5 Photoinduced electron transfer from Phycoerythrin to colloidal TiO$_2$ and metal-TiO$_2$ nanoparticles

5.1. Introduction

Solar energy conversion based on dye-sensitization of wide band gap nanocrystalline semiconductor is an area of intense investigation [1–5]. The most efficient dye-sensitized solar cells (DSSC) to date are based on ruthenium-containing metallorganic dyes adsorbed on nanocrystalline TiO$_2$, the best of which have been reported to convert solar energy to electrical energy with an efficiency of 10–11% [6,7]. Photoexcitation of N3 dye [cis-di-(thiocyanato)bis(4,4’-dicarboxy-2,2’-bipyridine) ruthenium(II)], results in an intramolecular metal-to-ligand charge-transfer transition. The photoexcited electrons located in the bipyridyl ligands can be efficiently injected into the conduction band of TiO$_2$ electrode on an ultrafast time scale via carboxyl groups anchored to the TiO$_2$ surface. Conversely, recombination between the electrons injected into TiO$_2$ and the cations of the N3 dye is a slow process [8], apparently due to the large separation between TiO$_2$ and Ru$^{3+}$ imposed by the bipyridyl ligands. Organic dyes have also been utilized as photosensitizers in DSSC. Organic dyes have several advantages as photosensitizers: (a) they are cheaper than Ru complexes, (b) they have large absorption coefficients due to intramolecular $\delta$-$\delta^*$ transitions and (c) there are no concerns about limited resources, because they do not contain noble metals such as ruthenium. DSSC based on metal-free organic dyes [9-26], porphyrin dyes [27-32] and natural dyes [33-35] have been studied and developed. Since the efficiencies of DSSC have not yet approached the theoretical limit and are not competitive with the more expensive silicon-based solar cells, their main advantage of cost-effectiveness depends on the utilization of cheap and readily available sensitizer dyes. So, the use of nontoxic natural pigments as sensitizers would definitely enhance the
environmental and economic benefits of this alternative form of solar energy conversion.

The phycobiliproteins are antennae-protein pigments involved light harvesting in cyanobacteria, rhodophytes, cryptomonads and cyanelles [36]. In cyanobacteria and red algae, the phycobiliproteins are organized in supramolecular complexes called phycobilisomes which are assembled in regular arrays on the outer surface of the thylakoid membranes. Phycobiliproteins are oligomeric and built up from chromophore bearing polypeptides belonging to the α and β families of polypeptides [37]. The colors of phycobiliproteins originate mainly from covalently bound prosthetic groups that are open-chain tetapyrrole chromophores namely phycobilins (possessing A, B, C and D rings). They are either blue colored phycocyanobilin (PCB), red colored phycoerythrobilin (PEB), yellow colored phycourobilin (PUB) and purple colored phycobiliviolin (PVB), also named cryptoviolin. These chromophores are generally bound to the polypeptide chain at conserved positions either by one cysteinyi thioether linkage through the vinyl substituent on the pyrrole ring A of the tetapyrrole or occasionally by two cysteinyi thioether linkages through the vinyl substituent on both A and D pyrrole rings [38]. Four main classes of phycobiliproteins are exist in nature: Allophycocyanin (APC, bluish green), phycocyanin (PC, blue), phycoerythrin (PE, purple) and phycoerythrocyanin (PEC, orange) having absorption in the range of 650-655 nm, 615-640 nm, 565-575 nm and 575 nm respectively and emit light at 660 nm, 637 nm, 577 nm and 607 nm respectively [39]. Phycobiliproteins are used as colorants in food, cosmetic and pharmaceutical industry [40], possess curative properties and used as fluorescence tags in biomedical research [41,42].

