CHAPTER V

Change in conformation of J-aggregate 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS) by addition of nonionic surfactant (Triton X-100)

5.1 Introduction

Molecular aggregates are of particular interest because of their unique electronic and spectroscopic properties [118-120]. There are two important kinds of molecular aggregation: J- and H-aggregates arranged in different way. J-aggregate exhibited a red-shift in absorption spectra, and was one-dimensional molecular arrangement in which the transition moments of individual monomers are aligned parallel to the line joining their centers (end-to-end arrangement or side-by-side arrangement). H-aggregate exhibited a blue shifted absorption band, and was aligned parallel to each other but perpendicular to the line joining their centers (face-to-face arrangement). 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS) is one of water-soluble porphyrins that can form aggregates in acidic solution [121-124] or in high ion strength containing inorganic cations [125]. For the recent years, there were many papers studied on aggregation by addition of some cationic molecules, e.g. cationic surfactants (i.e. cetyltrimethyl ammonium bromide (CTAB) [126,127]), and cationic dyes cyanide dyes (i.e. 3,3'-diethyloxacarbocyanine iodide {2[3-(3-ethyl-2,3-dihydrobenzooxolylidene)-propenyl]-3-ethylbenzoxazoliumiodide}(DiOC2(3) and 3,3'-dihexyloxacarbocyanine iodide {2-[3-(3-hexyl-2,3-dihydrobenzooxazolylidene) propenyl]-3-hexyl-benzoxazolium iodide} (DiOC6(3))) [128]. The J-aggregate of H_2TPPS was also induced by some polymers, e.g. polylysine, that used as a template for aggregate [129]. H_2TPPS is well known as a achiral compound in the monomer form while the J-aggregate, $(H_4TPPS)_n$, is a chiral compound that can induce CD spectra. The previous studies suggested that the sign of the CD spectra of the J-aggregate was changed by the stirring direction of solution [130,131] or by addition of enantiomer compounds, e.g. D- or L-typtophan [132]. The nonionic surfactant (Triton X-100,) altered CD spectra of J-aggregate to opposite sign was observed. In

this study, the interaction between the J-aggregate, $(H_4TPPS)_n$, and Triton X-100 were investigated. The mechanisms of the conformation changing of the J-aggregate, $(H_4TPPS)_n$, with different concentrations of Triton X-100 were also described.

5.2 Theory and literature survey

5.2.1 5,10,15,20-Tetrakis 4-sulfonatophenyl porphyrin (H_2TPPS)

H_2TPPS is a water soluble tetrapyrollic dye of well-defined chemical structure. Recently, H_2TPPS was widely used in clinical experiments as a potential sensitizer for the photosensitized therapy. The photophysical properties of a sensitizers, which predetemine its photochemical activity, are great important to its successful application for the clinical purposed.

H_2TPPS is known to form J-aggregate in acidic conditions or at very high ionic strength. Formation of aggregate changed in the absorption spectra of porphyrins, aggregates exhibited different lifetimes of singlet and triplet states in comparison to monomers, and as a rule lower constant of intersystem crossing and singlet oxygen generation quantum yields. This could affect the photosensitizing activity of porphyrins accumulated in cancerous tissues. The experimental investigations suggested that medium in cancerous tissue is often more acidic than in normal tissues. A hydrophilic molecule with negatively charged substitutes, H_2TPPS, accumulates in cells mainly in lysosomes, is acidic. Recently, applied research effects have endeavored to develop and exploit artificial systems of molecular aggregates for device application, since close-stacked molecular structures possessed properties suitable for superconductivity, optical frequency conversion as well as information processing, transmission, and storage. Despite of what is known about the spectroscopic features and excitonic in molecular aggregates, the mechanism and conditions of aggregation formation and their geometrical structure are continuing interest.

5.2.2 Literature survey

Self assembled molecular aggregates, formed by non-covalent

interactions, are much interest because of the special properties and possible technological applications of the mesoscopic materials.

The monomeric units of the chromophores might be arranged in different ways in an aggregate structure which may of may not be ordered. The aggregates, in which the molecular arrangement was highly ordered, namely H- and J-aggregates, was of special interest because of their unique electronic and spectroscopic properties.

H-aggregate was one dimensional arrangement of strongly coupled monomer such that the transition moments of the monomers are perpendicular to the line of centers (face-to-face arrangement). J-aggregate was also formed with the monomeric molecules arranged in one dimension, however, the transition moment of the monomers are parralled and the angle between the transition moment and the line joining the molecular centers is zero (side-by-side arrangement). J-aggregate was first described by Jelley and Scheibe in 1936. The optical properties of these molecular systems have attracted great interest. Cyanine dyes are typical compounds to form J-aggregate. The photophysical properties of porphyrin aggregate were extensively investigated by various spectroscopic techniques because of the structural and spectral similarities between the porphyrin and chlorophylls that played a vital role in nature such as photosynthetic systems. The water soluble anionic porphyrin, H_2TPPS, is known to form J-aggregate in acidic conditions [121-124] or at very high ionic strength [121] which has been confirmed by resonance light scattering[134], circular dichroism and polarization[121], fluorescence lifetime and anisotropy [135], and absorption and raman scattering [136]. Ohno et. al. [121] reported that the increasing of ionic strength (sodium perchlorate), the increasing of aggregated was obtained. The results were confirmed by measuring the UV-vis spectra of monomeric diacid TPPS (H_4TPPS) in the presence of various concentration of sodium perchlorate. The UV-vis spectrum indicated that an enhancement of the absorbance due to the aggregate formation is observed with the increasing of ionic strength, while the H_4TPPS was detected only in the low ionic strengths. In 1998, Maiti and coworkers [126,127] reported that the cationic surfactant cetyltrimethylammonium bromide (CTAB) can 1also induced the aggregate of TPPS. The interaction of cationic surfactant with H_4TPPS, led to the formation of the aggregate when the concentration of CTAB below the cmc. (critical micelle concentration) with the stoichiometric ratios of porphyrin : surfactant at 1 : 2 for the J-aggregate and 1 : 4 for the H-aggregate.

