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*Full Length Research Paper*

# Photobleaching of Sn(IV) chlorine e6 dichloride trisodium salt in different environments

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**The photobleaching of Sn(IV) chlorine e6 dichloride trisodiuim salt in variety of solvents was investigated. The absorption of photosensitizer in polar solvent was much higher than in non-polar solvent. The results prove that this photosensitizer was high water soluble. The photosensitizer absorption decreased at all bands for all solvents. Increasing the irradiation time leads to gradual decrease in the absorption of photosensitizer in solvents. The photosensitizer binding to the protein will result in a reduction of the diffusional movement of the photosensitizer to equalize the protein. The presence of serum in the environment leads to increase in the photobleaching rate and response to light as well. A new peak position of absorption was observed in the ultra violet region with the presence of serum. Singlet oxygen was found to be responsible for photobleaching.**

**Key words:** Sn(IV) chlorine e6, photobleaching, photoproduct.

## INTRODUCTION

Photodynamic therapy (PDT) is based on the interaction between suitable wavelength of light and a photosensitizer with the presence of oxygen to produce toxicity. Photodynamic therapy protocol depends on three important parameters; the administration of a photosensitizer in tumor tissue, light with a suitable wavelength and the presence of oxygen in the tumor tissue (Schnider et al., 2008; Juarranz et al., 2008). When the photosensitizer is exposed to light with a particular wavelength, the molecule of photosensitizer absorbs the photon of light and excited from its ground state to the excited singlet state. The lifetime of the molecule in the excited state is very short. The decay of molecule to its ground state occurs more than one way. It may decay directly by emitting a photon (fluorescence) after an internal conversion with loss of excess energy or by taking the inter-system crossing and transferring into the lowest triplet state, which has a lower energy level than the first excited state and emitted phosphorescence. A therapeutic photodynamic effect can be obtained when the photosensitizer undergoes electron spin conversion lost in absorption or fluorescence at some wavelengths to triplet state (3P*) which has a lower energy but has longer lifetime than singlet state (1 to 10 µs) (Oshsner, 1997). The increase of the molecule lifetime increases the probability of transferring the energy to neighboring molecules and the probability of the molecule to interact with other molecules, such as molecular oxygen, would also increase (Neto et al., 2006). Most of the photosensitizers have phototransformed to light irradiation. These transformations are termed photobleaching. Photobleaching or photofading can be defined as the loss of absorption or emission intensity that is caused by light (Verhoeven, 1996). Photobleaching has a significant influence in photodynamic efficiency of the photosensitizers. Thus, the photobleaching of the photosensitizer is necessary in understanding this modality of treatment (Bonnett and Martinez, 2002).

Rapid photobleaching in photosensitizer decreases the efficiency of photodynamic therapy because the tumor destruction will not be complete. Photobleaching of photosensitizers in PDT was observed *in vitro* and *in vivo* through using the fluorescence mode of observation, both directly and after the drug extraction (Bonnett et al., 1999). Two types of photobleaching process should be considered: the first type is photomodification, which gets and it might form new visible spectral bands. The

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chromophore is retained in modified form of m-THPP photosensitizer in methanol-water (Bonnett et al., 1999). The second type is true photobleaching, which involves the photosensitizer fragmentation and result to loss in absorption for visible region of m-THPC photosensitizer which depends on various parameters such as: environment, pH value, light dose and with or without FBS and BSA in phosphate buffered saline (Bonnett and Martinez, 2002; Lassalle et al., 2004).

Metalloporphyrins are well known in photodynamic therapy and have had high absorption in the therapeutic band at more than 630 nm. The rates of photobleaching of hematoporphyrin, photofrin, tetraphenylporphyrin tetrasulfonic acid and uroporphyrin could be very sensitive to solvent changes (Spikes, 1992; Liang et al., 2009). This study was to investigate the lost in absorption of photosensitizer (Sn(IV) chlorine e6 dichloride trisodium salt) in different environments and the modality of photobleaching.

## MATERIALS AND METHODOS

### Chemicals

Photosensitizer (Sn(IV) chlorine e6 dichloride trisodium salt) was purchased from Frontier Scientific (USA). Phosphate buffered saline (PBS), fetal bovine serum (FBS) and bovine serum albumin (BSA), was obtained from (GIBCO, Malaysia). Analytical buffered solutions of pH 2 to 9 (Sigma, Malaysia) were used. Variety of solvents (PBS, methanol, ethanol and dimethyle sulfoxide DMSO) were used to study the effect of environments on the behaviour of the Sn(IV) chlorine e6 photosensitizer. Most of chemical products were supplied by the School of Chemistry, Universiti Sains Malaysia.

### Preparing the environments

A stock of the photosensitizer was prepared in an environment of neat PBS with concentration of 1 mg/ml and kept frozen before use. A photosensitizer concentration of 10 µg/ml is added to the different solvents environments: neat PBS, methanol, n-hexane, PBS with 10% FBS, PBS with 1% BSA, PBS with 10% FBS and 10 mM mannitol and PBS with 10% FBS and 1 mM histidine.

### Irradiation of samples

A diode laser at wavelength 635 nm was used to irradiate sample under power density of 225 mW/cm$^2$ and processing area of 10 × 4 mm. The samples were horizontally irradiated inside the cuvettes for different irradiation times up to 30 min. The absorption spectrum was recorded every 5 min using U-2000 Hitachi spectrophotometer. The measures were done at wavelength ranging from 300 to 700 nm.

## RESULTS AND DISCUSSION

The absorption spectrum of Sn(IV) chlorine e6 in methanol and n-hexane (10 µg/ml) is recorded and shown in Figure 1. The absorption of methanol is much higher than in n-hexane. This result can be explained by solving this photosensitizer in higher polar solvents such as methanol than in non-polar solvents like n-hexane.