The choice of phycoerythrin pigment as a sensitizer [Scheme 1] is due to its significant visible light absorption and the presence of −COO− group which
could serve as an anchoring group between the pigment and TiO$_2$ surface. The spectral properties, such as (i) it contains multiple bilin chromophores and hence high absorbance coefficients over a wide region of visible spectra ($\varepsilon = 2.5 \times 10^6$ M$^{-1}$ cm$^{-1}$ at 563 nm); (ii) high fluorescence quantum yield ($\Phi = 0.98$) independent of pH; (iii) strong absorption at 563 nm and strong emission at 580 nm. It extend well into the red region of the visible spectrum, where interference from biological molecules is minimal; (iv) large stokes shift that minimizes interferences from Rayleigh and Raman scattering and other fluorescing species; (v) highly soluble in aqueous solutions; and (vi) stable in solution as well as solid phase, thus it can be stored for long periods. The phycocyanin pigment have longer lifetime (nano seconds) than widely employed N3 or N719 Ru based dyes (femto seconds) [43-45].

Photosensitization of wide-band gap semiconductors such as TiO$_2$ by visible light absorbing dyes has become more practical for solar cell applications in the conversion of light into electricity [46]. Sensitization of colloidal TiO$_2$ has been studied extensively in the past [47-51]. Recently we have reported the sensitization of colloidal TiO$_2$ nanoparticles using porphyrins [52]. Semiconductor particles of colloidal dimensions are sufficiently small to yield transparent solutions, allowing direct analysis of electron transfer by fluorescence quenching technique [53].

Scheme 1: Structure of Phycoerythrin
Our interest is to investigate the process of electron transfer from excited phycoerythrin to the conduction band of TiO$_2$ by using absorption and fluorescence spectroscopic measurements. From such studies, we can understand the feasibility of flow of electrons from conduction band of TiO$_2$ into the metal core based on the energetic calculations. Electrons stored in metal core can be readily discharged or scavenged on demand by electron acceptors as illustrated in \textbf{Scheme 2}. Further, the mechanism for electron transfer process on the basis of energy level diagram has also been proposed in this chapter. To the best of our knowledge this is the first attempt of using phycoerythrin as a photosensitizer for colloidal AuTiO$_2$, AgTiO$_2$ and TiO$_2$ nanoparticles.

\textbf{Scheme 2}: Photoinduced charge injection and charge separation.

5.2. Experimental section
5.2.1. Materials

Titanium (IV) 2-propoxide, Uniblue, Acid blue, Alizarin red S and Alizarin were purchased from Aldrich. Phycoerythrin (PE) was obtained as gift sample from Dr. S. Sekar, Bharathidasan University, Trichy. The doubly distilled water was used for preparing the solutions. All measurements were performed at room temperature.
5.2.2. Methods
5.2.2.1. Preparation of colloidal TiO\textsubscript{2} nanoparticles

The colloidal TiO\textsubscript{2} suspension was prepared by the hydrolysis of titanium(IV) 2-propoxide [54] as described in chapter 4, section 4.2.2.1.

5.2.2.2. Preparation of colloidal AuTiO\textsubscript{2} nanoparticles

Colloidal AuTiO\textsubscript{2} nanoparticles in water were prepared by electrostatic adsorption of AuCl\textsubscript{4}\textsuperscript{−} ions on TiO\textsubscript{2} surface followed by its reduction with NaBH\textsubscript{4} [55] as described in chapter 4, section 4.2.2.2.

5.2.2.3. Preparation of colloidal AgTiO\textsubscript{2} nanoparticles

The method of preparation of colloidal AgTiO\textsubscript{2} nanoparticles in water was similar to the one employed earlier [55] as described in chapter 4, section 4.2.2.3.

5.2.2.4. Preparation of phycoerythrin
5.2.2.4.1. Organism and culture conditions

Phycoerythrin employed in this study was obtained from the cyanobacteria namely \textit{Anabaena} sp. (fresh water form) from the culture collections maintained in the Department of Biotechnology, Bharathidasan University, Tiruchirappalli. \textit{Anabaena} sp. was cultured in BG II fresh water medium [56] at 27±2° C with artificial illumination from cool white fluorescent lamps.

