International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 41 No. 2 Dec. 2023, pp. 390-412

http://www.ijias.issr-journals.org/

Interaction between PFASs and protein using Fluorescence spectroscopy

Andry Harinaina Rabearisoa[1-3], Hajandrainy Rabearisoa[2], and Xinghui Xia[3]

[1]Université de Fianarantsoa, Ecole Normale Supérieure, Sciences et technologies, Fianarantsoa, Madagascar

[2]Université de Fianarantsoa, Ecole Normale Supérieure, Sciences et technologies, Fianarantsoa, Madagascar

[3]School of Environment, Beijing Normal University, State Key Laboratory of Water Environment Simulation, Key Laboratory for Water and Sediment Sciences (Ministry of Education), Beijing 100875, China

ABSTRACT: This research in this aspect is necessary, because of PFASs are one kind of hydrophobic and hydrophilic chemicals, and the study of PFASs with protein used as a dissolved organic matter in this study is valuable to evaluate the ecological risk of this kind of chemicals. Fluorescence has been proven as a sensible method to provide qualitative and quantitative information on the PFAS-serum albumin interactions. This work will provide some information for appropriately understanding the interaction between PFASs and protein and illustrate its binding mechanisms at different concentration of protein, cations and pH. The results obtained from fluorescence spectra indicated that PFASs could quench the intrinsic fluorescence of protein through a static quenching procedure, with the effective quenching constants (K'_{sv}) varying from 0.44 10^5 L mol^{-1} to 5.73 10^5 L mol^{-1}. It infers that the complex of PFAS-protein was formed. In addition, the ionic strength and pH affected the effective quenching constant of PFASs bound to bovine albumin. Furthermore, with increase of pH from 6.5 to 8.5, reduction in the binding affinity of PFAS to bovine albumin and soy peptone were also observed. The interaction between perfluoroalkyl substances and protein using fluorescence spectroscopy, and the occurrence of electrostatic interactions with hydrophobic force in the binding also studied.

KEYWORDS: Perfluoroalkyl substances (PFASs), Bioaccumulation, Protein, Dissolved organic matter (DOM), Cations, Fluorescence quenching.

1 INTRODUCTION

Fluorescence spectroscopy is a valuable method in the study of proteins due to its great sensitivity and convenient method for the study of protein-ligand interactions [1]. Fluorescence has been proven as a sensible method to provide qualitative and quantitative information on the PFAS-serum albumin interactions. A variety of molecular interactions can result in serum albumin quenching. These include excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching. In this chapter we will be concerned primarily with quenching resulting from collisional encounters between the fluorophore and quencher. In the present study, we will discuss static quenching, which can be a valuable source of information about binding between the protein sample and PFASs.

Perfluoroalkyl substances (PFASs) could bind to protein and the protein like serum albumin is able to bind with different biologically active compounds (drugs, fatty acids, steroids, etc.) in the body of organisms [2], [3]. The advantage of using fluorescence spectroscopy for the study is the reactivity of chemical and biological systems, and it allows non-intrusive measurements of substances in low concentration under physiological conditions [4]. Using fluorimetry in the studies of perfluoroalkyl substances protein interactions in the binding can be studied at equilibrium without physical separation of the bound complex from the free ligand and the protein. It can reveal the accessibility of quenchers to bovine albumin and soy peptone fluorophores, confer a better understanding of the mechanism of PFASs to bovine albumin/ soy peptone, and provide some clues about the nature of the binding phenomenon.

The quenching can be mathematically expressed by the Stern-Volmer equation, which allows for the calculation of quenching constant, binding constants and binding sites [5], [6]. Measuring fluorescent change is a practical method to study protein interactions with other substances [7], [8]. There are many reports on the study of interactions between proteins with ligands using fluorescence spectroscopic methods [9], [10]. But there have been no reports on the interaction of bovine albumin and soy peptone with six kinds of PFASs. Thus, bovine albumin/ soy peptone and PFASs were selected in this study.

In this work, we use a spectroscopic approach to investigate the binding of PFASs to bovine albumin and soy peptone in aqueous solution under physiological conditions. Obtaining interaction information regarding quenching mechanisms, examine the binding effect of ions, and pH effect are our investigation aims. Our work will provide some information for appropriately understanding the interaction between PFASs and protein and illustrate its binding mechanisms at different concentration of protein, cations and pH.

2 MATERIALS AND METHODS

2.1 FLUORESCENCE QUENCHING MEASUREMENTS

All fluorescence spectra will measure on a F-4600 fluorophotometer (Hitachi, Japan) equipped with a 10 mm quartz cell and a 150 W xenon lamp. An excitation wavelength of 278nm was chosen and very dilute solutions were used in the experiment.