The absorption spectra of Sn(IV) chlorine e6 in neat PBS buffer, 10% FBS and 1% BSA, are shown in Figure 2. In comparison, the absorption in both Soret and Q band, which was accompanied with red shift in the presence of 10% FBS and 1% BSA, was almost lower than the absorption of neat buffer at the same photosensitizer concentration. The slight reduction of the absorption is probably due to the binding among photosensitizer molecules and the protein molecules, which leads to more aggregation (Sumiyoshi et al., 2006). The presence of more proteins in the solvent leads to more reduction in absorption spectra as shown in Figure 2, where the absorption spectrum in 10% FBS is less than in 1% BSA. The effect of light irradiation on the absorption spectra of Sn(IV) chlorine e6 in 10% FBS and 1% BSA mediums are described in Figure 3 a, b and c, which showed the difference in absorption spectra before and after irradiation (absorption before irradiation minus absorption after irradiation).

The samples that contain photosensitizer are exposed to the light and this leads to reduced absorption at all bands for all solvents. For neat PBS, decrease of absorption was recorded at 409 nm (Soret band) and at 633 nm (Q band) as shown in Figure 3a.

Figure 3b represents the spectra of the photosensitizer in solution of PBS with 1% BSA, lead to a decrease in absorption spectra observed at both 409 nm (Soret band) and 635 nm (Q band). For PBS solution containing 10% FBS, the decrease in absorption occurred at 409 nm (Soret band) and at 635, 525 and 590 nm (Q band) as shown in Figure 3c. The bands of the absorption reduction can be observed clearly in the Figure 3a, b and c. In the meantime, the absorption is increased at other bands, therefore, there is an increment at 315 nm (UV band) and at 655 nm (Q band) for 10% FBS and it is increased at 315 and 430 nm (Soret band) and at 505, 550, 600 and 655 nm (Q band) for 1% BSA.

Figure 4 shows the absorption spectra of the photosensitizer in neat PBS, PBS with 1% BSA and PBS with 10% FBS at different irradiation times from 0 to 30 min. There is an increment in absorption at UV band and 655 nm band with the increasing irradiation time. Figure 4a, b shows that the increment in absorption occurred at 315 nm and slightly at 350 nm. In the Soret band at 315 nm, there is an explicit increment in absorption with 10% FBS compared with neat PBS with 1% BSA as shown in Figure 4c, while the increment is not clear at the Q band. It means that the new photoproduct with high absorption at Soret band was modified as a result of the irradiation.

The difference between the absorption before and after irradiation is considered as function of time (min) as shown in Figure 5. Figure 5a shows that there is an obvious increment at UV band with 10% FBS compared 300 and 315 nm may be due to the photodestruction of with neat PBS with 1% BSA. On the other hand, there