5.2.2.4.2. Phycobiliprotein Extraction

Phycoerythrin was extracted from the freshly harvested biomass by the method of freeze thawing in distilled water [57]. The biomass was frozen at −20° C for 48 h and then thawed at room temperature with the addition of distilled water (1 ml of water / gm of biomass). This is followed by centrifugation at 10,000 rpm for 10 min at 4° C.
5.2.2.4.3. Ammonium sulfate fractionation

Finely powdered ammonium sulfate was gradually added into the crude extract to obtain 35% saturation with continuous stirring for one hour. The resulting solution was kept overnight in dark and the precipitation was collected by centrifugation at 10,000 rpm for 10 min at 4° C. The pellets obtained from ammonium sulfate precipitation was suspended in a small volume of distilled water and subjected to dialysis for 10 h against 100 times volume of distilled water [58].

5.2.2.5. Fluorescence quenching experiments

Samples were prepared by dissolving phycoerythrin (PE) in water and administering the appropriate amounts of colloidal TiO$_2$, AuTiO$_2$ and AgTiO$_2$ nanoparticles. The samples were deoxygenated by bubbling with pure nitrogen. Quartz cells (4 x 1 x 1 cm) with high vacuum Teflon stopcocks were used for bubbling.

5.2.2.6. Steady-state measurements

The steady-state fluorescence quenching measurements were carried out in a JASCO FP-6500 spectrofluorimeter. Excitation and emission wavelengths of PE are 563 nm and 580 nm respectively. The slit widths (each 5 nm) and scan rate (500 nm/min) were maintained constant for all the measurements. Absorption spectral measurements were recorded using Cary 300 UV-Visible spectrophotometer.

5.2.2.7. Time-resolved measurements

Fluorescence lifetime measurements were carried out in a picosecond time correlated single photon counting (TCSPC) spectrometer. The excitation source was the tunable Ti-sapphire laser (TSUNAMI, Spectra Physics, USA). The diode
laser pumped Millenia V (Spectra Physics) CW Nd-YVO4 laser was used to pump the sapphire rod in the Tsunami mode locked picosecond laser (Spectra Physics). The diode laser output was used to pump the Nd-YVO4 rod in the Millennia. The PE was excited by the laser pulse at 563 nm. The time resolved fluorescence emission was monitored at 580 nm. The emitted photons were detected by a MCP-PMT (Hamamtsu R3809U) after passing through the monochromator (f/3). The laser source was operated at 4MHz and the signal from the photodiode was used as a stop signal. The signal from the MCP-PMT was used as start signal in order to avoid the dead time of the TAC. The difference between the start and stop signal is due to the time taken by the pulses traveling through the cables and electronic relaxation of the excited state. The data analysis was carried out by the software provided by IBH (DAS-6). The kinetic trace was analyzed by non-linear least square fitting of mono exponential function.

5.3. Results and Discussion
5.3.1. Absorption characteristics of PE with TiO2 and metal-TiO2

Figures 1a, 1b & 1c shows the absorption spectra of phycoerythrin (PE) in the absence and presence of colloidal AuTiO2, AgTiO2 and TiO2 nanoparticles at different concentrations. In the presence of colloidal AuTiO2 nanoparticles the absorbance of PE at 563 nm was increased with the peak shift around 5 nm [Figure 1a]. This implies that there is a surface interaction of phycoerythrin with colloidal AuTiO2 through carboxyl group [Scheme 3], similar to the interaction of fluorescein molecules with colloidal TiO2 reported by Marcus Hilgendorff and co-workers [59]. The changes in intensity of the absorption peak at 563 nm indicate the formation of surface complex. Similar type of spectral behaviour has been observed for PE–AgTiO2 and PE–TiO2 systems [figures 1b & 1c].
**Figure 1a:** Absorption spectrum of PE in the presence of colloidal AuTiO$_2$ nanoparticles in the concentration range of $0-5 \times 10^{-4}$ M in water. The inset is the straight line dependence of $1/A_{\text{obs}} - A_0$ on the reciprocal concentration of AuTiO$_2$.