A 3 mL solution were added in turn to a 10 mL colorimetric tube and made up to the mark with 0.2 mol L^{-1} $NaH_2PO_4 \bullet H_2O$-$Na_2HPO_4 \bullet H_2O$ buffer (as dissolved 35.61 g of $Na_2HPO_4 \bullet 2H_2O$ and 27.6 g of $NaH_2PO_4 \bullet H_2O$ separately in H_2O; Adjusted the volume of each solution to 1000 mL, and mixed the stock solutions as follows, for 50 mL: 38.5 mL of $Na_2HPO_4 \bullet 2H_2O$ and 11.5 mL of $NaH_2PO_4 \bullet H_2O$) containing an appropriate concentration of 20 mgL^{-1} and 30 mgL^{-1} (100 mg L^{-1}) for bovine albumin and soy peptone respectively, and it was titrated by successive additions of a 1 µmol $L^{-1,}$ 2 µmol $L^{-1,}$ 4 µ mol $L^{-1,}$ 6 µmol $L^{-1,}$ 8 µmol $L^{-1,}$ 10 µmol L^{-1}, 20 µmol L^{-1}, 30 µmol L^{-1}, 50 µmol L^{-1}, 100 µmol $L^{-1,}$ 200 µmol $L^{-1,}$ 500 µmol $L^{-1,}$ 800 µmol L^{-1} and 1000 µmol L^{-1}) of PFAS. The titrations were carried out at pH 7.00 (phosphate buffer). The fluorescence spectra were recorded after 3 minutes and each spectrum was an average of 3 accumulations. The excitation wave length was 278 nm, the corresponding scanning mission spectra were recorded in the range of wavelength 287 to 450nm and 295 to 450nm with slit width of 10nm increments (1nm increments, 0.1 s integration time) and scan speed was set at 120 nm min^{-1} for bovine albumin and soy peptone respectively. Then, the Perfluoroalkyl substances solution was gradually titrated to the cell using a micropipette.

2.2 PRINCIPLES OF FLUORESCENCE QUENCHING

Quenching data for all the quenchers used in this study were analyzed by the modified Stern-Volmer equation (1-1), which allows resolution of the static components.

$$F0/ (F0-F) = 1/ (K'_{sv}\, fa\, [C]) + 1/fa \quad (1\text{-}1)$$

where F0 and F are the fluorescence intensities of protein in the absence and presence of quencher, respectively. [C] is the concentration of the quencher, K'_{sv} is the effective quenching constant for the accessible fluorophores, and fa is the fraction of accessible fluorescence.

Otherwise, according to equation (1-2), the quenching rate constant k_q (L $mol^{-1}s^{-1}$) could be obtained.

$$K_q = K'_{sv}/\tau_0 \quad (1\text{-}2)$$

Where τ_0 is average life time of the molecule without the quencher and is generally taken as10^{-8} s.

2.3 EFFECT OF PH ON PFASS-PROTEIN INTERACTION

The effect of pH on the binding constant of the PFASs–bovine albumin/soy peptone system was prepared like fluorescence spectra analysis, but the titrations were carried out at pH 6.5, pH 7.00 and pH 8.5 (phosphate buffer). The concentration of protein was fixed at 20 mg L^{-1} for bovine albumin and soy peptone.

2.4 EFFECT OF Ca^{2+} AND Na^{+} ON PFASS-PROTEIN INTERACTION

The effect of $Ca^{2+,}$ and $Na^{+,}$ on the binding constant of the PFASs–bovine albumin/soy peptone system was the same as the fluorescence measurements for bovine albumin and soy peptone procedure. Solutions of cations were prepared from the calcium chloride ($CaCl_2$) and sodium chloride (NaCl) of their respective cations, and no cation gave a precipitate under the experimental conditions. The titrations were carried out at pH 7.00 with 0.2 mol L^{-1} $NaH_2PO_4 \bullet H_2O$-$Na_2HPO_4 \bullet H_2O$. The overall concentration of protein was fixed at 20 mg L^{-1} for bovine albumin and soy peptone respectively, and the added concentration of the Na^{+} and Ca^{2+} were fixed at 1 and 2 µmol L^{-1} (as5 x 10^{-4} mol L^{-1} $CaCl_2$ and NaCl solution).