Recently, Koti and Periasamy [129] introduced the two cyanine dyes, 3,3'-diethyloxacarbocyanine iodide {2-[3-(3-ethyl-2,3-dihydrobenzoxazolydene) propenyl]-3-ethylbenzoxalium iodide} ($DiOC_2(3)$) and 3,3'-dihexyloxacarbocyanine iodide {2-[3-(3-hexyl-2,3-dihydrobenzoxazolylidene) propenyl]-3-hexyl-benzoxazolium iodide} ($DiOC_6(3)$), that are symmetrical cationic cyanine dyes consisting of two *N*-ethyl and *N*-hexyl substituted benzoxazole groups linked by a trimethine bridge, respectively. The interaction between the H_2TPPS and the $DiOC_6$, with the ratio of porphyrin : cyanine of 1 :4, formed the stable aggregates of H_2TPPS. On the other hands, the cyanine dye, $DiOC_2(3)$, was not formed an aggregate with the porphyrin because the ethyl chain in the cyanine, $DiOC_2(3)$ was too small for the stabilizing the aggregate. Polylysine was firstly introduced by Koti and Periasamy to use as template for the efficient formation of J-aggregate of H_2TPPS. The results indicated that polylysine induced the J-aggregate of H_2TPPS more efficiently than monomeric lysine.

The H_2TPPS in the monomer form is well known as an achiral compound while in the aggregate form is a chiral compound that gives the CD spectra. In 1993, Ohno and coworker [121] reported that the CD sign of J-aggregate varies depending upon the direction of stirring, clockwise or counterclockwise, during the growth of aggregate. Crusats et. al. [130] also reported the chirality of aggregate could be selected by the direction of vertical stirring during the formation of the aggregate. It was conceivable to admit that the growth kinetics of the suspended chiral particles could be affected by stirring and that this would finally result in the enantioselective growth of the aggregates. The CD sign of J-aggregate can also be selected by addition of some enantiomeric molecules. Zhang and coworkers [132] suggested that the D- or L-tryptophan could be used to control the CD sign of the J-aggregate of the H_2TPPS. The CD sign of J-aggregate followed the CD sign of the enantiomeric film. Recently, Jiang and Liu [133] reported the aggregation of H_2TPPS on the layer-by-layer of DNA/PAH film. H_2TPPS could be directly penetrated into a DNA/PAH film and assembled into both H-aggregate and J-aggregate. H_2TPPS could also be penetrated into the DNA/PAH film using of cationic meso-tetrakis-(4-N-methylpyridyl) porphyrin (TMPyP), as a spacer. The results suggested that the chirality of J-aggregate TPPS gave the opposite sign in the presence of TMPyP in comparison to the chirality in the absence of TMPyP.

5.3 Experimental

5.3.1 Chemicals and reagents

5.3.1.1 5,10,15,20-Tetrakis 4-sulfonatophenyl porphyrin (TCI)

5.3.1.2 Triton X-100 (ICN Biochemicals, Inc.)

5.3.1.3 Polyethylene glycol 300 (PEG 300, TCI)

5.3.1.4 Acetic acid (Wako Chemical)

5.3.1.5 Sodium perchlorate (Wako Chemical)

5.3.1.6 Double distilled water (Milli-Q, Millipore).

5.3.1.7 N-(2-hydroxyethlyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES, Fluka)

5.3.1.8 Hydrochloric acid (Wako Chemical)

5.3.2 Instruments

5.3.2.1 UV-vis spectrophotometer (JASCO V-550). The UV-vis spectra were measured at 25 ^{0}C.

5.3.2.2 CD spectrophotometer (JASCO Spectrophotometer Power Supply 91N, Japan). The CD spectra were measured three times and averaged.

5.3.2.3 Fluorescence spectrophotometer (Hitachi F-4500).

5.3.2.4 pH meter (Methorm)

5.3.3 Preparation of J-aggregate, $(H_4TPPS)_n$

J-aggregate, $(H_4TPPS)_n$, was prepared by mixing of the following solution i) the appropriate volume of $5x10^{-3}$ M ii) 1 ml 0.01 M CH_3COOH and iii) 0.1 M sodium perchlorate. Then, the volume was adjusted by deionized water and kept in an incubator at 25 $^{\circ}$C for 1 day before measurements.

5.4 Results and discussion

5.4.1 Characteristic feature of J-aggregate, $(H_4TPPS)_n$

Fig. 5.1 shows UV-vis (left) and fluorescence spectra (right) of different species of H_2TPPS. The absorption spectrum of H_2TPPS in aqueous buffer pH 7 consists of four Q-band located at 515, 550, 578 and 633 nm. The absorption spectrum of H_2TPPS exhibited a maximum spectrum at 413 nm and the fluorescence spectrum of H_2TPPS exhibited maximum emission spectrum at around 650 nm. The previous study reported that the addition of acid (pH < 5) to a solution of H_2TPPS free base was generally believed to lead directly to the formation of diacid with proton attached to each pyrrole N-atom (Fig. 5.2) which dramatically changed its spectrum. The H_4TPPS exhibited a new absorption band that was red shift from the parent free base. The maximum absorption spectrum at 434 nm and the maximum emission spectrum at around 680 nm were observed. When the solution containing the high concentration ($\geq 10^{-5}$ M) in the buffer solution pH 3.3, the positive charge at protonated pyrroles of H_4TPPS could be interacted with the peripherally sulfonatophenyl anionic groups that induced the aggregation of H_4TPPS by ion-pair formation or electrostatic interaction that causes charge-neutralization. The UV-vis absorption spectra of J-aggregate,$(H_4TPPS)_n$, exhibited the characteristic peaks of J-aggregate,$(H_4TPPS)_n$, at 490 and 706.5 nm in acetic acid solution. Moreover, the J-aggregate exhibited peaks at around 434 and 645 nm that were attributed to monomer H_4TPPS. The fluorescene spectrum of J-aggregate, $(H_4TPPS)_n$, showed the maximum emission spectrum at around 716 nm. The J-aggregate, $(H_4TPPS)_n$, and H_2TPPS were also measured by polarized fluorescence (Fig. 5.3). The polarized fluorescence spectra of J-aggregate, $(H_4TPPS)_n$, and H_2TPPS were measured as a function of excitation wavelength. In the case of J-aggregate, $(H_4TPPS)_n$, a great positive polarization peak was observed upon excitation in the aggregate absorption maxima at around 490 nm for J-aggregate, $(H_4TPPS)_n$, (Fig 4.3a) while no polarization peak was observed for H_2TPPS (Fig 4.3a).