**Figure 1b:** Absorption spectrum of PE in the presence of colloidal AgTiO$_2$ nanoparticles in the concentration range of $0-5 \times 10^{-4}$ M in water. The inset is the straight line dependence of $1/A_{\text{obs}} - A_0$ on the reciprocal concentration of AgTiO$_2$. 
**Figure 1c:** Absorption spectrum of PE in the presence of colloidal TiO\textsubscript{2} nanoparticles in the concentration range of 0–5 \times 10^{-4} M in water. The inset is the straight line dependence of 1/A\textsubscript{obs}−A\textsubscript{0} on the reciprocal concentration of TiO\textsubscript{2}.

\[ R{-COO}^{-} + \text{TiO}_2_{\text{Ag/Au}} \rightleftharpoons R{-COO}......\text{TiO}_2_{\text{Ag/Au}} \]

*Scheme 3:* Electrostatic interaction of PE with positively charged colloidal TiO\textsubscript{2} surface

R = Phycoerythrin

Adsorption through electrostatic interaction

The change in absorption spectra indicates that PE molecules adsorbed on the surface of semiconductor nanoparticle to form a complex of the type PE...M-TiO\textsubscript{2}.

\[
\text{PE} + \text{M-TiO}_2 \xrightarrow{K_{\text{app}}} \text{PE}......\text{M-TiO}_2
\]

\[
K_{\text{app}} = \frac{[\text{PE}......\text{M-TiO}_2]}{[\text{PE}][\text{M-TiO}_2]}
\]
The apparent association constant for the formation of this type of surface complex (K\text{app}), can be estimated from the changes in absorbance at 563 nm by using Benesi–Hildebrand equation (2) [60].

\[ A_{\text{obs}} = (1-\alpha)C_0\varepsilon_{\text{PE}}l + \alpha C_0\varepsilon_c l \rightarrow (2) \]

where \( A_{\text{obs}} \) is the observed absorbance of the PE solution containing different concentrations of colloidal M-TiO\textsubscript{2} at 563 nm; \( \alpha \) is the degree of association between PE and M-TiO\textsubscript{2}; \( \varepsilon_{\text{PE}} \) and \( \varepsilon_c \) are the molar extinction coefficients at the defined wavelength for PE and the formed complex, respectively, ‘\( C_0 \)’ is the initial concentration of free PE. Equation (2) can be expressed by equation (3), where \( A_0 \) and \( A_c \) are the absorbance of PE and the complex at 563 nm, respectively, with the concentration of \( C_0 \):

\[ A_{\text{obs}} = (1-\alpha)A_0 + \alpha A_c \rightarrow (3) \]

At relatively high M-TiO\textsubscript{2} concentrations, \( \alpha \) can be equated to \( (K_{\text{app}}[\text{M-TiO}_2])/(1+K_{\text{app}}[\text{M-TiO}_2]) \). In this case, equation (3) can be expressed as equation (4):

\[ \frac{1}{A_{\text{obs}}-A_0} = \frac{1}{A_c-A_0} + \frac{1}{K_{\text{app}}(A_{\text{obs}}-A_0)[\text{M-TiO}_2]} \rightarrow (4) \]

The inset of figures 1a-c shows the Benesi-Hildebrand plot and there is a good linear dependence of \( 1/(A_{\text{obs}}-A_0) \) on the reciprocal concentration of semiconductor nanoparticles. The values of K\text{app} determined from the plots were shown in Table 1. Further, the reason for higher association constant value for PE–AuTiO\textsubscript{2} compared to AgTiO\textsubscript{2} and TiO\textsubscript{2} may be due to the larger surface area of AuTiO\textsubscript{2}. When the surface area is decreased, the effective electron acceptor nature should be decreased [61].
5.3.2. Fluorescence quenching characteristics

Figures 2a, 2b & 2c shows the effect of increasing concentration of colloidal AuTiO$_2$, AgTiO$_2$ and TiO$_2$ nanoparticles on the fluorescence emission spectrum of phycoerythrin. Addition of colloidal AuTiO$_2$ nanoparticles to the solution of phycoerythrin resulted in the quenching of its fluorescence emission [Figure 2a]. This quenching behaviour is similar to the earlier reported studies [62]. Similar type of spectral behaviour has been noticed for PE–AgTiO$_2$ and PE–TiO$_2$ [figure 2b & 2c].

