6
Solvent dependent interaction of OP with BSA: A comparative fluorescence quenching analysis

6.1 INTRODUCTION

The current Chapter describes the application of Fluorescence quenching study complemented with UV-vis absorption and Circular Dichroism (CD), enabling comparative analysis of the effect of solvent on organophosphate pesticide (OP)-protein interaction. The major focus of the present Chapter is to quantify the binding interaction of BSA with the widely used organophosphate pesticide (OP) CPF and its stable metabolite 3, 5, 6-trichloro-2-pyridinol (TCPy) in two different solution conditions to shed light on the effect of solvents on the OP-protein interaction. The multi-spectroscopic approach attempted here has proven to be appropriate for deciphering the molecular mechanism of OP-protein interaction.

As already discussed in Chapter 1, several experimental animal models and *in vitro* analysis have pointed out those adverse effects of OP pesticides, and the possible toxic effect of a combination of pesticides and their degradation products are not well interpreted. Hence the issue still requires further understanding both at the molecular as well as at the cellular levels. The fluorescence quenching studies of BSA emission in two different solvents *viz.*, water, and methanol in the presence of CPF and TCPy have led to the revelation of several interesting facts about the pesticide-protein interaction. The degradation of CPF to TCPy is shown in Chapter 1 (Figure 1.11). At neutral pH, TCPy is a charged species and has more water solubility compared to CPF [Anwar et al, 2009]. TCPy is excreted in urine with an average half-life of about 27 hrs as TCPy-glucuronide [Morgan et al, 2005]. Toxicity of TCPy is still debatable as it does not inhibit cholinesterase enzymes, unlike its parent [NPIC, 2010]. Conversely, on various other occasions, it is considered as more toxic due to its greater water solubility causing contamination of both soil and aquatic environments [John & Shaike, 2015a]. Even though toxicity studies of CPF is a commonly addressed subject, only a few reports are available on the toxicity of TCPy, which may have a distinct toxicity profile. Thus, a comparative analysis of these two pollutants in terms of their binding efficacy, possible diverse binding interaction as well as the effect of binding on conformation (if any) of serum protein is meaningful [Jun Wang et al, 2014]. Figure 6.1 illustrates the graphical representation of the methods used for the present Chapter.

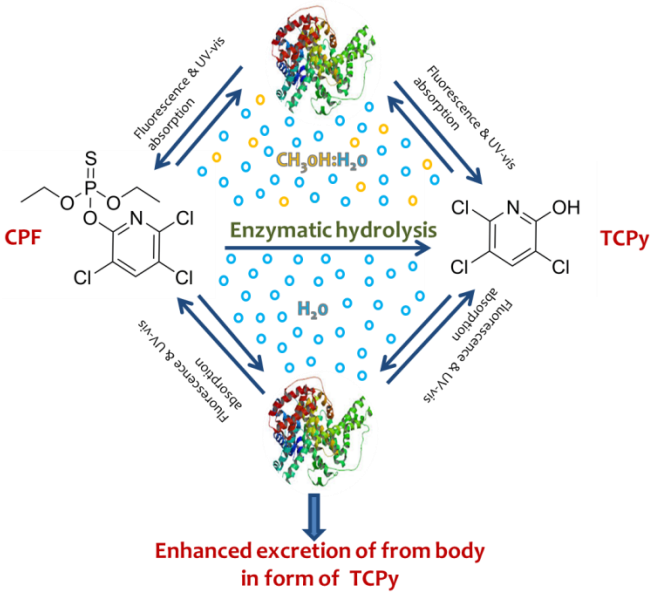

Figure 6.1 : Schematic representation of CPF-BSA and TCPy-BSA interaction.

6.2 SAMPLE PREPARATION

BSA and TCPy stock solutions were prepared by dissolving in phosphate buffer (PB: 0.05 M; pH 7.4). The stock concentrations were determined to be 20 μM for BSA and 100 μM for TCPy and used for both fluorescence and UV spectroscopy. Chlorpyrifos (CPF) is sparingly soluble in water, *i.e.*, maximum up to 5 μM, and hence, the concentration of CPF is maintained to 5 μM for stock preparation in PB. For studying fluorescence quenching mechanism at higher concentrations, another 100 μM stock solutions of CPF and TCPy were prepared in anhydrous methanol. Aliquots of BSA, TCPy, and CPF solutions were used to prepare fresh solutions before each measurement.

6.3 EXPERIMENTAL DETAILS

6.3.1 Fluorescence Measurements

Fluorescence emission of BSA was measured by scanning the solutions using a Perkin Elmer, LS 55 fluorescence spectrophotometer in the range of 250-450 nm using a 1.0 cm quartz cuvette. The excitation and emission slit widths were set to 5 nm throughout the experiments with a scanning speed of 200 nm/min. The fluorescence emission maximum was determined by keeping the excitation monochromator at 280 nm, and it was found to be at 347 nm for solutions in PB and at 344nm for solutions in PB with 10% methanol. To differentiate the quenching effect on the intrinsic tryptophan fluorescence from the global protein fluorescence, emission spectra were also recorded at 295 nm. All the experiments were carried out at room temperature keeping the concentration of protein constant at 0.5 μM in the final solution while increasing the concentration of both pesticides starting from 0–10 μM in case of 10% methanol in PB and up to 5 μM in aqueous PB (pH 7.4) to study both the systems BSA-CPF and BSA-TCPy.

6.3.2 UV-Vis Absorption Measurements

The UV-vis absorption spectra were recorded on a VARIAN Carry 400 UV-vis spectrophotometer in a 10 mm quartz cuvette and scanned from 195 to 500 nm at room temperature. The solutions for UV measurements were prepared in a way to have a final concentration of BSA as 5 μM in solution while the concentration of pesticides was increased from 0 μM to 10 μM resulting five different pesticides to protein concentration ratios of 0:1, 0.5:1, 1:1, 1.5:1 and 2:1. In the case of CPF-BSA, the final solutions contained 5% of methanol. This was obtained by making dilutions using PB.