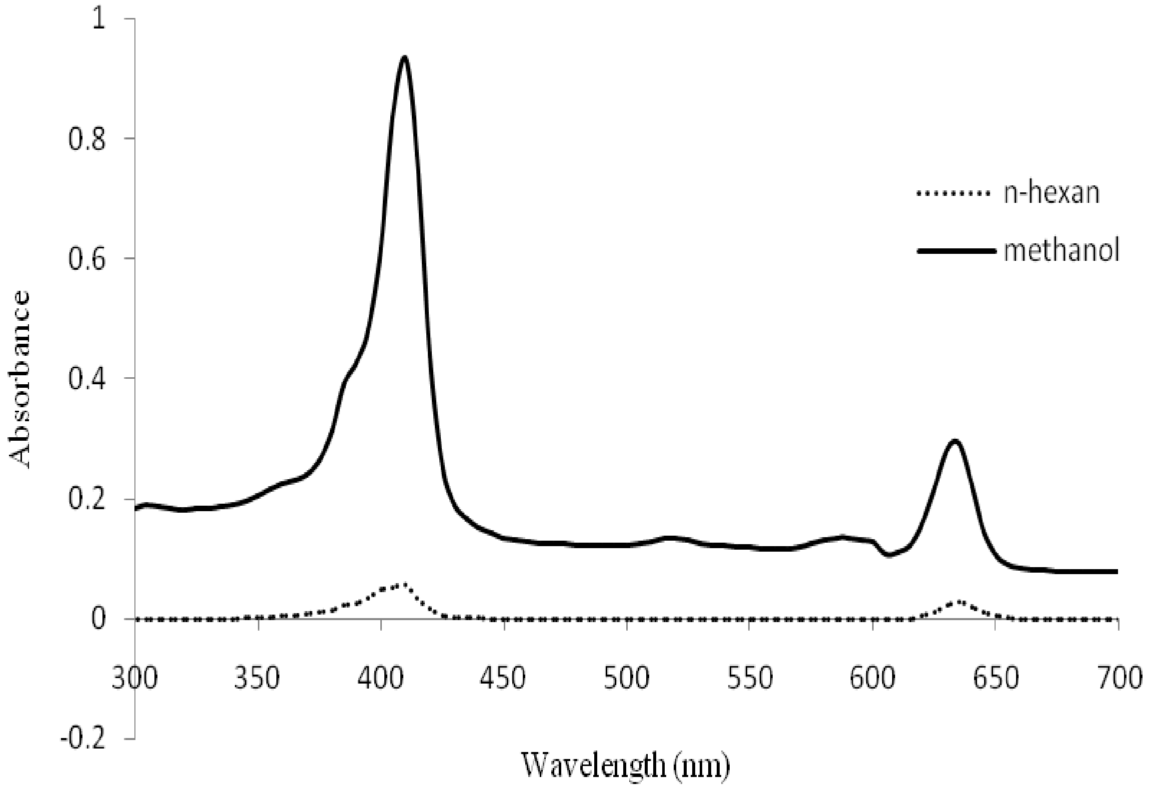

**Figure 1.** Absorption spectra of photosensitizer in methanol and in n-hexane

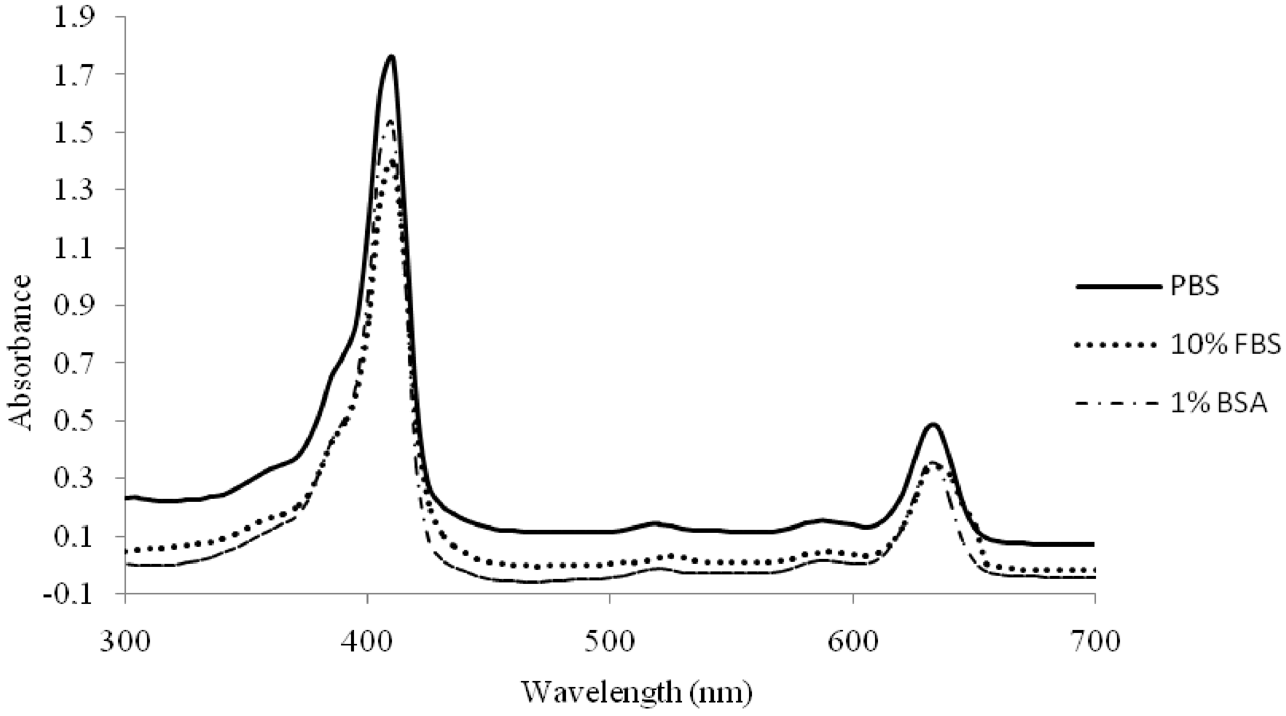

**Figure 2.** Absorption spectra of photosensitizer of concentration 10 µg/ml in Neat PBS, PBS with 10% FBS and in PBS with 1% BSA.