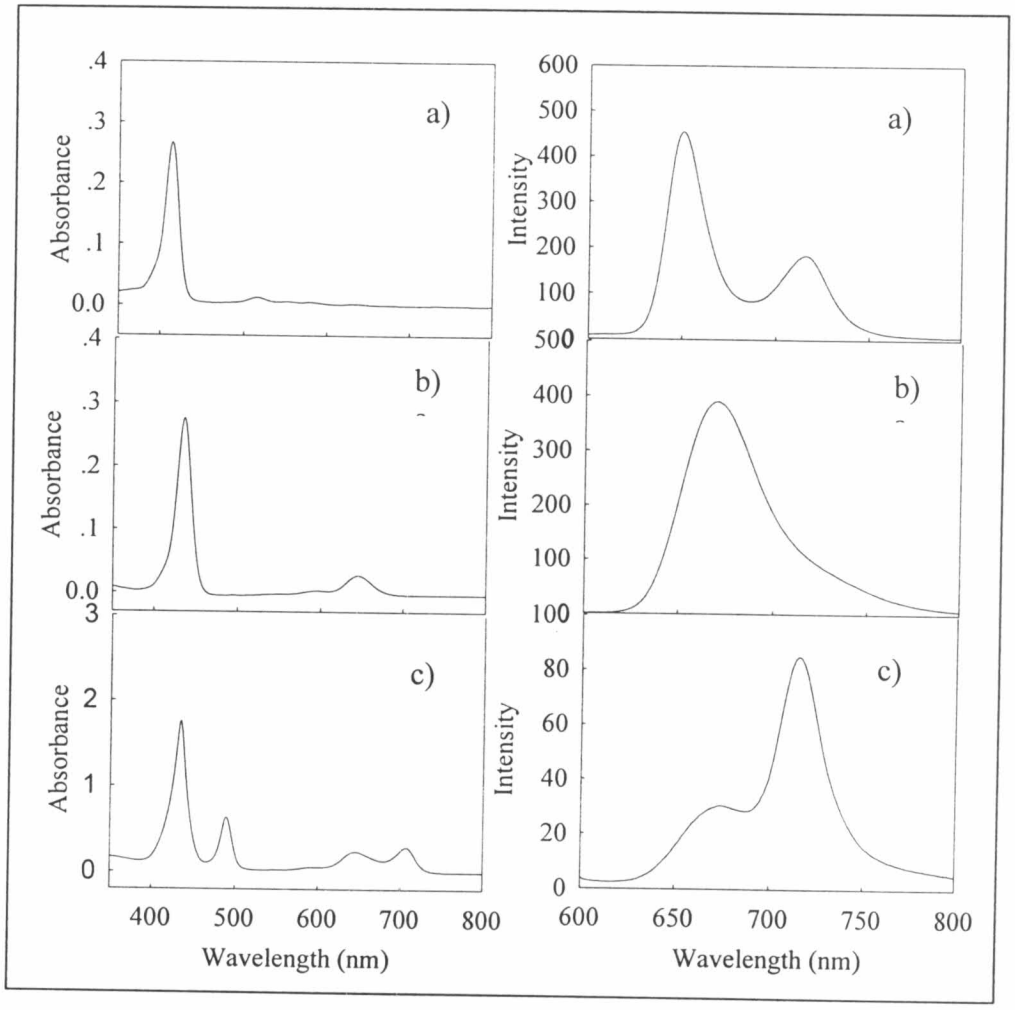

Figure 5.1 UV-vis (left) and fluorescence spectra (right) of: a) $1x10^{-6}$ M H_2TPPS in pH 7, b) $1x10^{-6}$ M H_2TPPS in pH 3.3, and c) $1x10^{-5}$ M H_2TPPS in pH 3.3.

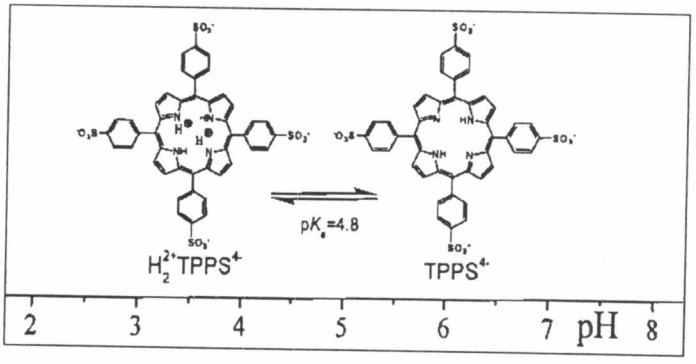

Figure 5.2 Protonation and depronotonation structure of H_2TPPS [135].

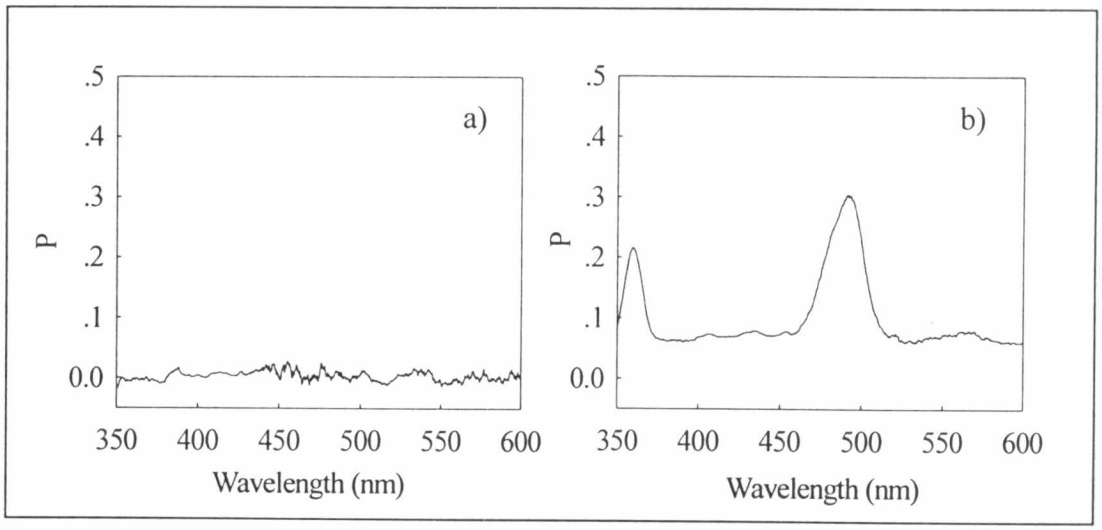

Figure 5.3 Fluorescene polarization of $1x10^{-5}$ M H_2TPPS in a) pH 7 and b) pH 3.3.