6.3.3 Circular Dichroism Spectroscopy

The CD spectra were recorded using JASCO, J-815 CD-Spectrometer in nitrogen (N_2) atmosphere in the wavelength range of 200–300 nm. An N_2 purging rate of 5 LPM (5l/min) was maintained throughout the experiments. A scan rate of 20 nm/min was used.

6.4 RESULTS AND DISCUSSION

6.4.1 Fluorescence quenching study

Fluorescence quenching is either a result of the formation of a quencher-fluorophore complex or due to the collision of the two. The former is known as static quenching, while the latter is called dynamic quenching [Joseph R. Lakowicz, 2006]. Any structural or conformational changes and complex formation that a protein can undergo in the presence or absence of a foreign molecule can be investigated by monitoring quenching of its fluorescent amino acid constituents (tryptophan, tyrosine, and phenylalanine). Amongst these three amino acids, tryptophan (Trp) is the dominant source of intrinsic protein fluorescence [Vivian & Callis, 2001]. Particularly in the case of albumins, tyrosine, and tryptophan serve as the intrinsic natural probe to study fluorescence of the proteins [Sułkowska, 2002]. BSA has two classes of tryptophan residues (Trp134 and Trp214) in subdomains IA and IIA respectively, and among these two, Trp134 is more exposed to the solvent environment, and Trp214 is deeply buried in subdomain II [Xiang et al, 2007].

(a) BSA fluorescence quenching in the presence of 0-10 μM TCPy and 0-5 μM CPF in aqueous phosphate buffer (PB) measured at 280 nm as well as at 295 nm

Figure 6.2 displays the stack plot of fluorescence spectra of BSA at room temperature with varying concentrations of TCPy and CPF from 0 to 5 μM in aqueous PB as the solvent, recorded with excitation wavelength 280 nm as well as 295 nm. From these fluorescence spectra, it can be seen that the decrease in fluorescence intensity is far greater for TCPy (*ca*. 41%) than CPF (*ca*. 18%) at an analyte concentration of 0.5 μM suggesting superior quenching effect of TCPy at a much smaller concentration range compared to CPF (Figure 6.3). Unlike TCPy, in the case of CPF, below 0.5 μM the change in emission maxima is also insignificant. Similar stack plots for TCPy and CPF in 10% methanol for a concentration range of 0-10 μM have been provided in Figure 6.4. The strong fluorescence emission band at 347 nm of BSA has shown a remarkable decrease in intensity with increasing concentration of pesticides, indicating the formation of pesticides-BSA complex. Since there is no emission band identified for the test pesticides in the range of 300-500 nm, their contribution to emission spectra has been neglected.

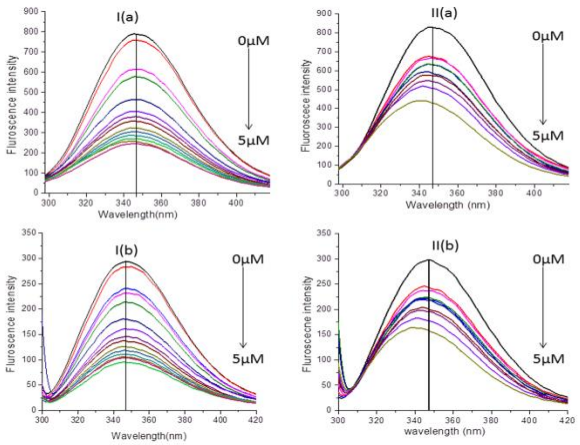

Figure 6.2 : Fluorescence emission spectra of BSA at room temperature in presence of varying concentration of (I) TCPy: [0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.5, 2, 2.5, 3, 4 ,5 μM] (II) CPF: [0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 μM] at (a) λ_{ex} = 280 nm (b) λ_{ex} = 295 nm. [BSA] = 0.5 μM; pH = 7.40

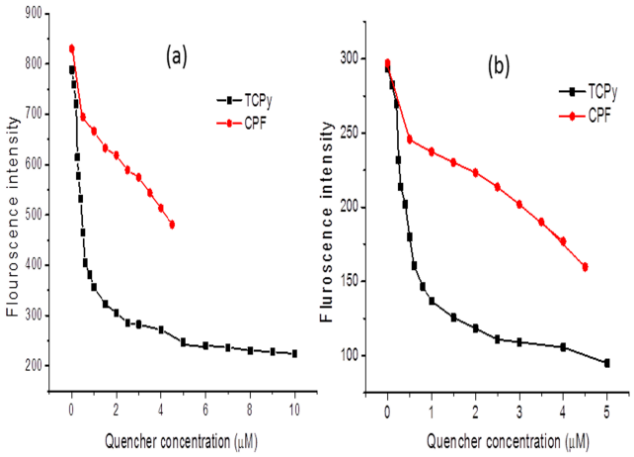

Figure 6.3 : Plot of fluorescence intensity maxima for BSA against [TCPY] and [CPF] in aqueous PB obtained at (a) λ_{ex}:280 nm (b) λ_{ex}:295 nm. [BSA] = 0.5 μM; pH = 7.40. [CPF] in aqueous solution cannot exceed 5 μM.