3 RESULTS AND DISCUSSION

3.1 FLUORESCENCE CHARACTERISTICS OF BOVINE ALBUMIN AND SOY PEPTONE AND THE QUENCHING MECHANISM

Bovine albumin is considered to possess intrinsic fluorescence originating from the tryptophan residues. When excited at 278nm, the maximum emission band of bovine albumin located at 340.4nm (Fig. 1) and soy peptone was at 344.8nm (Fig. 3). At low PFAS concentration, $\lambda_{EM,\ max}$ remains essentially constant. However, a clear blue shift is observed at higher PFAS concentration, indicating a change in the environment around the Trp residue (s) of bovine albumin. The fluorescence quantum yield of a Trp residue may be as low as 0.07-0.10 in a non polar interior site and as high as 0.32-0.44 in a more polar near-surface. The blue shift and decrease in intensity thus suggest the Trp transitions from a more polar to a less polar environment. [11] also observed that fluorescence intensity decreased with increasing quencher concentrations and it was the result of factors including molecular rearrangements, excited-state reactions, ground-state complex formation, molecular collisions, and energy transfer. (Fig. 1, 2) shows the fluorescence spectra result of bovine albumin in the presence of PFASs. With gradual increase in the PFAS concentrations, the fluorescence intensities of protein were found to decrease gradually in their maxima emission. In addition, the result from the (Fig.1-4) indicated that the interaction between PFAS and protein increased with increasing carbon chain length, suggesting that long carbon chains length was strong fluorescence than short carbon chains length. This is explained by the fact that a long carbon chain is more hydrophobic than a short one and therefore has a greater affinity to be adsorbed hydrophobically. For example, PFOA and PFNA has 8 and 9 carbon atoms were found to have little effect on bovine albumin and soy peptone, respectively based on both the fluorescence spectra; apparently long carbon chains (PFUnA and PFDoA) had more effect and the difference fluorescence intensity between spectrum fluorescence located at 340.4nmfor bovine albumin.