5.4.2 Effects of Triton X-100 on pK_a of H_2TPPS

The solution containing the $1x10^{-6}$ M H_2TPPS in pH less than 5 was used in this study. The pK_a value of H_2TPPS was about 4.8 (pK_{a3} = 4.76; pK_{a4} = 4.8 [138]) that was attributed to the proton attached to pyrrole N-atom of H_2TPPS. The pKa values were evaluated in the presence of various concentrations of Triton X-100. The spectra of H_4TPPS in the solution containing Triton X-100 exhibited the intensity of soret-band, which was located at 418 nm (Fig. 5.4). The absorbance at the wavelength of 418 and 434 was plotted against with pH and used for the calculation of pKa using specfit 32 program (Fig 5.5). The results showed that the pKa value decreased when the presence of the concentration of Triton X-100 increased because the interaction between H_2TPPS and Triton X-100 was faster than the protonation of H_4TPPS.

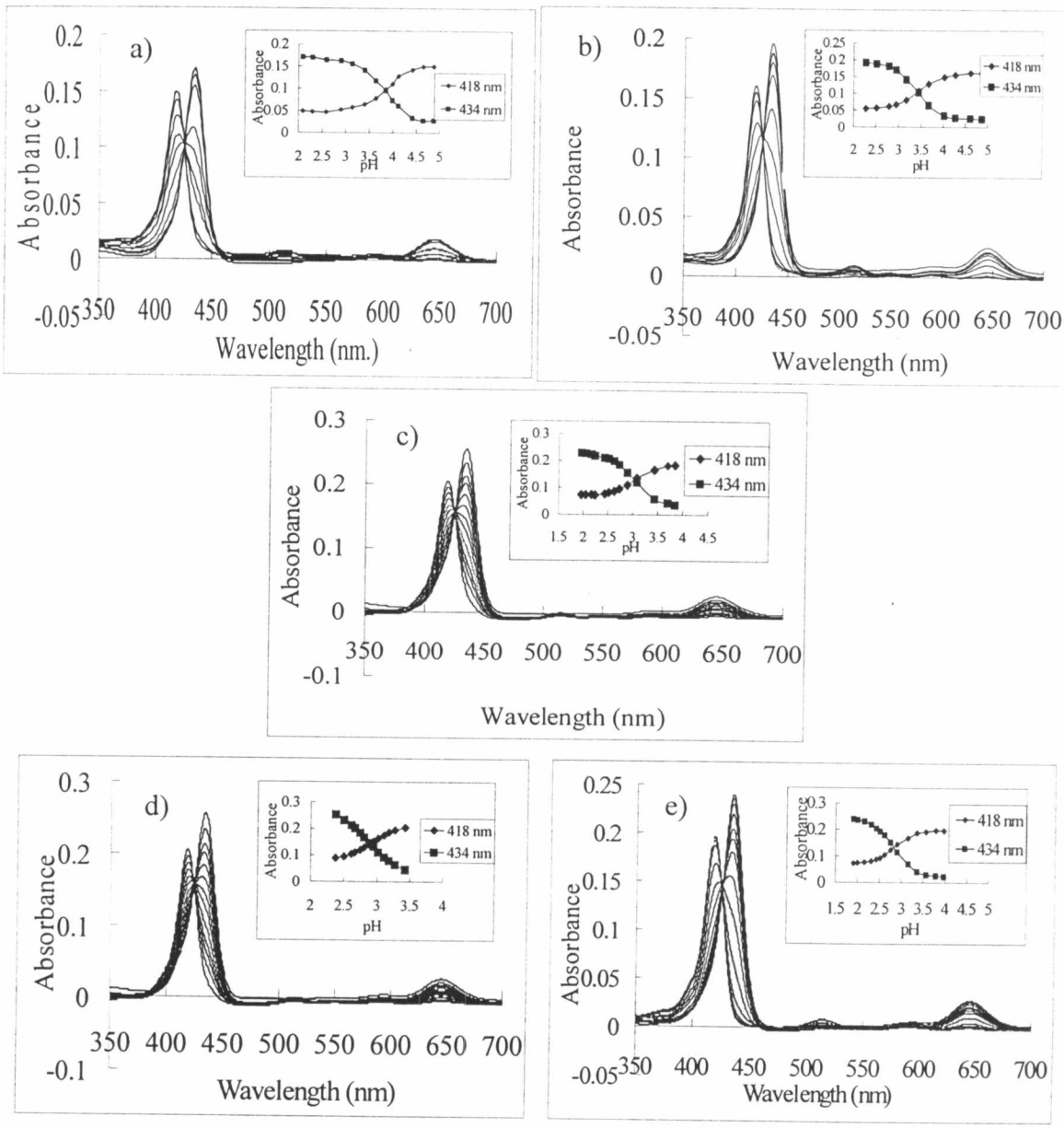

Figure 5.4 UV-vis titration curves between the $1x10^{-6}$ M H_2TPPS in the presence of Triton X-100 a) 0.05%, b) 0.1%, c) 0.5 %, d) 1%, and e) 2% v/v. The inset Figures are the curves between the absorbance and pH.

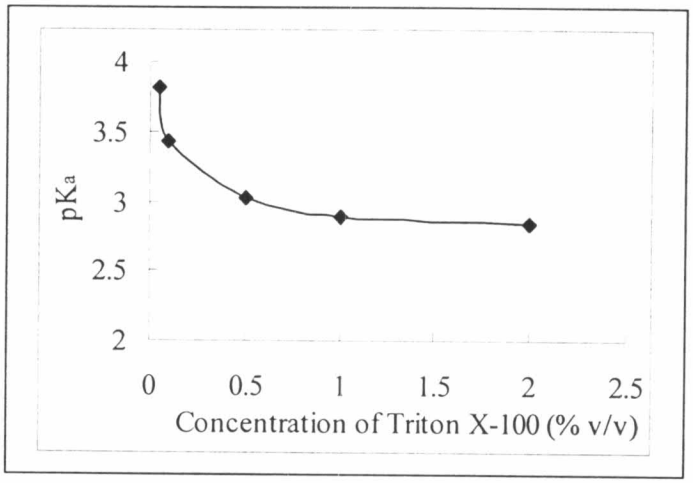

Figure 5.5 Relationship between the concentration of Triton X-100 and pK_a.