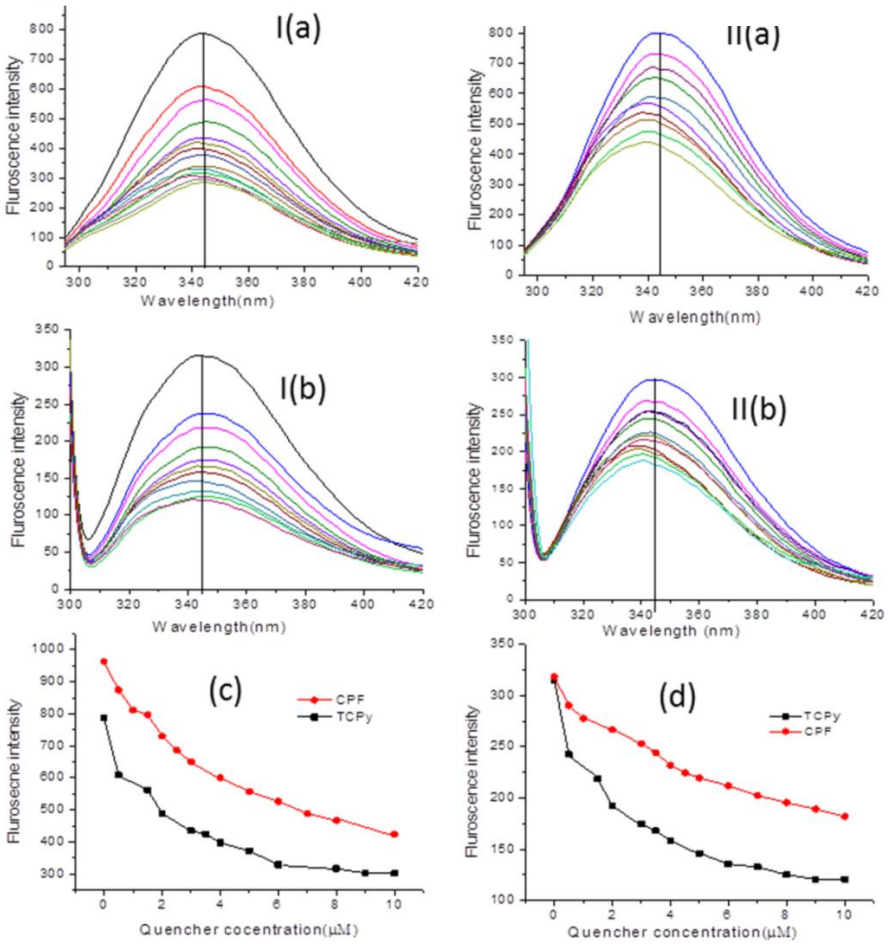

Figure 6.4 : Fluorescence emission spectra of BSA at room temperature in presence of varying concentration (0-10μM) of (I) TCPy (II) CPF in 10% methanol in PB at (a) λ_{ex}:280nm (b) λ_{ex}:295nm.
Plot of fluorescence intensity maxima for BSA against [TCPY] and [CPF] in 10% methanol in aqueous PB obtained at (c) λ_{ex}:280 nm (d) λ_{ex}:295 nm [BSA] = 0.5 μM; pH = 7.40

Furthermore, the emission maxima of BSA have also exhibited a significant blue shift *ca*. 6 nm in case of increasing concentration of CPF while a slight blue-shift of 1.5 nm in case of TCPy. In general, the shift in emission maximum corresponds to changes in polarity around the chromophore molecule. In the present case, more specifically, the observation has suggested the occurrence of a less polar or more hydrophobic environment around the tryptophan residues in the presence of the quencher. Also, the progressive shift of the emission spectrum to shorter wavelengths with increasing quencher concentrations have indicated that those tryptophan residues emitting at longer wavelengths are quenched more readily than the tryptophan residues emitting at shorter wavelengths [Lakowicz, 2006]. It is well-known in macromolecule literature that a tryptophan residue buried inside the hydrophobic core of protein exhibits blue shifted emission compared to the surface exposed tryptophan residues [Albani, 2004]. Hence it is clearly understood from the experimental data that in case of CPF, quenching of the surface exposed tryptophan residues are far greater compared to the buried tryptophan residue. However, TCPy might be able to access both the residues resulting in a lower blue-shifted emission maximum. To analyse the nature of the quenching of BSA fluorescence in presence of TCPy and CPF, a series of intensities measured have been plotted against the quencher concentration, as shown in Figure 6.5 (a and b).

The experimental data have been then evaluated by employing the Stern-Volmer equation [Eftink, 1991] as given in Eq.(2.25) in Chapter 2. The Stern-Volmer plots for CPF and TCPy have revealed that in the case of CPF, the Stern-Volmer plot remains linear in the concentration range of 0-5 μM while in the case of TCPy a clear negative deviation is observed after 1 μM. The clear downward curvature in the case of TCPy suggests the presence of heterogeneity in the microenvironment around the tryptophan populations that have been differentially accessed by the quencher resulting in a biphasic quenching process. Here, a linear Stern-Volmer plot has been employed to evaluate the Stern-Volmer constant for CPF (0-5 μM), and for TCPy (0 -1 μM) at 280 nm as well as at 295 nm and the results have been tabulated in Table 6.1. For dynamic quenching, the maximum quenching rate constant of various quenchers with biopolymers has been reported to be of the order of 2.0×10^{10} $Lmol^{-1}s^{-1}$[Lakowicz and Weber, 1973]. In the present study, the quenching rate constants of BSA fluorescence induced by CPF and TCPy are three orders of magnitude greater than the above-mentioned value, which clearly suggests the quenching process be static in nature. Hence the main mechanism of quenching is due to the formation of a complex between BSA and the quenchers' *viz*., CPF, and TCPy. Further, to quantify binding parameters, Eq.(2.26) has been used to calculate number of binding sites (n) from the slope and the binding constant (K_A) from the intercept of the plot, as given in Chapter 2 and shown in Figure 6.5 (c and d) for both TCPy and CPF at 280 nm and at 295 nm. A comparison of the binding constants, as summarized in Table 6.1 for both the quenchers, clearly demonstrated TCPY as a stronger binder to BSA than CPF.