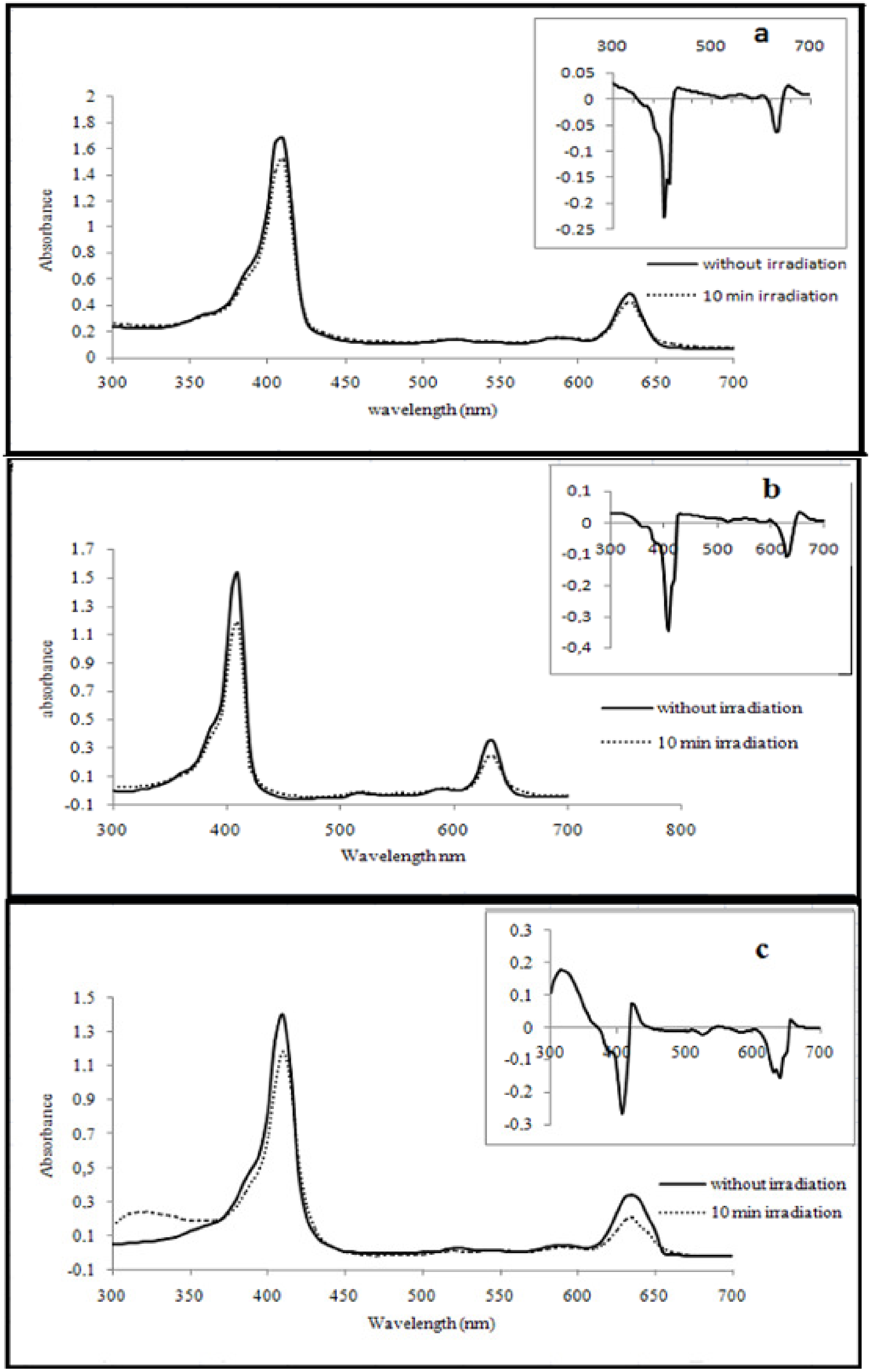

**Figure 3.** Photobleaching of the photosenistizer in diffferent solvents: Absorption spectra of non irradiated and after 10 min of irradiation. Inset: absorption difference spectra.