5.4.3 Effect of time on the J-aggregate, $(H_4TPPS)_n$

The J-aggregate, $(H_4TPPS)_n$,was study as a function of time. Fig 5.6 shows a UV-vis spectra of the solution containing $1x10^{-5}$ M H_2TPPS at the different of time. The results showed that the increasing of the time, the increasing of J-aggregate, $(H_4TPPS)_n$, as the evidence in the increasing of absorbance at the wavelength of 490 and 706.5 nm were obtained. While the H_4TPPS was decreased as the evidence in the decreasing of the absorbance at the wavelength of 434 and 644 nm. The decreasing of the absorbance at 434 nm was match well by the growth of the absorbance of 490 nm correspond to the mechanism of the formation of J-aggregate, $(H_4TPPS)_n$. The J-aggregate, $(H_4TPPS)_n$, formation was completed in 1 day.

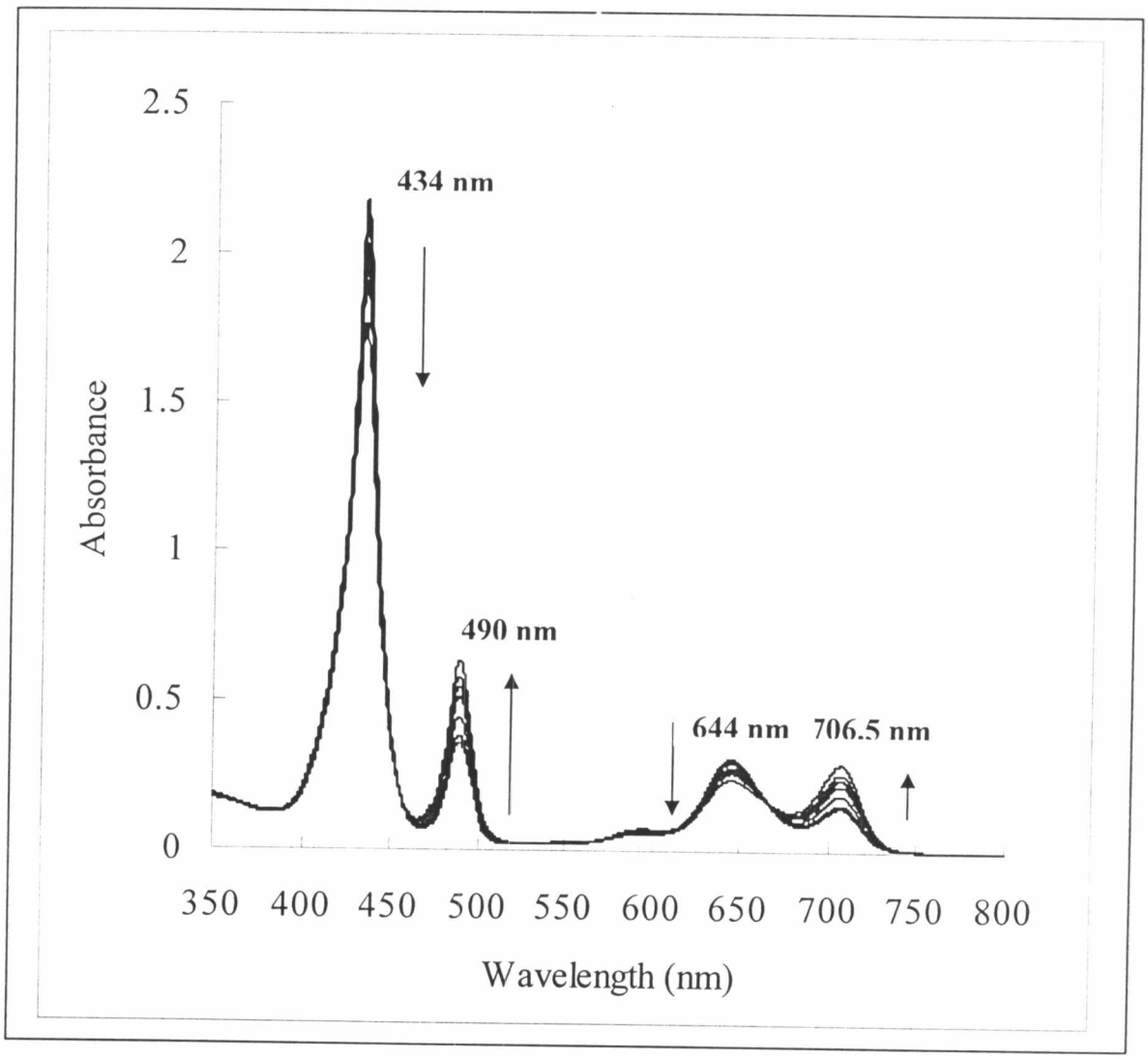

Figure 5.6 UV-vis spectra of H_4TPPS (1 X 10^{-5} M) in 0.01 M acetic acid and 0.1 M sodium perchlorate.

5.4.4 Effect of time of the interaction between J-aggregate, $(H_4TPPS)_n$ and Triton X-100

The effect of time on the interaction between the J-aggregate, $(H_4TPPS)_n$, and Triton X-100 was investigated. In this study, the concentration of Triton X-100 above critical micellar concentration (cmc., 5% v/v) was used.

The UV-vis spectra of TPPS in the presence of Triton X-100 above the cmc. exhibited a new peak at the wavelength 418 nm while the wavelength at 434 nm disappeared (Fig 5.7). The increasing of the time, the increasing of the peak at 418

nm was obtained While, the peak at 490 nm that were attributed to the J-aggregate, $(H_4TPPS)_n$, decreased with the time until the peak disappeared (2 days). The results suggested that J-aggregate, $(H_4TPPS)_n$, dissociated into the micelle and was soluble as the form of deprotonated free base porphyrin (H_2TPPS).