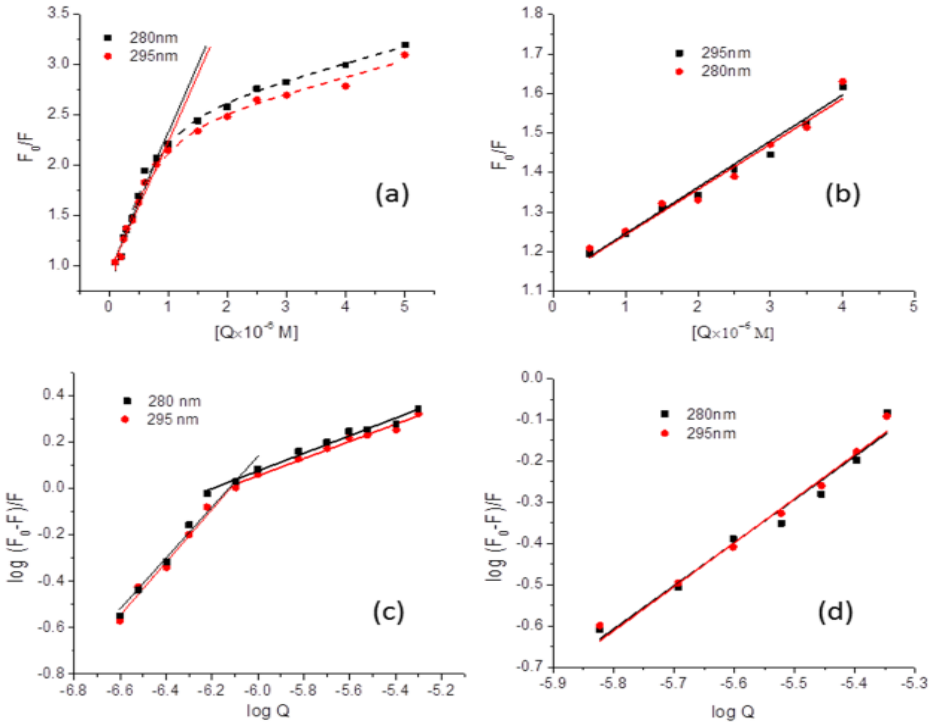

Figure 6.5 : Stern-Volmer plot for quenching of BSA fluorescence in presence of (a) TCPy (b) CPF and Double log plot for calculating binding parameters of the (c) BSA-TCPy and (d) BSA- CPF in aqueous PB at room temperature with concentration varying from 0-5 μM; [BSA] = 0.5 μM; pH= 7.40.

A close inspection of Table 6.1 also reveals that the values calculated for K_{sv} at 280 nm and at 295 nm are of the same order for both CPF and TCPy, signifying that it is mainly the tryptophan residues that are affected while tyrosine has a minor contribution. The biphasic behaviour in the double log plots for a lower concentration range (0-5 μM) motivated us to analyse the quenching mechanism of TCPy for a complete concentration range of 0-10 μM. The modified version of the Stern-Volmer equation (Equation 6.1) has been used to further analyse the quenching mechanism of TCPy for the complete concentration range.

$$\frac{F_0}{\Delta F} = \frac{1}{f_a} + \frac{1}{f_a} K_A[Q] \qquad (6.1)$$

where f_a is the fraction of the initial fluorophore that is accessible to the quencher, and K_A is quenching constant of accessible fluorophore [Singh, Sharma, & Awasthi, 2015]. All the relevant quenching parameters extracted using Eq.(6.1) are tabulated in Table 6.1. Figure 6.6 (a and b) represents Stern-Volmer as well as the modified Stern-Volmer plot for BSA fluorescence quenching in the presence of TCPy. In an aqueous medium, the Stern-Volmer plot clearly exhibits three distinct concentration ranges *viz*. 0-1 μM, 1-5 μM, and 5-10 μM for TCPy. The change in fluorescence intensity is negligible with the increase in quencher concentration in the third concentration range, as already seen in Figure 6.6 (a) for TCPy. Hence the third region is identified as a saturation phase where no further binding takes place [Anand and Mukherjee, 2013]. From the modified Stern-Volmer plot, initial fluorophore accessibility of 70% has been quantified, while from the double log plot, the binding constants and the number of binding sites are calculated for the first two concentration ranges. Since all the binding events are completed up to the second region, the binding constant has not been evaluated for the third region.

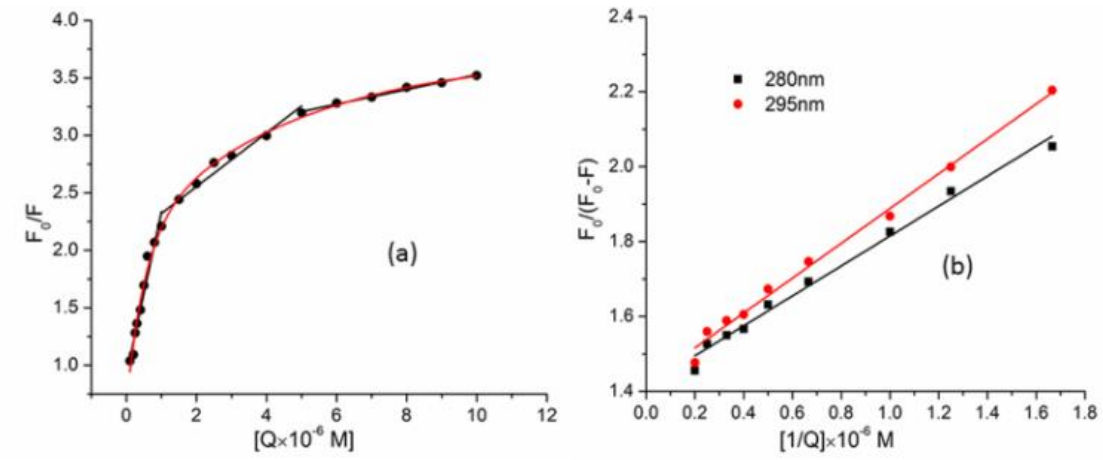

Figure : 6.6 (a) Stern-Volmer plot for quenching of BSA fluorescence in the presence of TCPy (0-10 µM) (b) Modified Stern–Volmer plot of BSA in the presence of TCPy in aqueous PB at room temperature; [BSA] = 0.5 µM; pH = 7.40.