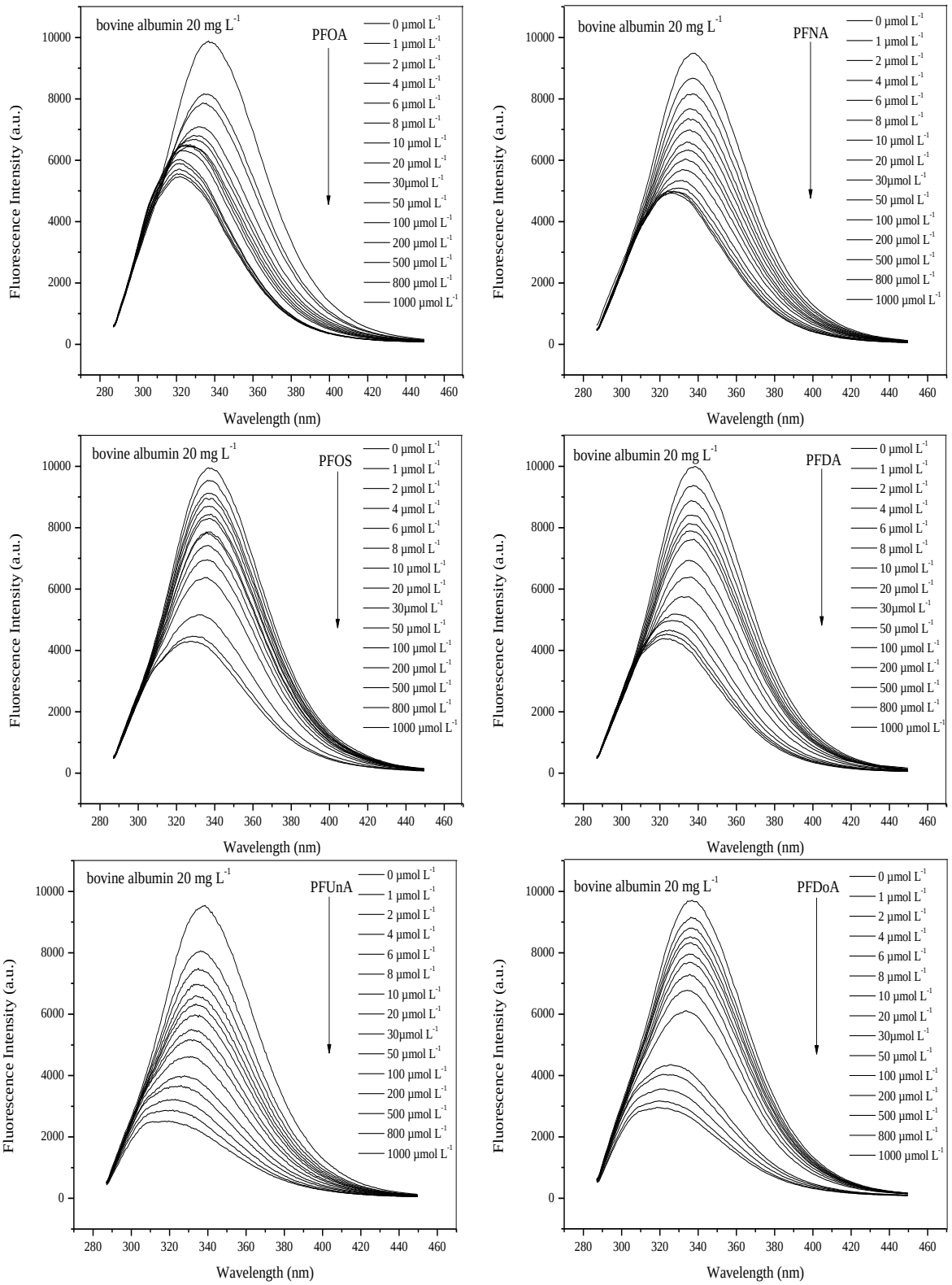

Fig. 1. Emission spectra of bovine albumin in the presence of various concentrations of PFAS (λex=278 nm). c (bovine albumin) = 20 mg L-1

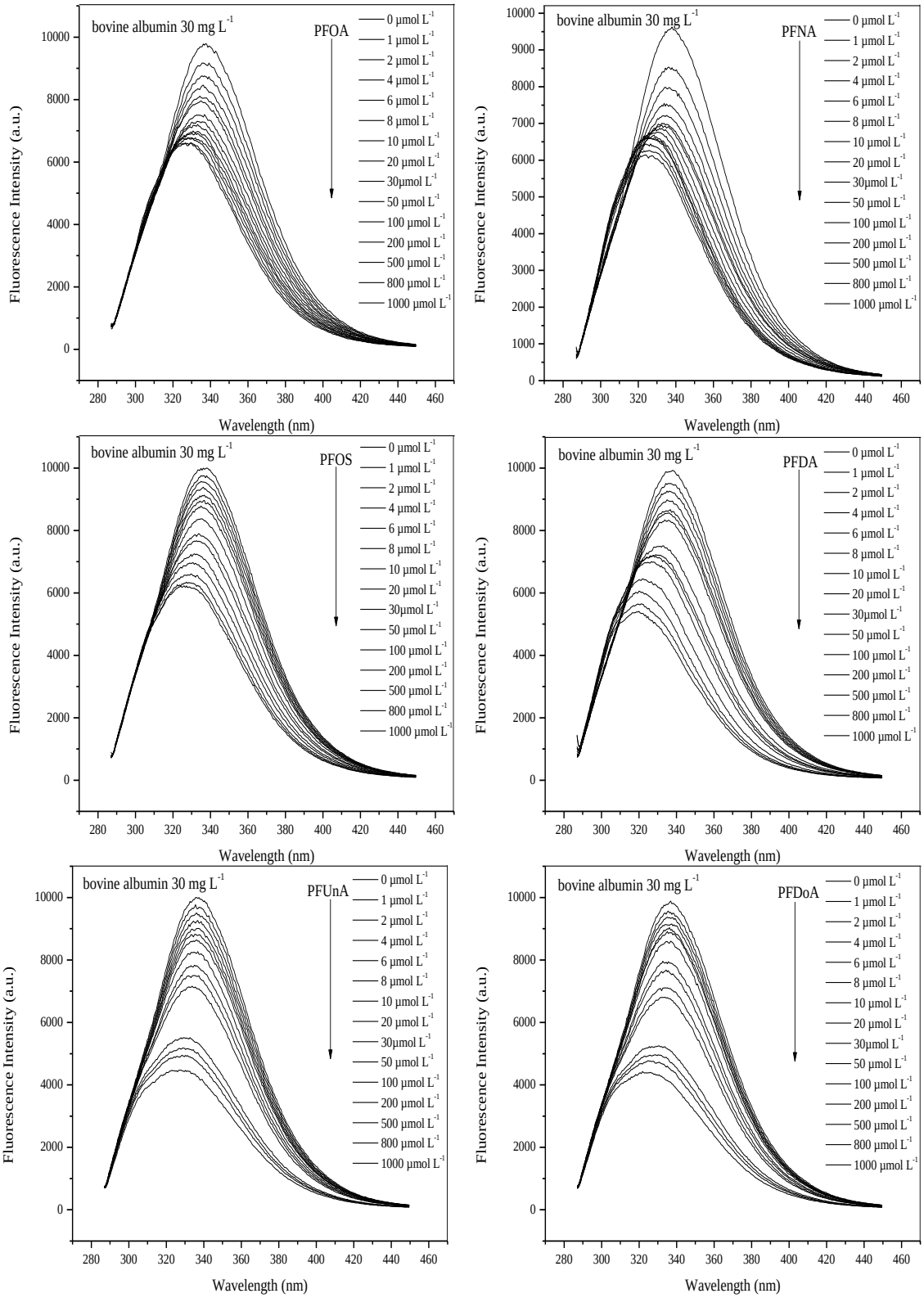

Fig. 2. Emission spectra of bovine albumin in the presence of various concentrations of PFAS (λex=278 nm). c (bovine albumin) = 30 mg L^{-1}

