}\text{cm}^{-1}$  and aspect ratio of  $\sim 3.8$  was estimated to be  $\sim 4.75 \times 10^9 \text{ M}^{-1}\text{cm}^{-1}$  following the procedure given in reference 103.

For absorption and fluorescence measurements AuNR concentration of  $\sim 60$  picomolars was used for both the rods. The complex of  $Cp_6$  and AuNR were made by simply adding them together and mixing for a short while. Absorption and fluorescence measurements were done as described in chapter 2, section 2.3. The photobleaching of the drug and the drug nanorod conjugates was studied by photoillumination centered at  $660 \pm 20 \text{ nm}$  at a light dose rate of  $0.26 \text{ J/cm}^2/\text{min}$ .

### 7.3 Results

Typical TEM images of the PAH and PDDAC coated AuNR are given in chapter 2, (Fig. 2.4). After PAH or PDDAC coating about 95% of the nano structures were observed to be rod shaped and rest were either spherical or irregular shaped. The coated AuNRs had an average aspect ratio of  $2.5 \pm 0.2$  and  $3.8 \pm 0.3$ . The absorption spectra of the AuNRs used in this study are shown in Fig. 7.2.



**Figure 7.2** Absorbance spectra of CTAB (red), PAH (green) and PDDAC (blue) coated AuNRs at physiological pH. The two sets of rods are with L-SP peak at  $\sim 660$  and  $\sim 800$  nm. See also Table 7.1 for additional details.

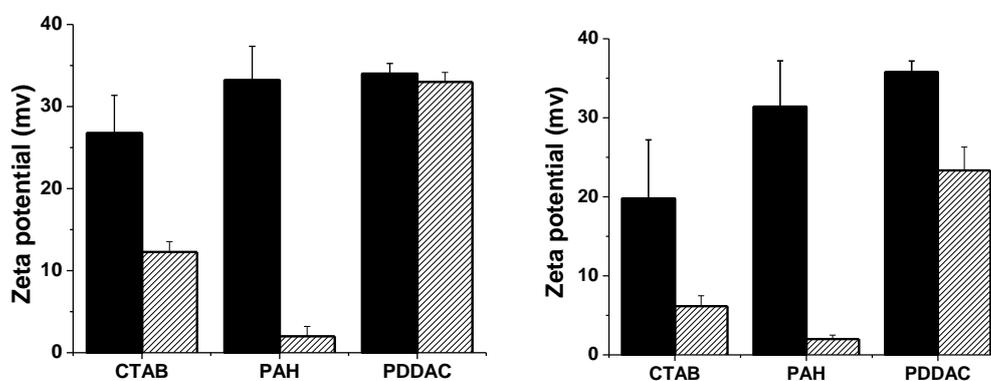
For the shorter aspect ratio, the L-SPR peak was centred on  $\sim 660$  nm and the effect of PAH or PDDAC coating on the L-SPR band position were observed to be insignificant. However for the longer aspect ratio AuNRs the PAH or PDDAC coating resulted in a significant blue shift of the L-SPR band (from 800 to 770 nm, see Table 7.1). The zeta potential of the coated AuNRs at physiological pH was observed to be positive. For the CTAB coated short aspect ratio AuNRs the measured zeta potential was  $27 \pm 5$  mV. When these CTAB coated AuNRs were over coated with the negatively charged polymer PSS the zeta potential values became  $-44 \pm 4$  mV respectively. Upon further coating with positively charged polymer PAH (or PDDAC) the zeta potential values

became  $33 \pm 4$  (or  $34 \pm 1$  mV). For the long aspect ratio rods the trends in the zeta potential values upon successive coating with PSS and PAH (or PDDAC) are similar (Table 7.1).