Table 6.1 : Relevant parameters of the BSA-quencher complex formation in aqueous PB for 0-5 µM range *viz.*, Stern-Volmer constant (K_{sv}), fraction of accessible fluorophore (f_a), quenching constant (K_q), association constant (K_A) and no. of binding sites (n) as calculated from Stern-Volmer, modified Stern-Volmer and double log plot recorded at room temperature. *, ° Denotes biphasic curves having two regions *(0-1 µM) region 1 & ° (1-5 µM) region 2.

System	**Stern-Volmer plot**			**Double log plot**		
	K_{sv} (L mol^{-1})	$K_q \times 10^{13}$ (L mol^{-1} s^{-1})	R^2	$K_A \times 10^5$ (L mol^{-1})	*n*	R^2
BSA_CPF						
280 nm	1.14×10^5	1.14	0.982	2.50×10^5	1.04	0.959
295 nm	1.18×10^5	1.18	0.959	3.52×10^5	1.06	0.958
BSA_TCPy						
280 nm	1.40×10^6	14.0	0.964	*5.12×10^6	1.09	0.959
				° 190.5	°0.38	0.977
295 nm	1.31×10^6	13.14	0.965	2.82×10^6	1.05	0.973
				° 186	° 0.37	0.979
BSA_TCPy	Modified Stern-Volmer for 0-10 µM range					
	K_{sv} (L mol^{-1})	f_a	R^2			
280 nm	3.53×10^6	0.71	0.989			
295 nm	3.06×10^6	0.70	0.992			

(b) BSA fluorescence quenching in the presence of 0-10 μM TCPy and CPF in phosphate buffer (PB) with 10% methanol (CH_3OH) measured at 280 nm as well as at 295 nm

In this section, BSA emission in PB with 10% methanol is measured. This particular solvent system has been chosen to increase the solubility of CPF in the solution so that similar concentration ranges (0-10 μM) for CPF can also be analysed analogously to the studies made for TCPy in the previous section. Also, 10% CH_3OH has no effect on the protein structure, as observed from absorption spectroscopy (data not shown). It must be mentioned here that a minute amount of CH_3OH could be present in blood plasma as a natural constituent and can also be absorbed via natural foods (*e.g.,* fruits and vegetables) which may alter the pesticide-protein interactions [Lindinger et al,1997; Lauwerys, 2001]. Hence a comparative analysis of such interactions in this solvent system is meaningful.

The emission maximum for BSA in the case of 10% CH_3OH has appeared at 344.5 nm, exhibiting a minor blue shift *ca.* 3 nm compared to BSA in aqueous PB due to change in solvent polarity [Gasymov & Glasgow, 2007] as depicted in Figure 6.4. Further, the decrease in fluorescence intensity maximum with increasing quencher concentration in 10% CH_3OH buffer system has also been shown in Figure 6.4 (c & d). In comparison to aqueous PB, the decrease in emission maxima in the case of TCPy for a concentration range of 0-5 μM is of a lesser extent. On the other hand, in the same concentration range, CPF exhibited a greater decrease of emission intensity that can be reasoned out due to better solubility of CPF in 10% CH_3OH buffer compared to aqueous PB. The Stern-Vomer and the Double Log plot for this particular solvent system have been represented in Figure 6.6. The relevant fluorescence quenching parameters for both TCPy and CPF are tabulated in Table 6.2.

In this case, once again, TCPy exhibits not only negative deviation from linearity in Stern-Volmer plot beyond 6 μM but also a biphasic double log plot is observed indicating differential accessibility of fluorophore populations. On the other hand, the Stern-Volmer plot remains linear in the case of CPF. In the case of TCPy, the double log plot has been analysed in terms of two linear regions. Further, the modified Stern-Volmer equation has also been used to evaluate the fractional accessibility of fluorophore populations by TCPy using Eq.(6.1) and plotted in Figure 6.8.

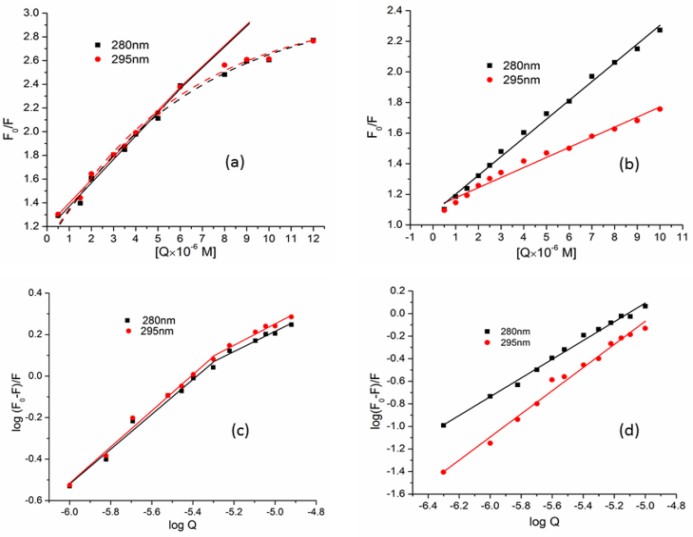

Figure 6.7 : Stern-Volmer plot for quenching of BSA fluorescence in the presence of (a) TCPy (b) CPF & Double log plot for calculating binding parameters of the (c) BSA-TCPy and (d) BSA- CPF in 10% methanol in PB at room temperature with concentration varying from 0-10 µM; [BSA] = 0.5 µM; pH = 7.40.