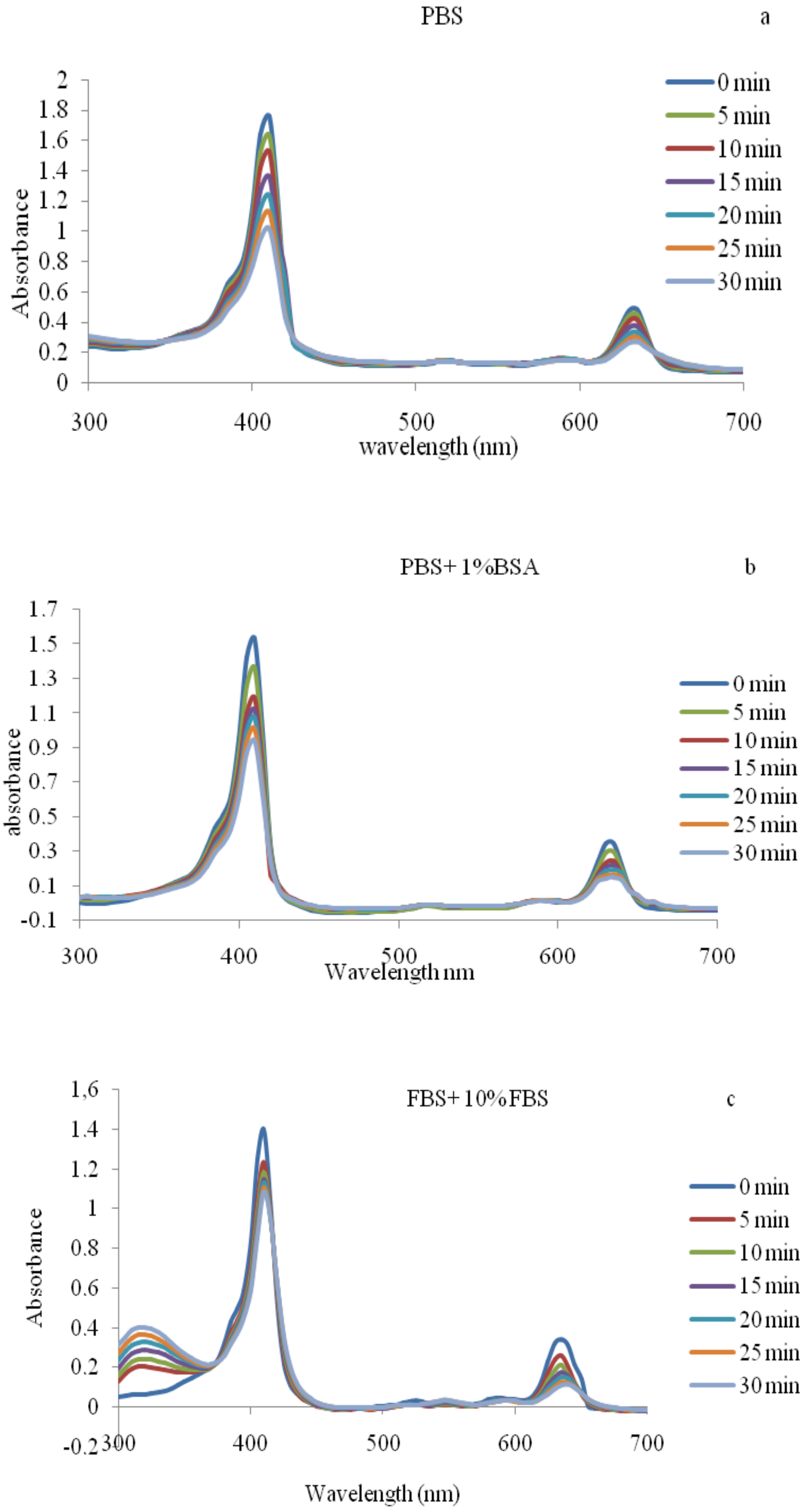

**Figure 4.** The absorption spectra for the photosensitizer in neat PBS, PBS + 1%BSA, and 10% FBS.

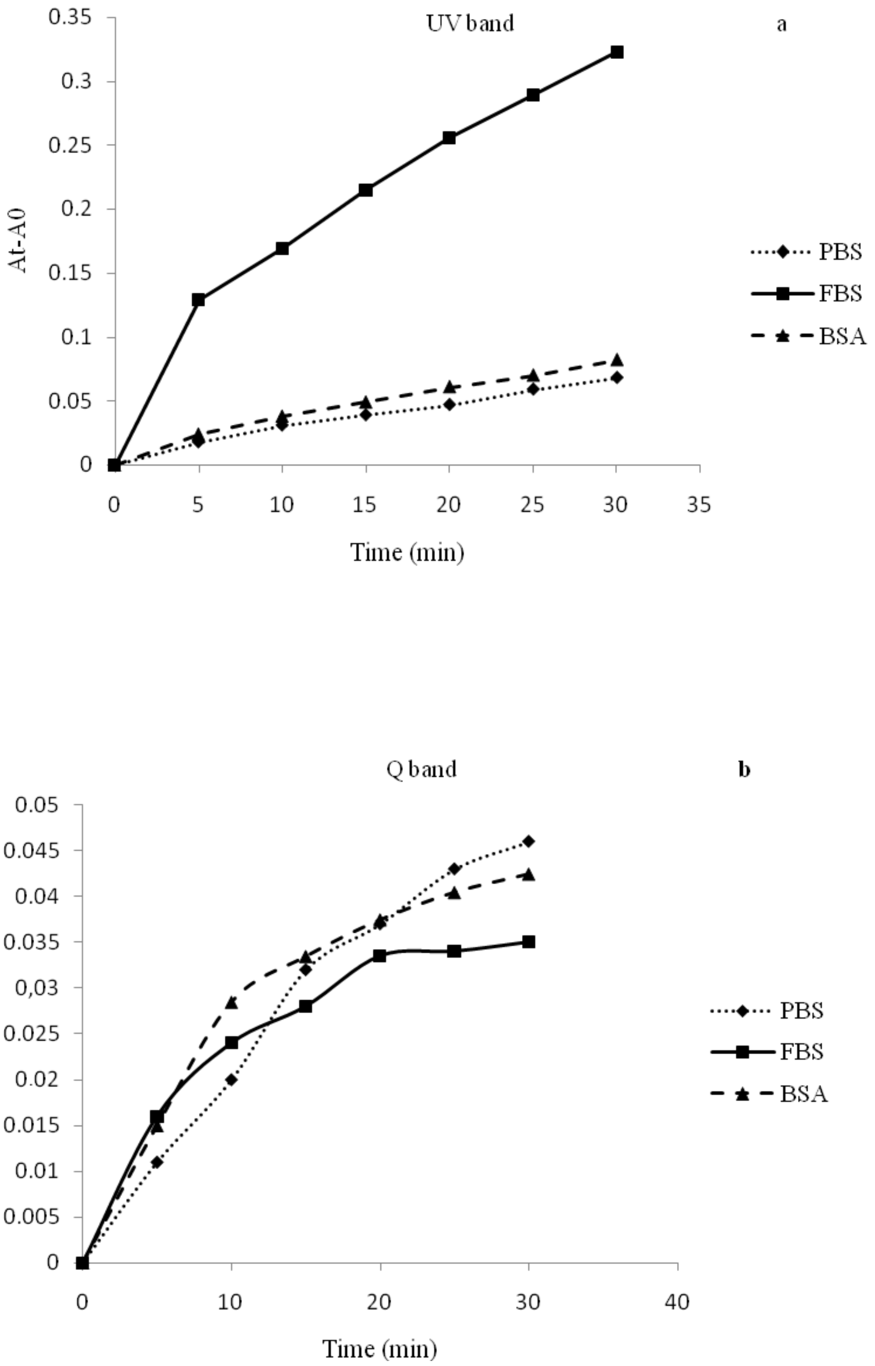

**Figure 5.** Photoproduct formation of Sn(IV) chlorin e6 at UV band and around 650 nm in indicated solvents.

was no significant difference in absorption at Q band among these three solvents, as shown in Figure 5b.

Changes in the photosensitizer spectra during irradiation are due to the use of different processes. The loss of absorption in the visible bands and the appearance of photoproducts absorbing in UV region at photosensitizer ring together with the formation of photoproduct at 650 nm (Rotomskis et al., 1996). However, in porphyrin, hematoporphyrin and chlorine derivatives, there is a reduction in visible spectra and an increase in absorption at UV and at 450, 500 and 640 nm upon similar irradiation (Rotomskis et al., 1996, 1997; Streckyte et al., 1993). This may indicate that some of the photoproducts are identical.