**Table 7.1** Size, zeta potential and SPR band position of different coated AuNRs

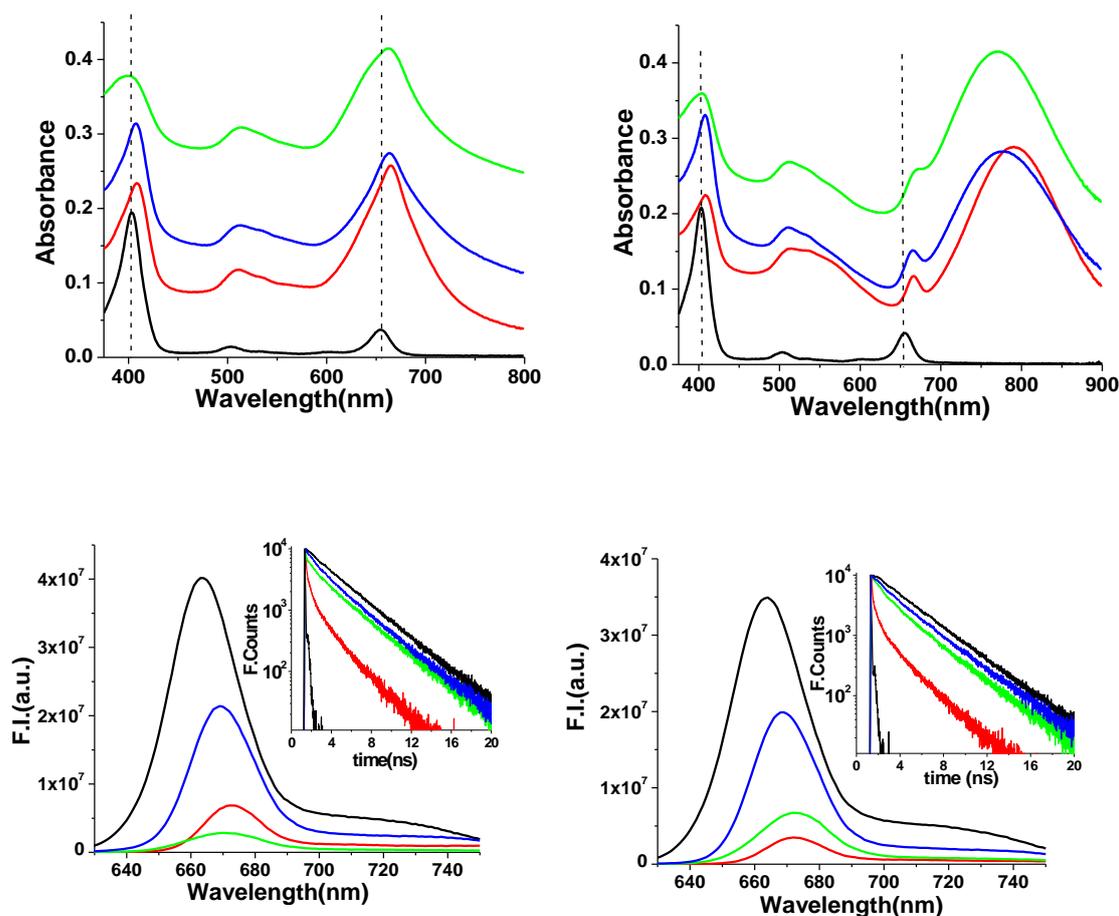
Coating	Aspect ratio $2.5 \pm 0.2$		Aspect ratio $3.8 \pm 0.3$	
	L-SPR band position (nm)	Zeta potential (mV)	L-SPR band position (nm)	Zeta potential (mV)
CTAB	653	$26 \pm 5$	798	$20 \pm 7$
PAH	654	$33 \pm 4$	770	$31 \pm 6$
PDDAC	662	$35 \pm 1$	777	$36 \pm 2$

Since the zeta potential of the AuNRs is positive and the drug is negatively charged at physiological pH, they can bind to each other by electrostatic interaction. The zeta potential of the drug-AuNR complex is an important parameter because it shows the stability of the complex. The observed zeta potentials of the various drug-nanorod complexes (drug: AuNR =  $\sim 1.5 \times 10^3$ ) are shown in Fig. 7.3.