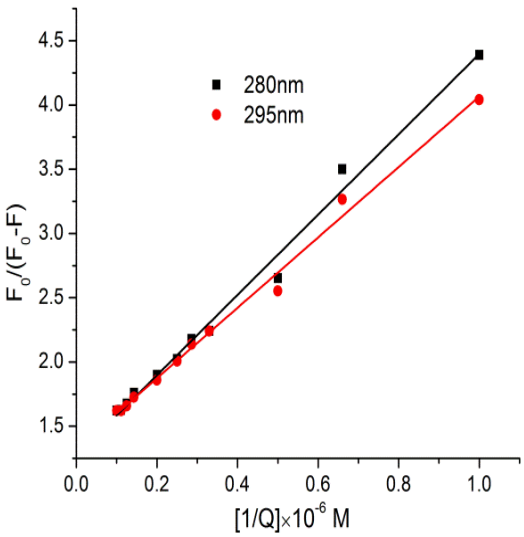

Figure 6.8 : Modified Stern–Volmer plot of BSA in the presence of TCPy in 10% methanol in PB at room temperature with concentration varying from 0-10 µM; [BSA] = 0.5 µM; pH = 7.40.

A close inspection of Table 6.2 reveals that in the case of CPF, a distinct change in Stern-Volmer constant, as well as binding constant, is observed at the two excitation wavelengths *viz*., 280 nm, and 295 nm compared to the aqueous PB case. This is also true for the lower concentration range of CPF shown in Figure 6.8. This is due to a better solubility of CPF in 10% CH_3OH that allows the quencher to access fluorophore residues such as tyrosine besides tryptophan resulting in a change in the magnitude of the binding constants measured at 280 nm and 295 nm respectively. In the case of TCPy from the modified Stern-Volmer plot, it is understood ~80% of the total fluorophores are initially accessible to TCPy. This is because of the conformational state of a protein that can have an influence on the exposure of its tryptophan residues to solvent.

From the double log plot, two regions of binding have been identified for TCPy, where region 1 has been denoted as the region of specific binding, while region 2 has been named as the non-specific binding region. In region 1 the number of binding sites (n) value depicts that TCPy binds exclusively to energetic sites of the protein and affects the tertiary structure of the protein. Moreover, unlike aqueous PB case, TCPy in 10% methanol in PB exhibits a linear Stern-Volmer plot (Figure 6.9) indicating possible accessibility of a single fluorophore population in region 1. While in region 2, the n value suggests that TCPy does not have any specific binding sites [Anand and Mukherjee, 2013]. Further, it has a much lower tendency to bind to BSA. This might be due to an increase in steric crowding as a result of higher concentration. However, the behaviour of CPF remains unchanged, as in the case of aqueous PB.

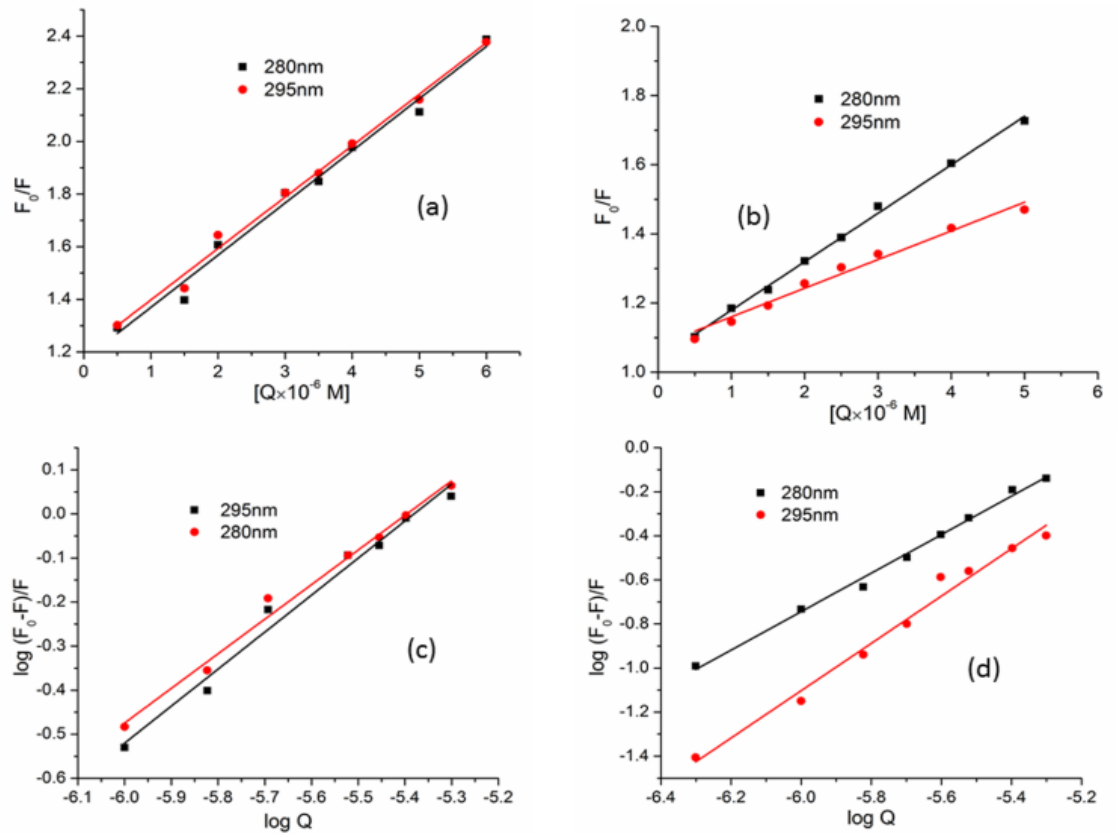

Figure 6.9 : Stern-Volmer plot for quenching of BSA fluorescence in presence of (a) TCPy (b) CPF & Double log plot for calculating binding parameters of the (c) BSA-TCPy and (d) BSA- CPF in 10% methanol in PB at room temperature with concentration varying from 0-5μM; pH = 7.40.