The CD spectra of TPPS at the same condition were also measured (Fig. 5.8). Firstly, the CD spectra decreased when addition the Triton X-100 to the solution. Then, the CD spectra changed to the opposite sign and decreased with the time. Finally, the CD spectra disappeared that are attributed to the completely solubilized of H_2TPPS in the micellar of Triton X-100 media.

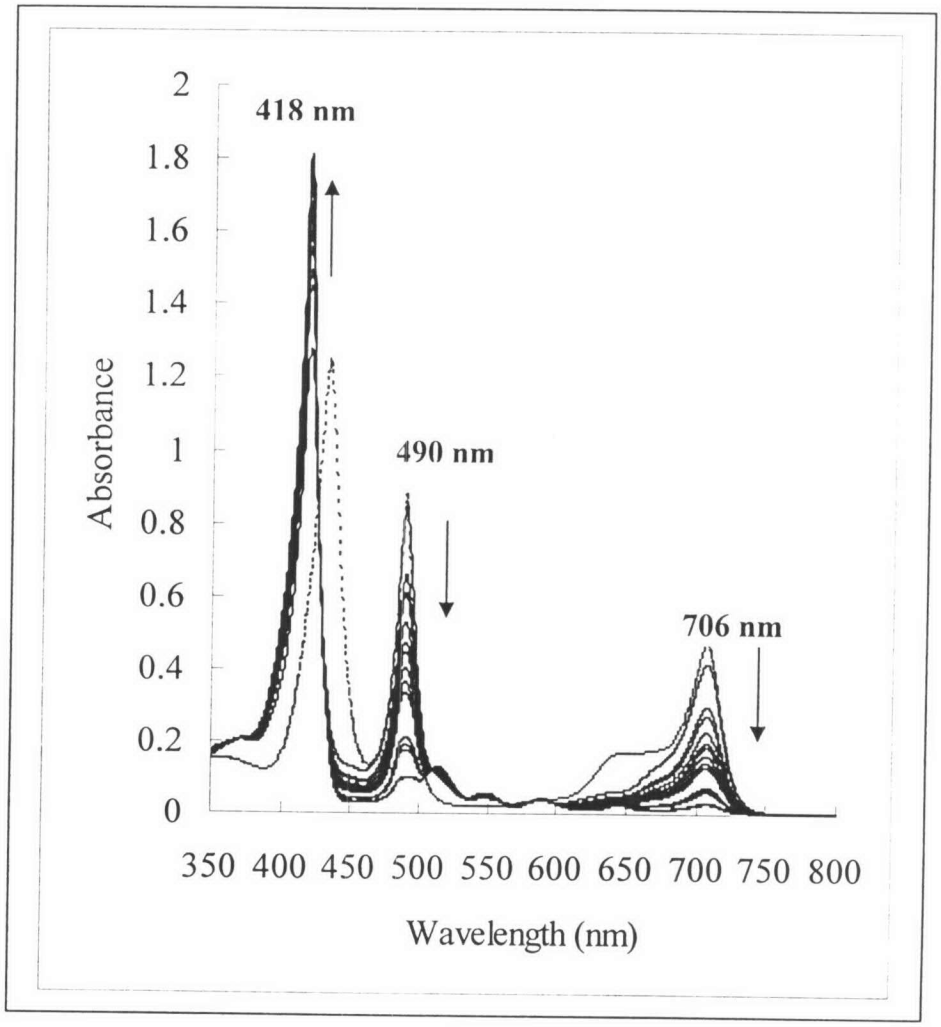

Figure 5.7 UV-vis spectra of H_4TPPS (1 X 10^{-5} M) in the presence of Triton X-100 of 5% in v/v (pH 3.6) in 0.01 M acetic acid and 0.1 M sodium perchlorate as a function of time. The UV-vis spectra (dash line) represented the spectra of H_4TPPS (1 X 10^{-5} M).

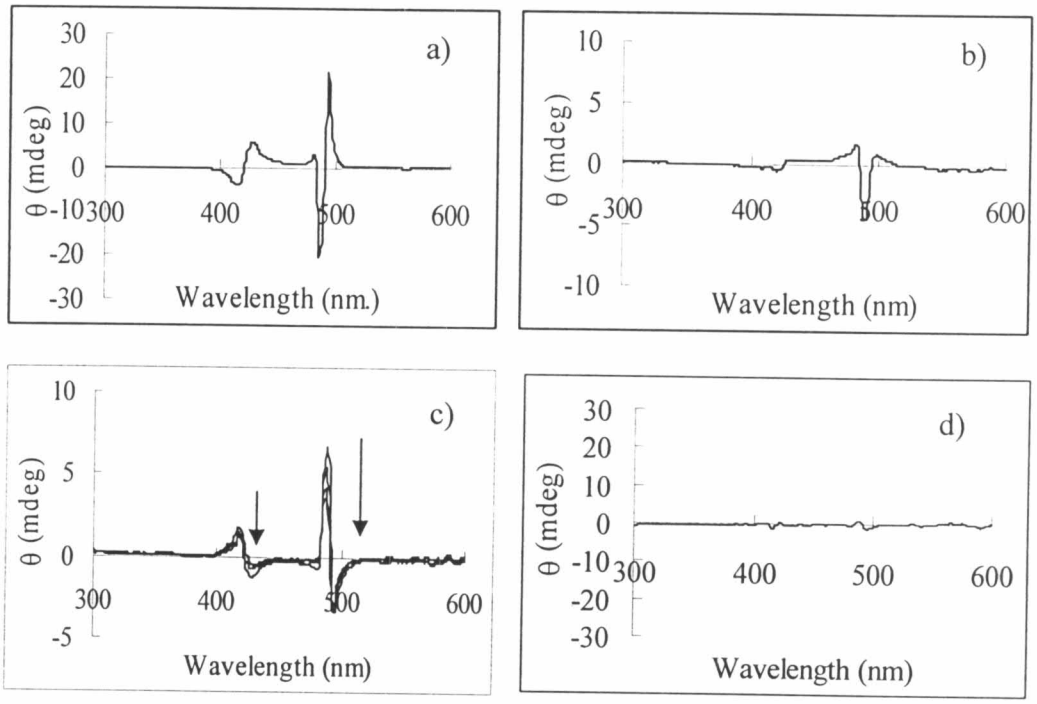

Figure 5.8 CD spectra of H_4TPPS (1 x 10^{-5} M) in the presence of Triton X-100 5 % in v/v (pH 3.6) in 0.01 M acetic acid and 0.1 M sodium perchlorate at a) 0 min, b) 15-60 min and c) 2 days.