**Figure 7.3** The observed zeta potentials of the coated AuNRs with L-SPR  $\sim 660$  nm (left) and  $\sim 800$  nm (right) and their complexes with  $Cp_6$  (shaded bars).

The figure shows that change in the zeta potential of the AuNRs after addition of  $Cp_6$  ( $2\mu\text{M}$ ) is significantly high in case of PAH coated AuNRs, where the measured zeta potential values are close to zero. The absorption, emission and fluorescence decay curves



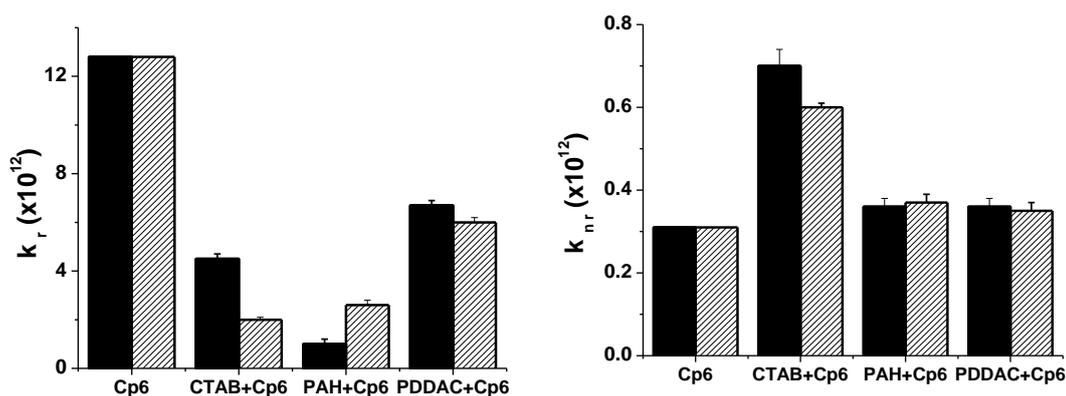
**Figure 7.4** Absorption (top) and fluorescence (bottom) spectra ( $\lambda_{ex} = 400$  nm) of  $Cp_6$  in presence of different coated AuNR at physiological pH. The left and right panel corresponds to AuNRs having L-SPR at  $\sim 660$  and  $\sim 800$  nm respectively. The inset at bottom panel shows the corresponding fluorescence decay of the drug. Dashed vertical lines correspond to the Soret and Q-band maxima of the free drug.

Colour code:  $Cp_6$  (black); CTAB coated AuNR- $Cp_6$  (red); PAH coated AuNR- $Cp_6$  (green) and PDDAC coated AuNR- $Cp_6$  (blue).

of free  $Cp_6$  and  $Cp_6$  in the presence of differently coated AuNRs (rod concentration  $\sim 60$  pM) at physiological pH are shown in Fig. 7.4. As already discussed in the earlier chapters, the absorption spectra of the free drug shows two distinct peaks at  $\sim 400$  (Soret band) and  $\sim 655$  nm (Q-band) [91]. The composite absorption spectra of the drug-AuNR system (Fig. 7.4, top) show two distinct peaks at  $\sim 400$  and  $\sim 660$  nm which can be attributed to the Soret and Q-band of the drug modified by the plasmon resonance of the