Table 6.2 : Relevant parameters of the BSA-quencher complex formation in (1:9) methanol: PB for 0-10 μM range *viz.*, Stern-Volmer constant (K_{sv}), quenching constant (K_q), fraction of accessible fluorophore (f_a), association constant (K_A) and no. of binding sites (n) as calculated from Stern-Volmer, modified Stern-Volmer and double plot recorded at room temperature. *, ° Denotes biphasic curves having two regions *(0-5 μM): region 1 and ° (6-10 μM): region2

System	**Stern-Volmer plot**			**Double log plot**		
	K_{sv} (L mol^{-1})	$K_q \times 10^{13}$(L mol^{-1} s^{-1})	R^2	K_A (L mol^{-1})	*n*	R^2
BSA_CPF						
280 nm	1.17×10^5	1.17	0.994	2.28×10^4	0.85	0.998
295 nm	0.73×10^5	0.73	0.981	6.64×10^4	0.98	0.986
Modified Stern-Volmer				**Double log plot**		
BSA_TCPy	K_A	f_a	R^2	K_A	*n*	R^2
280 nm	4.06×10^5	0.83	0.990	*3.16×10^4	0.84	0.979
				°398	0.48	0.951
295 nm	4.81×10^5	0.75	0.992	*1.69×10^4	0.78	0.987
				°316	0.46	0.968

A comparison of Table 6.1 and Table 6.2 for the same concentration range further reveals that the binding constants are of 1-2 order lower in 10% CH_3OH compared to aqueous PB. The lower binding constants measured for both the quenchers might be due to a competition of CH_3OH with the quenchers to interact with BSA and thereby prohibiting CPF and TCPy to bind as efficiently as in case of the aqueous PB. Also, it is well understood that the polarity of the medium plays an important role in dictating the quenching mechanism. The rate constants are mainly solvent polarity dependent [Guha et al, 1999]. In the present case, the binding interaction is far superior in the aqueous medium than in 10% CH_3OH.

6.4.2 UV-vis spectral analysis

UV-vis absorption spectroscopy is one of the most simple tool for a qualitative investigation of protein structural changes during the invasion of the foreign molecules. Figure 6.10 represents the UV-vis spectra of CPF, TCPy, and BSA, measured at room temperature for a 5 μM analyte concentration.

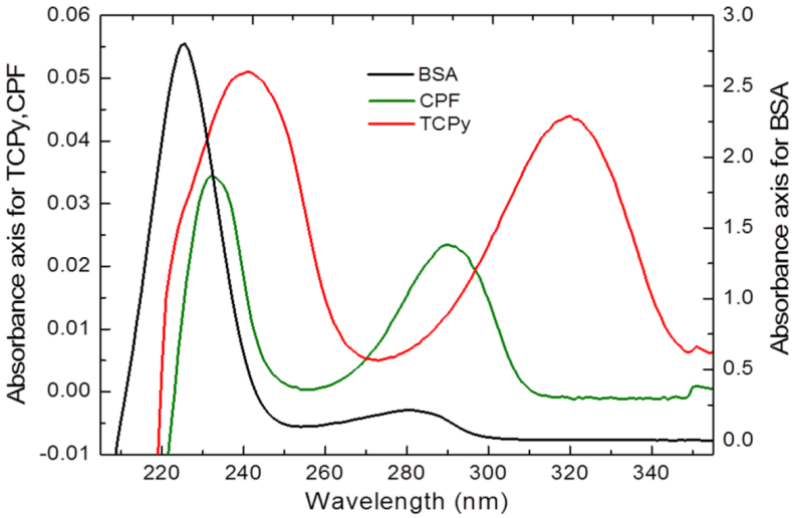

Figure 6.10 : UV-Vis absorption spectra of CPF (5μM), TCPy (5μM), BSA(5 μM)at room temperature.

CPF exhibits two absorption maxima at 230 nm and 290 nm while TCPy has absorption maxima at 240 nm and 320 nm. BSA, like other proteins, exhibits an extremely strong absorption band at 280 nm due to tryptophan residues besides an extremely intense band at 222 nm due to the peptide backbone of BSA at the same analyte concentration. Figure 6.11 (a & b) represents the UV-vis spectra of BSA with an increasing concentration of TCPy and CPF from 0–10 μM. A close analysis of the spectra has revealed that in the case of TCPy, the peptide band at 222 nm has undergone a hyperchromic shift along with a blue shift of about 1 nm while the tryptophan band at 280 nm [Xu et al., 2013] has exhibited only a minor increase in intensity.

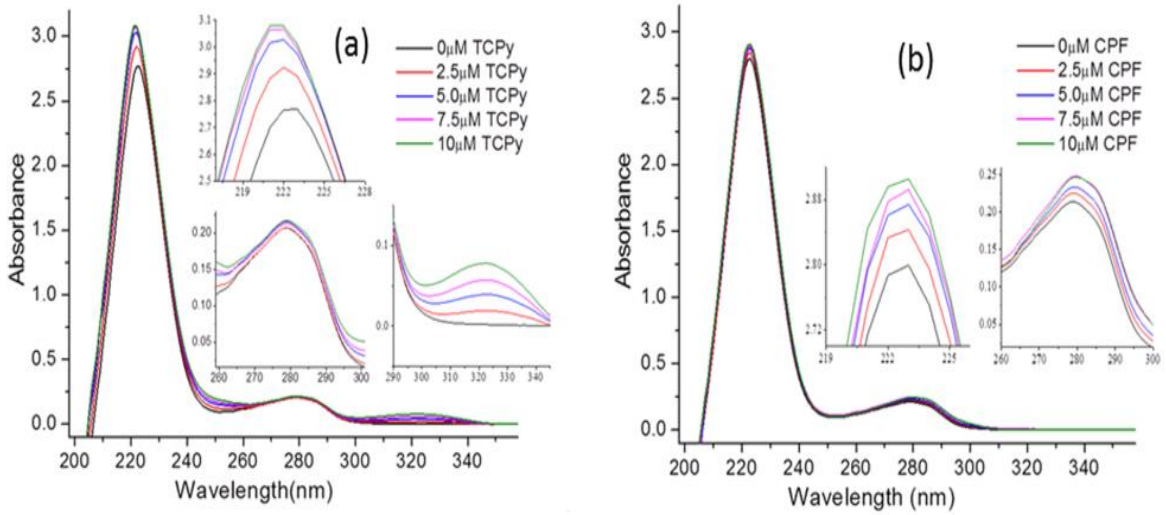

Figure 6.11 : UV-vis absorption spectra of BSA with increasing concentration of (a) TCPy and (b) CPF at room temperature. [BSA]=5 μM; pH = 7.40