Table 1 shows the absorption parameters of Sn(IV) chlorine e6 and full width half maximum (FWHM)

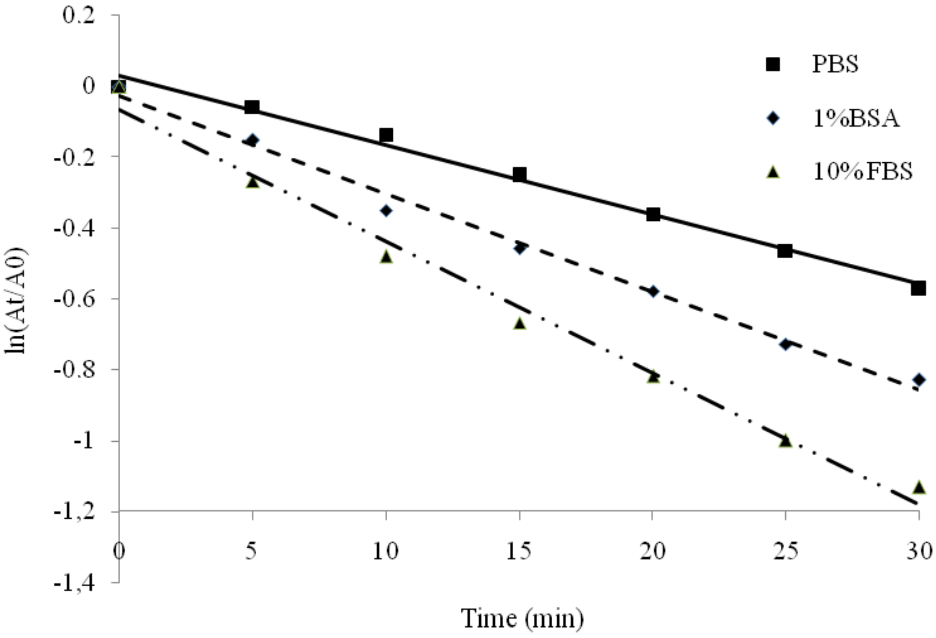

**Figure 6.** Rate of change of Q band absorption of Sn(IV) chlorin e6 during illumination in the different environments: neat PBS, PBS with 1% BSA, and PBS with 10% FBS.

including maximum absorption position in various solvents. There is a decrease in FWHM of the Soret band in the presence of 1% BSA and 10% FBS compared with neat PBS. In methanol, FWHM has higher value than in PBS. The decrease of FWHM in serum and the reduction in the absorption maximum with 1% BSA and 10% FBS compared with neat PBS indicates that the photosensitizer is more aggregated in these media. Table 1 illustrates that a slight red shift in Q band occurred when serum was added to the medium, while no shift appears in the Soret band. This red shift of the photosensitizer could be due to the partitioning of the drug between the bulk water and lipid bilayer or the matrix protein (Das et al., 2005). The rate of change of the absorption maxima of Q band with irradiation time for three different mediums is shown in Figure 6. It was observed that the photobleaching in all environments follows the first order kinetics. The photobleaching rate is more steep in 10% FBS (6.18 x $10^{-4}$ $s^{-1}$) than in both 1% BSA (4.6 x $10^{-4}$ $s^{-1}$) and neat PBS (3.28 x $10^{-4}$ $s^{-1}$) as shown in Table 2.

The rates of photobleaching of hematoporphyrin and photofrin depend on solvent type (Spikes, 1992). The solvent has some responsibility for this change. The effect of solvents on photobleaching is not yet clear but some researchers show that the polarity of the solvent could play an important role in photobleaching (Sumiyoshi et al., 2006).

The solvents of polarity tend to stabilize the polar transition states, which are involved in the reaction with the generated singlet oxygen or reactive oxygen species. In Table 2, the rate of photobleaching in serum is twice more fast than in neat buffer. In serum, there are hydrophobic regions distributed homogeneously inside the solvent. For serum solution, the photosensitizer is partially bound to the serum proteins, which means a non-polar or hydrophobic environment; hence, the rate of photobleaching should be lower than that in neat buffer (Das et al., 2005).

In conclusion, there is a significant increase in the photobleaching rate in serum by a factor 2 compared with that in neat buffers, which is contrary to the previous result. The photosensitizer binding to the protein will result in a reduction of the diffusional movement of the photosensitizer to equalize the protein. This reduction will lead to increase the reaction of singlet oxygen and reactive oxygen species with photosensitizer, which means more photobleaching (Hadjur et al., 1998).

The photobleaching rate was faster in serum by a factor of 1.3 than in 1% BSA. This finding supports the assumption of the photosensitizer binding to the proteins accompanied with reduction of diffusional movement. It is the decrease in serum concentration that led to increase in the diffusional movement and thus, less reaction of singlet oxygen or reactive oxygen species with the photosensitizer and finally, decreasing the photobleaching rate.

Mechanistic studies were carried out to determine the responsible mechanisms for photobleaching using known quenchers for singlet oxygen and free radicals. Two known quenchers were used in this study: L-histidine as a singlet oxygen quencher and D-mannitol as free radicals' quencher. Figure 7 shows the effect of the addition of 10 mM mannitol to the solvent. Comparing Figure 7a, b with Figure 3c and Figure 4c, respectively,