The apparent association constant ($K_{app}$) has been obtained from the fluorescence quenching data according to the following equation (5),

$$
\frac{1}{F_0-F} = \frac{1}{F_0-F'} + \frac{1}{K_{app}(F_0-F')[M-TiO_2]} \quad \rightarrow (5)
$$

where $K_{app}$ is the apparent association constant, $F_0$ is the initial fluorescence intensity of phycoerythrin, $F'$ is the fluorescence intensity of phycoerythrin adsorbed on colloidal M-TiO$_2$ and $F$ is the observed fluorescence intensity at its maximum. The plot of $1/F_0-F$ versus $1/[M-TiO_2]$ is shown in the inset of figures 2a-c representing a good linear relationship between $1/F_0-F$ and the reciprocal concentration of colloidal M-TiO$_2$. From the slope, the value of $K_{app}$ has been calculated and these values are shown in table 1.

**Table 1:** The apparent association constant ($K_{app}$) for the PE-MTiO$_2$ systems.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Systems</th>
<th>$aK_{app}$ (x 10$^2$ M$^{-1}$)</th>
<th>$bK_{app}$ (x 10$^2$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PE-AuTiO$_2$</td>
<td>4.39</td>
<td>3.43</td>
</tr>
<tr>
<td>2</td>
<td>PE-AgTiO$_2$</td>
<td>2.35</td>
<td>2.11</td>
</tr>
<tr>
<td>3</td>
<td>PE-TiO$_2$</td>
<td>1.72</td>
<td>1.67</td>
</tr>
</tbody>
</table>

$^a$ obtained from absorption studies

$^b$ obtained from fluorescence studies
Figure 2a: Fluorescence quenching of PE in the presence of colloidal AuTiO$_2$ nanoparticles in the concentration range of 0–5 x 10$^{-4}$ M in water. The inset is the straight line dependence of 1/(F$_0$-F) on the reciprocal concentration of AuTiO$_2$.

Figure 2b: Fluorescence quenching of PE in the presence of colloidal AgTiO$_2$ nanoparticles in the concentration range of 0–5 x 10$^{-4}$ M in water. The inset is the straight line dependence of 1/(F$_0$-F) on the reciprocal concentration of AgTiO$_2$. 
Figure 2c: Fluorescence quenching of PE in the presence of colloidal TiO₂ nanoparticles in the concentration range of 0–5 x 10⁻⁴ M in water. The inset is the straight line dependence of 1/(F₀-F) on the reciprocal concentration of TiO₂.

The Kₜₐₚ value is decreased in the following order:

\[ \text{AuTiO}_2 > \text{AgTiO}_2 > \text{TiO}_2 \]

Among the above three systems pure TiO₂ shows lesser Kₜₐₚ. While comparing AuTiO₂ and AgTiO₂ the former one shows more quenching efficiency than the latter due to the higher Fermi level of Au (0.75 V) than Ag (0.45 V).