This clearly indicates that although there could be a possible change in the polarity of the microenvironment of the peptide backbone [Trnkova, Bousova, Kubicek, & Drsata, 2010], no significant change has happened in the microenvironment of the aromatic acid residues due to BSA-TCPy interaction. On the other hand, in the case of BSA-CPF, both the bands at 222 nm and at 280 nm have exhibited a significant increase in intensity confirming the formation of a ground-state complex [Rasoulzadeh, Asgari, Naseri, & Rashidi, 2010] altering the protein microenvironment globally. To further comment on any structural changes that BSA might have undergone due to complex formation with the pesticides, the CD spectra of BSA with and without pesticides are recorded and compared, as given in Figure 6.12. The CD spectra of BSA exhibits two distinct negative bands in the UV region at 208 nm and 220 nm, which are the characteristic peaks of proteins due to $n \rightarrow \pi^*$ transition within the peptide bond of α-helix [qing Wang, ping Tang, mei Zhang, hua Zhou, & cheng Zhang, 2009]. In general, CD is an excellent tool to estimate protein secondary structure [Greenfield, 2006] by monitoring the changes in the negative bands (if any) due to complex formation. In the present case, minor but detectable changes have been observed in the BSA CD spectra when recorded in the presence of both CPF and TCPy. The upward shift of the negative bands, as seen in Figure 6.12 has clearly suggested that the test molecules are not only able to modify protein microenvironment polarity but also bring subtle conformational changes within the protein [Wang and Li, 2011]. The characteristic shape of the CD spectra for BSA remains the same in the presence and absence of quenchers indicating the absence of a denaturation event [Singh et al., 2015]. However, there is a slight decrease in α-helicity of protein, as confirmed from the upward shift in negative bands.

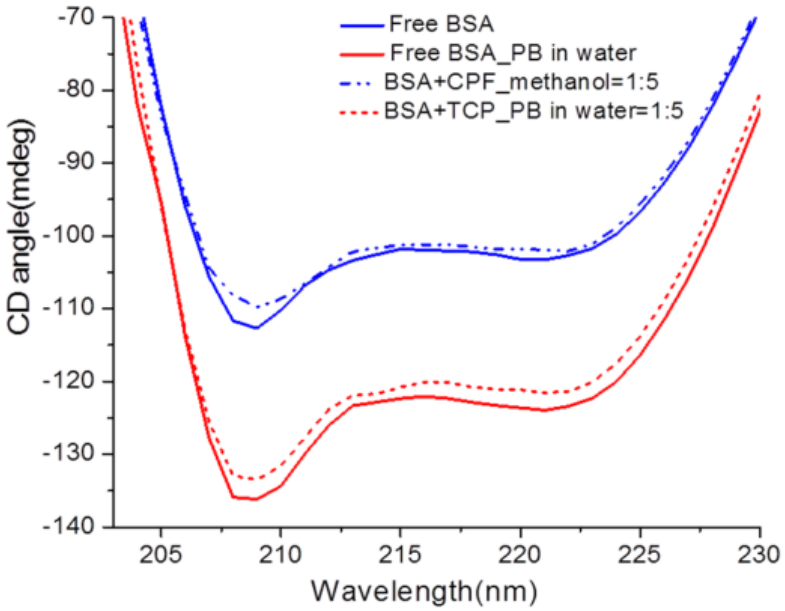

Figure 6.12 : CD spectra of BSA-TCPy and BSA-CPF system;[BSA]=10 µM; [TCPy]=50 µM; [CPF]= 50µM.

This is in agreement with UV and Fluorescence results that indicated only the possibility of conformational changes of the protein in the presence of the pesticides without complete denaturation. A point to be mentioned here is that the BSA-CPF CD spectra are recorded in the presence of methanol to increase the solubility of CPF in the solution. The change in solvent polarity has also changed the intensity of the negative bands of BSA.

6.5. CONCLUSION

Some of the previous literature reports have already addressed the toxicity of CPF and TCPy in light of enzyme activity, DNA damage, etc. whereas, in the current report, a comparative binding interaction of both CPF and TCPy with BSA is investigated. The major findings of this study are highlighted in the following:

1. Both CPF and TCPy exhibit static quenching of BSA fluorescence emission in solution, indicating the formation of a ground-state complex.
2. Solvent polarity, as well as pesticide concentration, plays a major role in dictating OP-protein interaction.
3. In aqueous medium, TCPy–BSA interaction is not only much stronger compared to CPF–BSA but also manifests differential mode of interaction with BSA by accessing different fluorophore populations.
4. In aqueous medium, BSA emission exhibits a greater blue shift in the presence of CPF that possibly indicates significantly higher conformational changes of BSA in the presence of the same compared to TCPy.
5. Fluorescence, UV, and CD data have exhibited that binding of CPF and TCPy perturbs the protein microenvironment significantly while conformational changes of BSA might be minimal.
6. Based on the binding interaction analysis, it might be speculated that free concentration of CPF in the plasma will be higher compared to TCPy, resulting in greater diffusion of CPF to target tissues while TCPy will be retained in plasma as TCPy-BSA complex.

As a whole, the current study provides important insight regarding the role of solvent in dictating the OP-protein interaction in solution. The study enables one to predict the distribution and effect of OP on various tissues within the body.

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