5.4.5 Effect of the concentrations of Triton X-100 on the J-aggregate, $(H_4TPPS)_n$

The UV-vis spectra of J-aggregate,$(H_4TPPS)_n$, were also investigated in the presence of various concentrations of Triton X-100 in order to gain the more insight in the mechanism of the interaction between the J-aggregate, $(H_4TPPS)_n$, and Triton X-100. The UV-Vis spectra depended on the concentrations of Triton X-100, especially, concentration below, near or above critical micelle concentration (cmc.) of Triton X-100 (Fig. 5.9). The details of each concentration were described as the following:

(a) Triton X-100 below cmc. (0.001% v/v). The UV-vis spectrum of H_4TPPS solution exhibited absorption maximum peaks at 434 and 490 nm. The peak intensity at the 490 nm decreased in the presence of Triton X-100 that was attributed the interaction of Triton X-100 with $(H_4TPPS)_n$ and to the dissociation of the J-aggregate, $(H_4TPPS)_n$, into monomer H_4TPPS.

(b) Triton X-100 near cmc. (0.1% v/v). Triton X-100 formed premicelle under this condition. The UV-vis spectrum exhibited a board peak at the maximum wavelength around 430 nm. This peak was a mixture of two species, J-aggregate $(H_4TPPS)_n$ and H_2TPPS into the micelle.

(c) Triton X-100 above cmc. (5 % v/v). Triton X-100 formed micelle in the solution under this condition. UV-vis spectra showed a maximum wavelength at 418.5 nm, while the peak at 434 nm disappeared and the peak at 490 nm decreased. The results suggested that J-aggregate, $(H_4TPPS)_n$, was dissociated into monomers and are solubilized into the micelle as the form of deprotonated free base H_2TPPS.

Fig. 5.10 shows the change of CD spectra of J-aggregate, $(H_4TPPS)_n$ in the presence of Triton X-100 at different concentrations under the same conditions of UV-vis spectral measurements. The CD spectrum of J-aggregate changed to opposite sign by addition of Triton X-100 at the concentration below cmc. (Fig. 5.10b) and the intensity decreased with increase of Triton X-100 (Fig. 5.10c) and finally disappeared at the concentration of Triton X-100 above cmc (Fig. 5.10d). The turnover of CD spectra by addition of Triton X-100 implied a specific interaction of Triton X-100 with $(H_4TPPS)_n$.

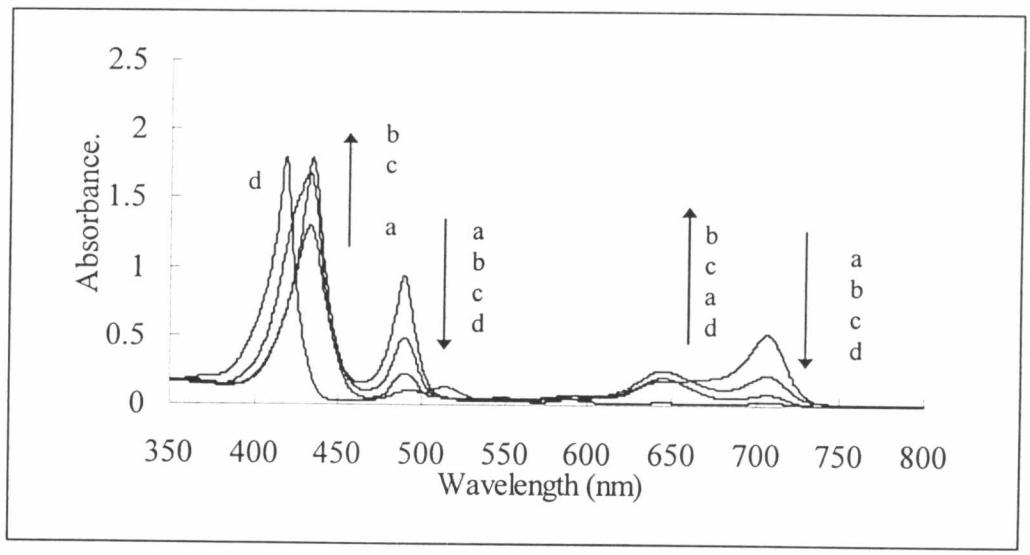

Figure 5.9 UV-vis spectra of H_4TPPS (1×10^{-5} M) in the presence of Triton X-100 of (a), 0% (pH 3.3); (b) 0.001% (pH 3.3); (c), 0.1% (pH 3.3) and (d), 5% in v/v (pH 3.6) in 0.01 M acetic acid and 0.1 M sodium perchlorate.