The ability of the excited state phycoerythrin to inject its electrons into the conduction band of TiO₂ is determined from the energy difference between the conduction band of TiO₂ and excited state oxidation potential of phycoerythrin. According to the equation \( E_{s^*/s^+} = E_{s/s^+} - E_s \), the oxidation potential of excited state phycoerythrin is calculated as −1.77 V vs SCE, where, \( E_{s/s^+} \) is the oxidation potential of phycoerythrin, 0.36 V vs SCE and \( E_s \) is the excited state energy, 2.13 eV (Excited state energy of the phycoerythrin is calculated from the fluorescence maximum based on the reported method [63]). The energy level of the conduction band of TiO₂ is −0.1 V vs SCE [64]. It suggests that the electron
transfer from excited state phycoerythrin to the conduction band of TiO$_2$ is energetically favorable [Scheme 4]. The electrons injected into the conduction band of TiO$_2$ are quickly transferred to the metal core and it leads to the suppression of back electron transfer process [Scheme 2&4]. Hence, AuTiO$_2$ is more efficient in suppressing the back electron transfer process while compared to AgTiO$_2$ and pure TiO$_2$ nanoparticles.

![Scheme 4: Schematic diagram describing the electron-donating energy level of PE.](image)

5.3.3. Fluorescence lifetime measurements

As shown by previous reports [65-67], the dye molecules adsorbed on the semiconductor surface had significantly shorter fluorescence lifetime than the unadsorbed molecules. The fluorescence decay of PE ($1 \times 10^{-6}$ M) in the absence and presence of metal-semiconductor nanoparticles are shown in figures 3 (a, b and c for PE with AuTiO$_2$, AgTiO$_2$ and TiO$_2$ respectively). In the absence of metal-semiconductor nanoparticles, the decay curve of PE is fitted with single
exponential decay \( (F(t) = A \exp(-t/\tau)) \) with lifetime of 7.2 ns. Upon addition of metal-semiconductor nanoparticles \((5 \times 10^{-4} \text{ M})\), the decay of PE is deviated from single exponential decay to bi-exponential decay \( (F(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)) \). It shows shorter-lifetime and longer-lifetime components. The shorter lifetime is attributed to the adsorbed PE \( (\tau_{ads}) \) and the longer one is for free/unadsorbed PE \( (\tau) \). The fluorescence lifetime of PE with metal semiconductor nanoparticles are given in Table 2. Further these lifetime data clearly indicates that more electron transfer is possible in the case of Au-TiO\(_2\) system compared to pure TiO\(_2\) system because of the flow of electrons from the conduction band of TiO\(_2\) into the metal core having low lying Fermi level which is energetically favorable (Scheme 4).

Figure 3a: Fluorescence decay of PE in the absence and presence of colloidal AuTiO\(_2\) nanoparticles \((5 \times 10^{-4} \text{ M})\) in water.
**Figure 3b:** Fluorescence decay of PE in the absence and presence of colloidal AgTiO$_2$ nanoparticles ($5 \times 10^{-4}$ M) in water.

**Figure 3c:** Fluorescence decay of PE in the absence and presence of colloidal TiO$_2$ nanoparticles ($5 \times 10^{-4}$ M) in water.
The observed decrease in lifetime could be correlated with the electron transfer process in the dye molecules adsorbed on the semiconductor nanoparticles by using equation (6),

\[ \text{k}_{et} = \frac{1}{\tau_{ads}} - \frac{1}{\tau} \rightarrow (6) \]

where, \( \tau \) and \( \tau_{ads} \) are the lifetime of free PE molecules in aqueous solution and adsorbed on the semiconductors surface and \( \text{k}_{et} \) is the specific rate of electron transfer process. By substituting the values of \( \tau \) and \( \tau_{ads} \) in the above equation (6) the values of \( \text{k}_{et} \) were calculated and shown in Table 2. The presence of noble metals promotes the interfacial electron transfer process in the excited state of PE to the metal-semiconductor nanoparticles [Scheme 2&4] and hence higher \( \text{k}_{et} \) was obtained for Au and AgTiO\(_2\) compared to pure TiO\(_2\).