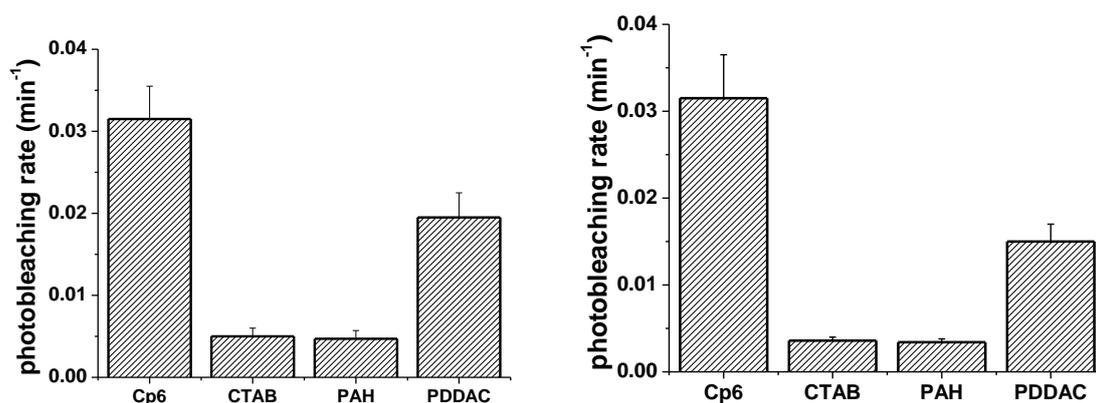
AuNR. The emission band maxima of free  $Cp_6$  is at  $\sim 663$  nm, but in the presence of various coated AuNRs the band maxima are observed to be significantly red shifted (Fig. 7.4, bottom). In addition, the fluorescence of the drug is observed to quench significantly in the presence of coated AuNRs.

Interestingly, the amount of red shift and the amount of quenching is observed to depend upon the coating on the AuNRs. As expected, the fluorescence lifetime of the drug is also observed to quench in the presence of AuNRs, the amount of quenching being highest for CTAB coated AuNRs. In order to understand the nature of fluorescence quenching of the drug in the presence of the various coated AuNRs the radiative ( $k_r$ ) and the nonradiative



**Figure 7.5** Radiative (left) and nonradiative (right) decay rates of the free  $Cp_6$  and  $Cp_6$  in the presence of coated AuNRs (black: L-SPR  $\sim 660$  nm; shaded: L-SPR  $\sim 800$  nm).

( $k_{nr}$ ) rates of the drug are calculated from the respective quantum yield ( $\Phi_f$ ) and average lifetime ( $\tau$ ) values (using the relations:  $\Phi_f = k_r\tau$  and  $k_{nr} = \tau^{-1} - k_r$ ). Fig. 7.5 shows the radiative and nonradiative decay rates of the free  $Cp_6$  and  $Cp_6$  in the presence of AuNRs. The figure shows that, in the presence of coated AuNRs, the radiative decay rates of  $Cp_6$  decreases significantly especially for the PAH coated AuNRs, whereas the nonradiative decay rates of the  $Cp_6$  are not affected by both PAH and PDDAC coating.



**Figure 7.6** Percentage photobleaching of  $Cp_6$  and its complexes with different coated AuNRs (left: L-SPR  $\sim 660$  nm; right: L-SPR  $\sim 800$  nm). See experimental section for more details.

The photobleaching of free  $Cp_6$  and  $Cp_6$  in presence of coated AuNR was studied by irradiating the samples at the Q-band absorption region of the drug. It was observed that photo illumination results in an overall decrease in the absorbance of the drug. This was quantified by monitoring the rate of change of the absorbance at the Soret peak ( $\sim 400$  nm) of the drug. The photobleaching rate of the free drug and drug-AuNR systems are shown in Fig. 7.6. Compared to  $Cp_6$ , the photobleaching rate was observed to be lower for all drug-AuNR systems. The photobleaching rate of the free drug and drug-AuNR systems are shown in Fig. 7.6.