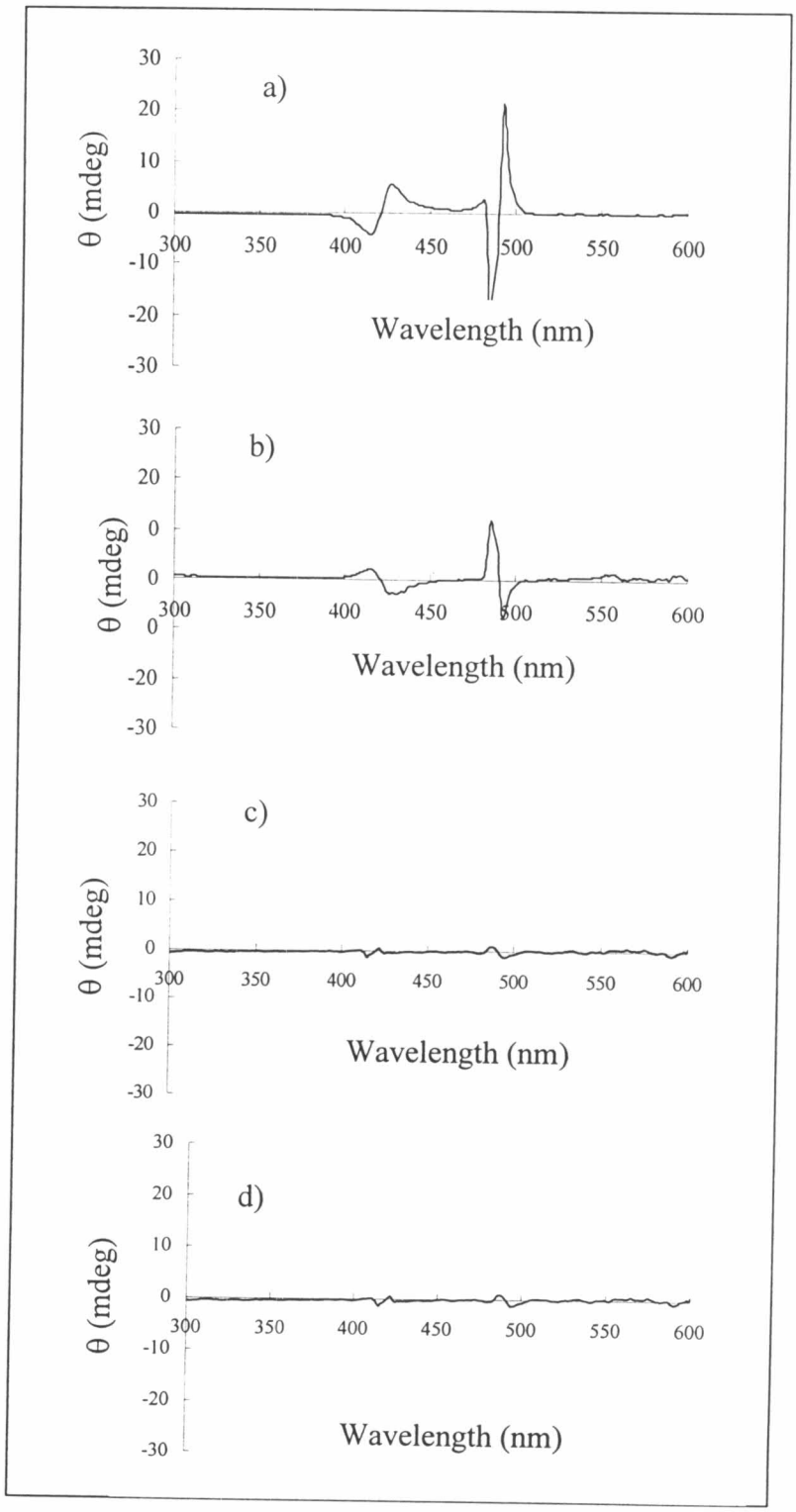

Figure 5.10 CD spectra of H_4TPPS (1×10^{-5} M) in the presence of Triton X-100 of (a), 0 % (pH 3.3); (b), 0.001% (pH 3.3); (c), 0.1 % (pH 3.3) and (d), 5 % in v/v (pH 3.6) in 0.01 M acetic acid and 0.1 M sodium perchlorate.

5.4.6 Effect of polyethylene glycol on the J-aggregate, $(H_4TPPS)_n$

Due to the finding of the new phenomena as described in previous section, the nonionic polymer, Polyethylene glycol 300 (PEG 300), that has a functional group and the molecular weight near the Triton X-100 was investigated by the measurement of CD-spectra. Fig. 5.11 shows the CD spectra of J-aggregate, $(H_4TPPS)_n$, in the presence of various concentrations of PEG 300. PEG 300 interacted toward J-aggregate, $(H_4TPPS)_n$, resulting the changed the CD-spectra to opposite sign. The higher the concentrations of PEG 300, the higher the intensities of CD spectra (opposite sign) were obtained. Moreover, the reaction between J-aggregate, $(H_4TPPS)_n$, and PEG 300 (1 hr.) was faster than the reaction between J-aggregate, $(H_4TPPS)_n$, and Triton X-100 (2 days).

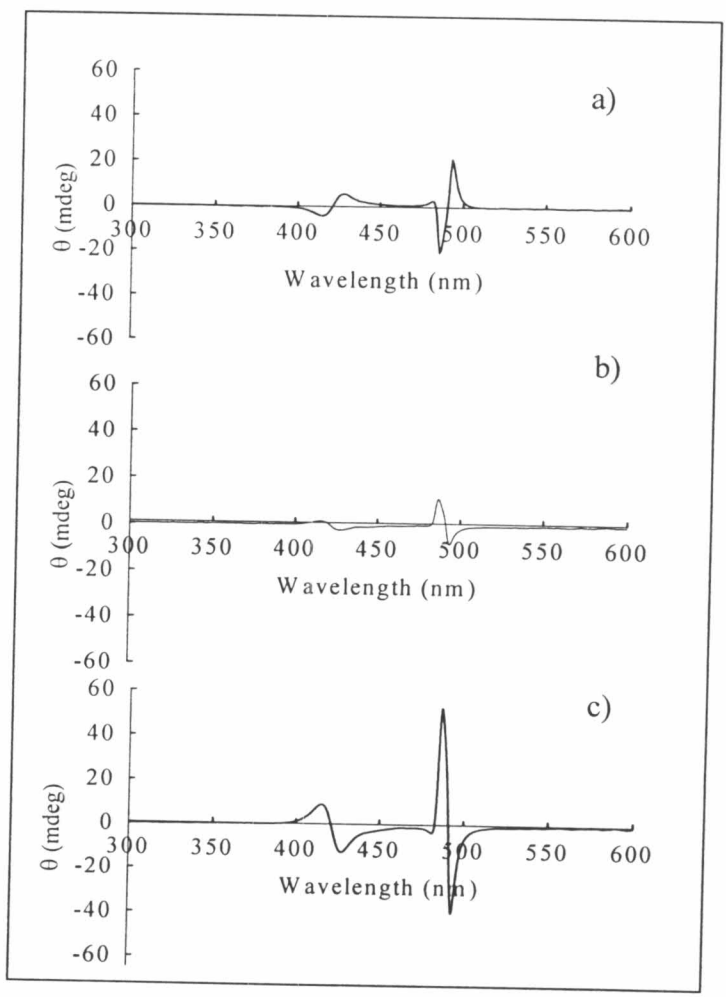

Figure 5.11 CD spectra of H_4TPPS (1×10^{-5} M) in the presence of PEG 300 of (a), 0 % (pH 3.3); (b), 0.01% (pH 3.3); and (c), 5 % in v/v (pH 3.6) in 0.01 M acetic acid and 0.1 M sodium perchlorate. The time of reaction was 1 hr.