**Table 2:** Fluorescence lifetime of PE (1 x 10\(^{-6}\) M) in presence of metal-semiconductors (5 x 10\(^{-4}\) M) and the rate of electron transfer (\( \text{k}_{et} \)).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sensitizer</th>
<th>( \tau ) (ns)</th>
<th>( \tau_{ads} ) (ns)</th>
<th>( \text{k}_{et} ) (x 10(^9) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PE</td>
<td>7.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>PE+TiO(_2)</td>
<td>7.1</td>
<td>0.22</td>
<td>4.40</td>
</tr>
<tr>
<td>3</td>
<td>PE+AgTiO(_2)</td>
<td>7.1</td>
<td>0.19</td>
<td>5.12</td>
</tr>
<tr>
<td>4</td>
<td>PE+AuTiO(_2)</td>
<td>7.0</td>
<td>0.12</td>
<td>8.19</td>
</tr>
</tbody>
</table>

5.3.4. Calculation of free energy change (\( \Delta G_{et} \)) for electron transfer reactions

The bandgap energy of TiO\(_2\) (3.2 eV) is greater than the excited state energy (2.13 eV) of phycoerythrin and there is no overlap between the fluorescence emission of phycoerythrin (580 nm) with the absorption of colloidal...
TiO$_2$ (350 nm). Thus energy transfer from excited state phycoerythrin to colloidal TiO$_2$ has been ruled out.

Therefore it is concluded that the fluorescence quenching shown in figure 2a, 2b & 2c is caused by electron transfer. The thermodynamic feasibility of excited state electron transfer reaction was confirmed by the calculation of free energy change by employing the well known Rehm-Weller expression [68].

$$\Delta G_{st} = E_{\frac{1}{2}}^{(ox)} - E_{\frac{1}{2}}^{(red)} - E_s + C \rightarrow (7)$$

where, $E_{\frac{1}{2}}^{(ox)}$ is the oxidation potential of phycoerythrin (0.36 V), $E_{\frac{1}{2}}^{(red)}$ is the reduction potential of TiO$_2$ (i.e.) conduction band potential of TiO$_2$, −0.1 V, $E_s$ is the excited state energy of phycoerythrin and $C$ is the coulombic term. Since one of the species is neutral and the solvent used is polar in nature, the coulombic term in the above expression can be neglected [69]. The value of $\Delta G_{st}$ is calculated as −1.67 eV and this higher negative value indicates that the electron transfer process which occurred in this system is thermodynamically favorable [70,71].

5.3.5. Photoreduction of Anthraquinone dyes

The electron transfer process has also been proved by the comparative study of phycoerythrin with certain anthraquinone dyes. Anthraquinone dyes are very good electron acceptors [Scheme 5] [72,73], so they have used to probe the electron transfer process in phycoerythrin. Figures 4a, 4b, 4c & 4d shows the fluorescence spectrum of PE in the absence and presence of anthraquinone dyes in different concentration range. From the fluorescence study we observed that the emission intensity of PE gradually decreases with increasing the dye concentration, this clearly indicates the quenching of PE was occurred.

The fluorescence quenching of PE can be explain based on the well known Stern-volmer relationship as shown in equation (8).

$$I_0/I = 1 + K_{sv} [Q] \rightarrow (8)$$

where $I_0$ and $I$ are the intensity of PE in the absence and the presence of dye,
\( K_{sv} \) is Stern-Volmer constant and \([Q]\) is the concentration of the dye. The bimolecular quenching rate constant \((k_q)\) was calculated [Table 3] using equation (9).

\[
K_{sv} = \tau \cdot k_q \rightarrow (9)
\]

where, \(\tau\) is the fluorescence lifetime of PE in the absence of quencher, \(k_q\) is the bimolecular quenching rate constant. The plot between \(I_0/I\) vs \([Q]\) were linear for all dyes, indicating dynamic nature of quenching process [Figure 5].
Figure 4a: Fluorescence quenching of PE in the presence of Uniblue in the concentration range of $0-5 \times 10^{-4}$ M in water.

Figure 4b: Fluorescence quenching of PE in the presence of Acid blue in the concentration range of $0-5 \times 10^{-4}$ M in water.
Figure 4c: Fluorescence quenching of PE in the presence of Alizarin Red S in the concentration range of $0–5 \times 10^{-4}$ M in water.