**Table 7.2** Photophysical and photobleaching parameters of  $Cp_6$  bound to different coated AuNRs at physiological pH<sup>1</sup>

System	Emission parameters					Photobleaching rate (min <sup>-1</sup> )
	Maxima (nm)	$\Phi_f$ ( $\times 10^3$ )	$\tau_{av}$ (ns)	$k_r$ ( $\times 10^6 s^{-1}$ )	$k_{nr}$ ( $\times 10^9 s^{-1}$ )	
$Cp_6$	663	40*	3.1 $\pm$ 0.1	12.8 $\pm$ 0.3	0.31 $\pm$ 0.2	0.0315
CTAB-AuNR- $Cp_6$	673	7 $\pm$ 1	1.5 $\pm$ 0.2	4.5 $\pm$ 0.2	0.70 $\pm$ 0.04	0.0050
	673	4 $\pm$ 1	1.5 $\pm$ 0.2	2.5 $\pm$ 0.1	0.64 $\pm$ 0.01	0.0042
PAH-AuNR- $Cp_6$	673	3 $\pm$ 0.5	2.8 $\pm$ 0.1	1.0 $\pm$ 0.2	0.36 $\pm$ 0.02	0.0034
	671	7 $\pm$ 0.5	2.6 $\pm$ 0.2	2.6 $\pm$ 0.2	0.37 $\pm$ 0.02	0.0031
PDDAC-AuNR- $Cp_6$	669	18 $\pm$ 3	2.7 $\pm$ 0.1	6.7 $\pm$ 0.2	0.36 $\pm$ 0.02	0.0195
	669	18 $\pm$ 1	2.8 $\pm$ 0.1	6.0 $\pm$ 0.2	0.35 $\pm$ 0.02	0.0150

<sup>1</sup>The values in italics corresponds to longer aspect ratio rods

\* Fluorescence quantum yield of  $Cp_6$  at physiological pH has been taken from ref. 137.

Compared to  $Cp_6$ , the photobleaching rate was observed to be lower for all drug-AuNR systems. In particular, the photostability of the drug was observed to increase by ~six and ~ten times respectively in presence of CTAB and PAH coated AuNRs. The photophysical as well as photobleaching parameters of the  $Cp_6$ -AuNR systems as well as free  $Cp_6$  are provided in Table 7.2.

## 7.4 Discussion

$Cp_6$ , a porphyrin based photosensitizer, has three ionizable carboxylic acid groups and at neutral pH it is tri-anionic in nature. The molecule absorbs over the entire visible spectrum and has two prominent bands at ~400 (Soret) and ~655 (Q-band) nm [156]. It has been reported earlier that the binding of the drug with other macromolecular systems affects the position of the Q-band maxima [91, 134, 136, 137, 156].

The major objective of this study was to investigate how the chemical nature of the coated material as well as the SPR of the positively charged AuNRs affects the photophysical properties of a negatively charged photosensitizer,  $Cp_6$ . A change in the coating material (from CTAB to PAH or PDDAC) resulted in a slight increase of the zeta potential of the AuNRs (30-35 mV compared to 20-25 mV as shown in Table 7.1). For short aspect ratio AuNRs, a change in coating material hardly affected the SPR position but for longer aspect ratio AuNRs this resulted in a significant blue shift of the L-SPR band position (Fig. 7.2). This is expected because the dielectric sensitivity of the L-SPR band increases with increasing aspect ratio [76, 77, 98]. The zeta potential of the AuNR were observed to decrease upon addition of  $Cp_6$  ( $Cp_6$ : AuNR  $\sim 1.5 \times 10^3$ ) indicating binding between the drug and AuNR. Interestingly, the relative decrease was observed to depend upon the nature of the coating (Fig. 2). On addition of  $Cp_6$ , the maximum change in the zeta potential was observed for PAH coated AuNRs and the minimum for PDDAC coated AuNRs. The Soret band of the drug at 400 nm shows a slight red shift in the presence of coated AuNRs, in addition for PAH coated AuNR, it was observed to be

significantly broader. Results obtained from earlier studies indicate that this might be due to aggregation of the drug. The Q-band region of the drug (~660 nm) is convoluted with the L-SPR band of the AuNR and hence it is difficult to ascertain any changes in the band parameters compared to that of the free drug.