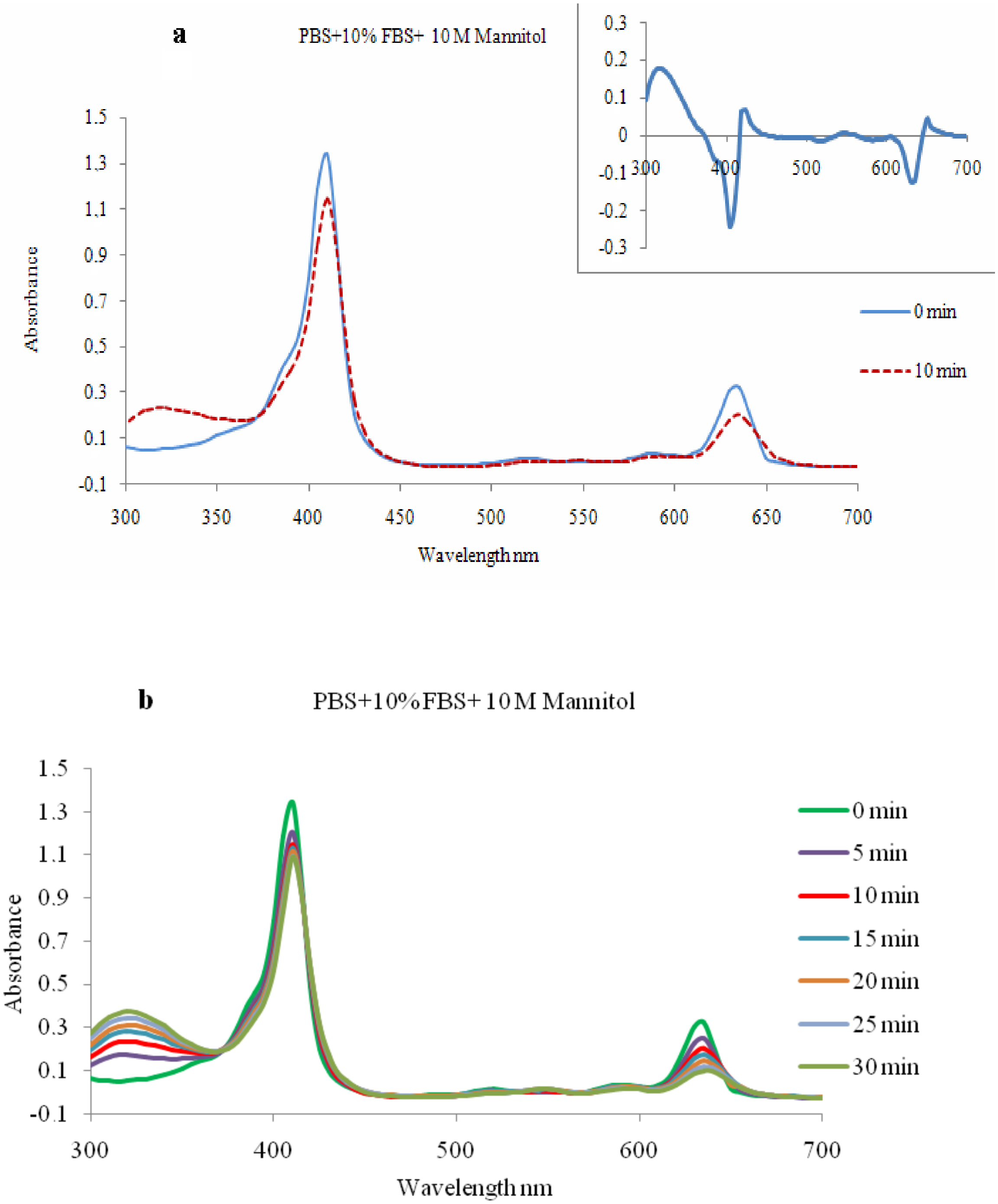

**Figure 7.** Absorption spectra of the photosensitizer in PBS with 10% FBS with addition of 10 mM mannitol; the inset in a represent the difference between the spectra before and after irradiation.

they are almost similar. On the other hand, Figure 8 shows the effect of addition 1 mM histidine to the solvent. Comparing Figure 8a, b with Figure 3c and Figure 4c, respectively, there is a significant difference between them. To investigate the effect of these two quenchers, the rate of photobleaching has been represented in Figure 9. The addition of 10 mM of D-mannitol to 10% FBS solution resulted in a non significant difference in the photobleaching rate compared with 10% FBS. This means that there is a negligible effect of free radicals on the photobleaching rate. On the other hand, the addition of 1 mM of L-histidine resulted in a reduction of