Figure 4d: Fluorescence quenching of PE in the presence of Alizarin in the concentration range of $0–5 \times 10^{-4}$ M in water.
The quenching rate constant decreased in the following order:

\[
\text{Uniblue} > \text{Acid blue} > \text{Alizarin Red S} > \text{Alizarin}
\]

Among the dyes, Uniblue possess highest quenching rate constant than acid blue. This is due to the fact that uniblue has one additional vinyl sulfone group which is an electron withdrawing group that makes the uniblue more electron deficient compared to acid blue and hence enhancing it’s ability to quench the PE.

Alizarin Red S has higher quenching rate constant than Alizarin. These two dyes are almost structurally similar, but in the case of Alizarin Red S, one sulfonate group is attached in the anthraquinone system, so the electron density in the quinone group is reduced, making it relatively more electron deficient and hence enhancing its ability to quench the PE via electron transfer.
The nature of electron transfer pathway (i.e., oxidative or reductive quenching of the PE excited state) can be understood by examining the free energy of the corresponding electron transfer reactions. Thermodynamics of electron transfer from PE to the quencher can be calculated by the well known Rehm-Weller equation (7).

The calculated \( \Delta G_{et} \) values are given in Table 3. The negative values of free energy change indicated the electron transfer from PE to dyes is thermodynamically favorable [Scheme 6].

\[
\text{Scheme 6: Mechanism of fluorescence quenching of PE by dyes}
\]

where, \( k_d \) and \( k_{-d} \) are the rate constants of diffusion and dissociation of encounter complex, respectively. \( k_{et} \) and \( k_{-et} \) are the activation controlled rate constants of electron transfer, and \( k_{esc} \) is the rate constant for the separation of radicals. \( k_b \) is the rate constant for the recombination of radical pair. \( k_R \) is the rate constant for decay of PE radical.
Table 3: Fluorescence quenching rate constants and thermodynamic data of PE by anthraquinone dyes.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Quencher</th>
<th>$k_q \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$</th>
<th>$E_{1/2}$ vs SCE (V)</th>
<th>$\Delta G_{et}$ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uniblue</td>
<td>20.6</td>
<td>0.65</td>
<td>-2.42</td>
</tr>
<tr>
<td>2</td>
<td>Acid blue</td>
<td>15.9</td>
<td>0.66</td>
<td>-2.43</td>
</tr>
<tr>
<td>3</td>
<td>Alizarin red S</td>
<td>12.6</td>
<td>0.67</td>
<td>-2.32</td>
</tr>
<tr>
<td>4</td>
<td>Alizarin</td>
<td>8.7</td>
<td>0.55</td>
<td>-2.44</td>
</tr>
</tbody>
</table>

\(^{a}\) determined by steady state fluorescence quenching in water  
\(^{b}\) reduction potential of dyes in V vs SCE in water.  
\(^{c}\) calculated by Rehm-Weller equation.

5.4. Conclusions

The interaction of phycoerythrin with colloidal metal-semiconductor nanoparticles has been studied by absorption, steady state and time resolved fluorescence spectroscopic methods. Phycoerythrin adsorbed on the surface of metal semiconductor nanoparticles through its carboxyl group, as evidenced by the effect of colloidal metal semiconductor nanoparticles concentration on the absorption study. The apparent association constants were calculated from both the absorption and fluorescence changes and they were agreed well. Based on the energy level diagram and more negative free energetics, it is suggested that the metal core has effect on the electron transfer from excited state phycoerythrin to the conduction band of TiO$_2$. On the other hand, presence of metal core suppresses the charge recombination process. Electron accumulation within the metal core is likely to influence the overall charge separation in the composite system. The electron transfer process has also been proved by the comparative study of phycoerythrin with certain anthraquinone dyes. Insinuation of the metal semiconductor nanoparticles is improving the performance of dye-sensitized solar cells.
5.5. References