The fluorescence properties of the drug were observed to depend significantly upon the nature of the coating on the AuNR. While the PDDAC coating decreases the fluorescence quantum yield by half, it does not significantly affect the fluorescence lifetime. In case of PAH coating a significant quenching of the drug's fluorescence is observed but change in its lifetime is not significant. For CTAB coating significant quenching in both quantum yield and lifetime of the drug is observed. From Table 7.2 we see that polymer (PDDAC & PAH) coatings affect only the radiative rates of the drug but for CTAB coating, both radiative and nonradiative rates decrease significantly. Fluorescence quenching near gold nanoparticles have been studied earlier [202, 205] and it has been observed that quenching results from two effects, a decrease in the transition probability for radiative transitions [202] and due to energy transfer [203]. It has been observed that in certain cases both may be present [204, 205]. Thus the observed quenching of  $Cp_6$  fluorescence in the presence of CTAB coated AuNRs may be attributed to both of these effects. However, no significant changes in the nonradiative rates of the drug in the presence of polymer (PDDAC or PAH) coated AuNRs suggest that the distance between  $Cp_6$  and AuNR surface is large enough (due to the presence of three spacer layers: CTAB, PSS and PDDAC or PAH) to prevent energy transfer between the molecule and the metal. Between PDDAC and PAH coated AuNRs the observed changes in the fluorescence property of the drug were also different due to different magnitudes of decrease in the radiative rates of the drug.

At this point it is worthwhile to look at the chemical structures of the repeating units of the polymers PAH and PDDAC (Fig.7.1). The repeating unit of PAH contains one primary amine group while the repeating unit of PDDAC contains three tertiary

amino group.  $Cp_6$  is expected to bind to these polymers by electrostatic interaction between the carboxyl group of the drug and the amino group of the polymers. It is clear that distances between binding sites will be larger for PDDAC. The zeta potential values of the drug-polymer coated AuNR complex shows that compared to PDDAC coating the PAH coating results in zeta potential values of almost zero for same drug:AuNR ratio indicating that charge neutralization is almost complete for the  $Cp_6$ -PAH-AuNR complex. As a result, the observed shape change of the Soret band of the drug in the absorption spectra as well as its higher decrease in radiative rate may be attributed to the relatively close packing of the drug at the PAH coated AuNR surface.

Another important aspect of this study was to investigate how the photobleaching properties, of an adsorbed photosensitizer, are affected by the surface plasmon of AuNR as it could affect the photodynamic efficacy of the drug. We have observed that the photobleaching of the drug gets significantly reduced when it is bound to these coated AuNRs (Fig. 7.6). In particular the photobleaching rate is suppressed by ~six times in the presence of CTAB and PAH coated AuNRs. This shows that photostability can depend upon the nature of the coated material and hence it is important to choose the coating material accordingly when utilizing these for combined hyperthermia and PDT applications.

## 7.5 Conclusion

We prepared three types of coated AuNRs with two different aspect ratios. While the zeta potential did not change with changes in aspect ratios, the L-SPR band position changed significantly with changes in the coating material for the larger aspect ratio AuNRs. This has been attributed to the larger sensitivity of larger aspect ratio rods towards the dielectric medium. The binding of a negatively photosensitizer  $Cp_6$  with these AuNRs resulted in coating specific reduction in the zeta potential. The spectroscopic properties of the drug also depended upon the nature of the coating. While the polymer (PDDAC &

PAH) coating affected only the radiative rates of the drug the CTAB coating resulted in decrease of both radiative and nonradiative rates. Interestingly, the decrease in radiative rates was observed to be much higher in presence of PAH coated rods as compared to PDDAC coated rods. The observed changes have been attributed to the difference in the distance between the  $Cp_6$  and the AuNR surface which gets modified with additional layers of coated materials and also to the different chemical structure of the polymers PAH and PDDAC. The nature of the coated material was also observed to modulate the photostability of the drug. However, the effect of changing the L-SPR spectral position did not affected the photophysical and photobleaching properties of the drug.