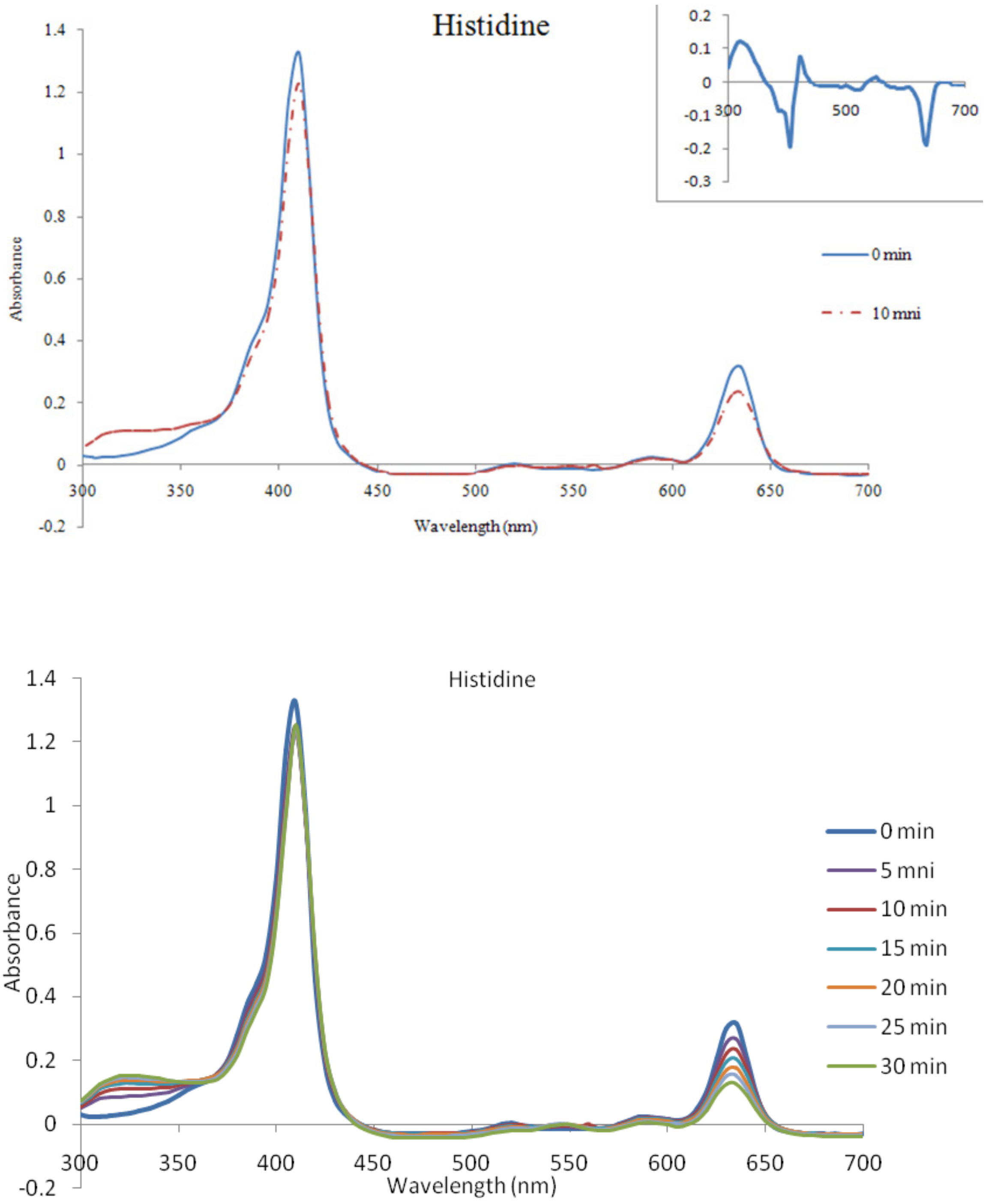

**Figure 8.** Rate of change of Q band absorbance of Sn(IV) chlorin e6 during photoillumination in different environments and effect of addition of histidine and mannitol: neat PBS, with histidine, with mannitol, and with 1% FBS only.

photobleaching rate. This observation proves that singlet oxygen generated by type I process plays an important role in photobleaching of Sn(IV) chlorine e6 (Das et al., 2005).

Porphyrin's derivatives, which are hydrophobic in nature, showed a strong decrease in the absorption at all bands in buffer (Belitchenko et al., 1998; Maestrin et al., 2004). The reduction in absorption at all absorption bands can be due to the aggregation of the photosensitizer in the polar environment. Consequently, the

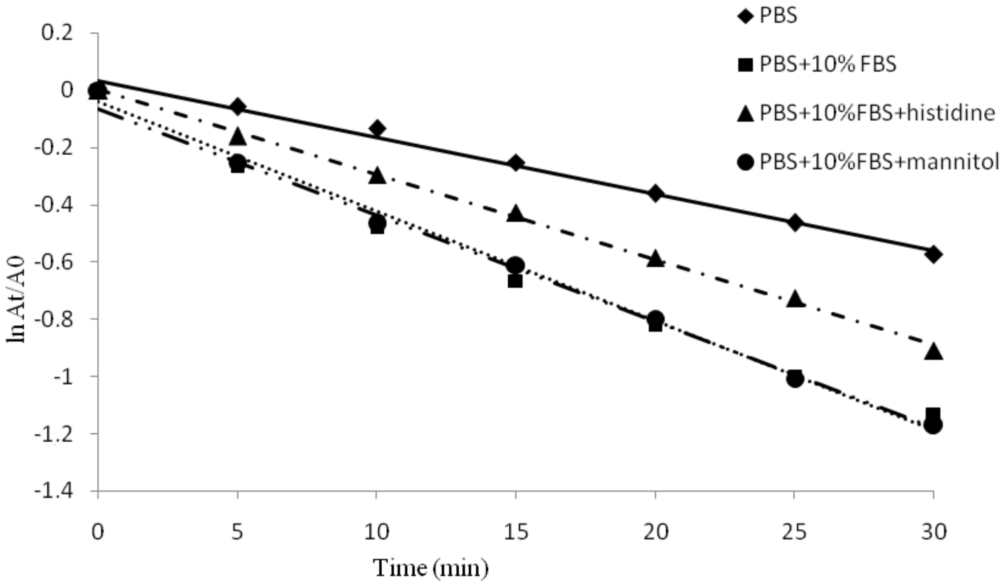

**Figure 9.** Rate of change of Q band absorption of Sn(IV) chlorin e6 during photoillumination in different environments and effect of addition of histidine and mannitol: neat PBS, with histidine, mannitol and 1% FBS only.

presence of proteins in media leads to mono-merization.

## Conclusions

The photosernsitizer have a high solubility in polar solvents and less in non-polar solvents, which is due to amphiphilic nature for this photosensitizer. The modifications effect on photosintizer were observed as spectra changes and shift in maxima positions of absorption spectra due to protonation. The photobleaching rate of the photosensitizer with serum is faster than with neat buffer, which can be explained that the reduction of the diffusional motion of the drug allows for more reactions with singlet oxygen to get more photobleaching. The decrease in aggregation in neat buffer may be related to aggregation in the photosensitizer.

The generated singlet oxygen upon irradiation has had major responsibility for the photobleaching of this photosensitizer. The reduction in absorption at all absorption bands can be due to the aggregation of the photosensitizer in the polar environ-ment. The photoproducts resulted during irradaition are similar to the photoproducts that was achieved from porphyrin, hematoporphyrin and chlorine's derivatives. The efficiency of photobleaching and the formation photoproducts in aqueous solution were conditioned by the aggregation state and chemical structure of photosensitizer. A new peak position of absorption was observed in the ultra violet region with the presence of serum. Singlet oxygen was found to be the main one responsible for photobleaching.

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