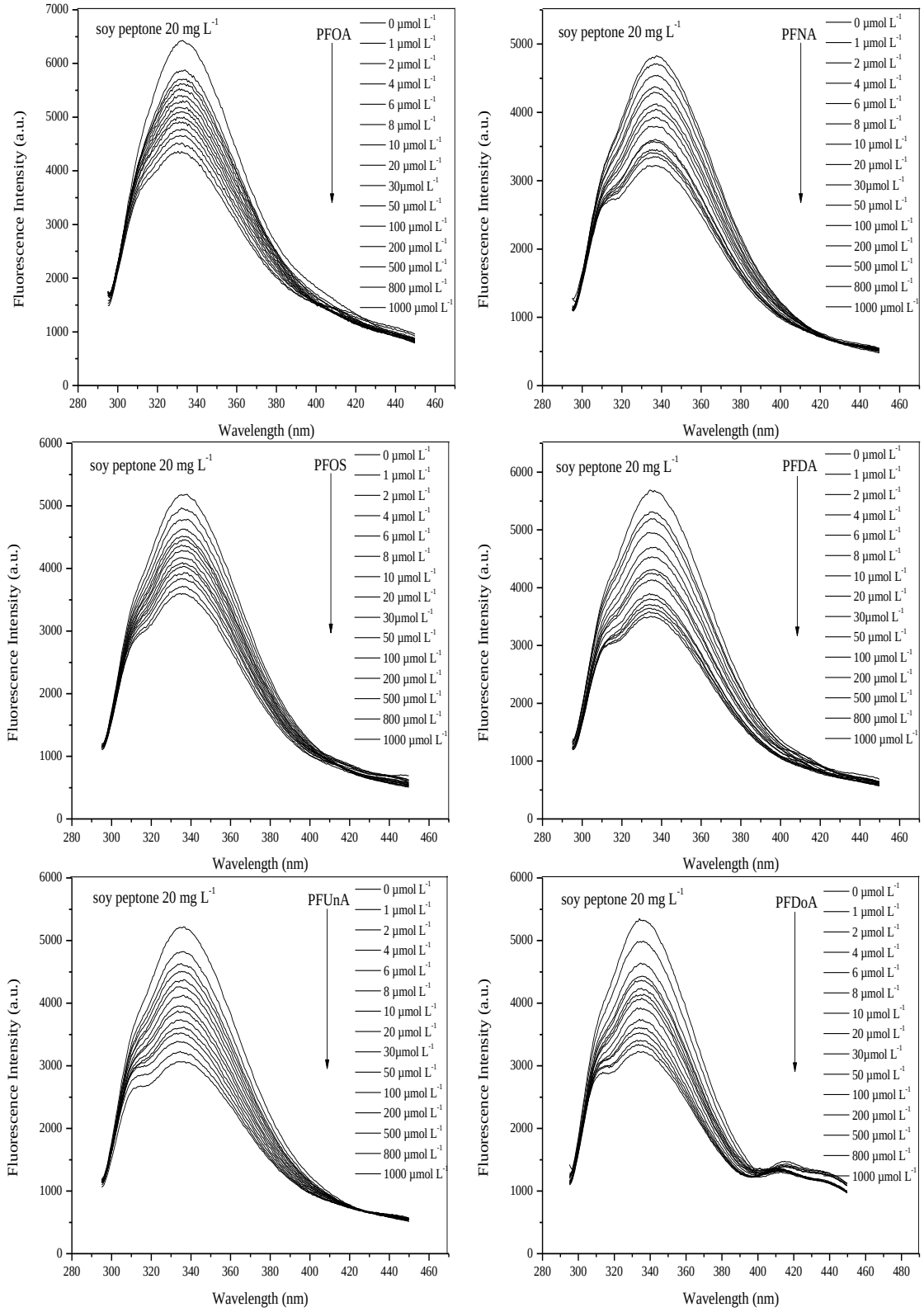

Fig. 3. Emission spectra of soy peptone in the presence of various concentrations of PFAS (λex=278 nm). c (soy peptone) = 20 mg L-1

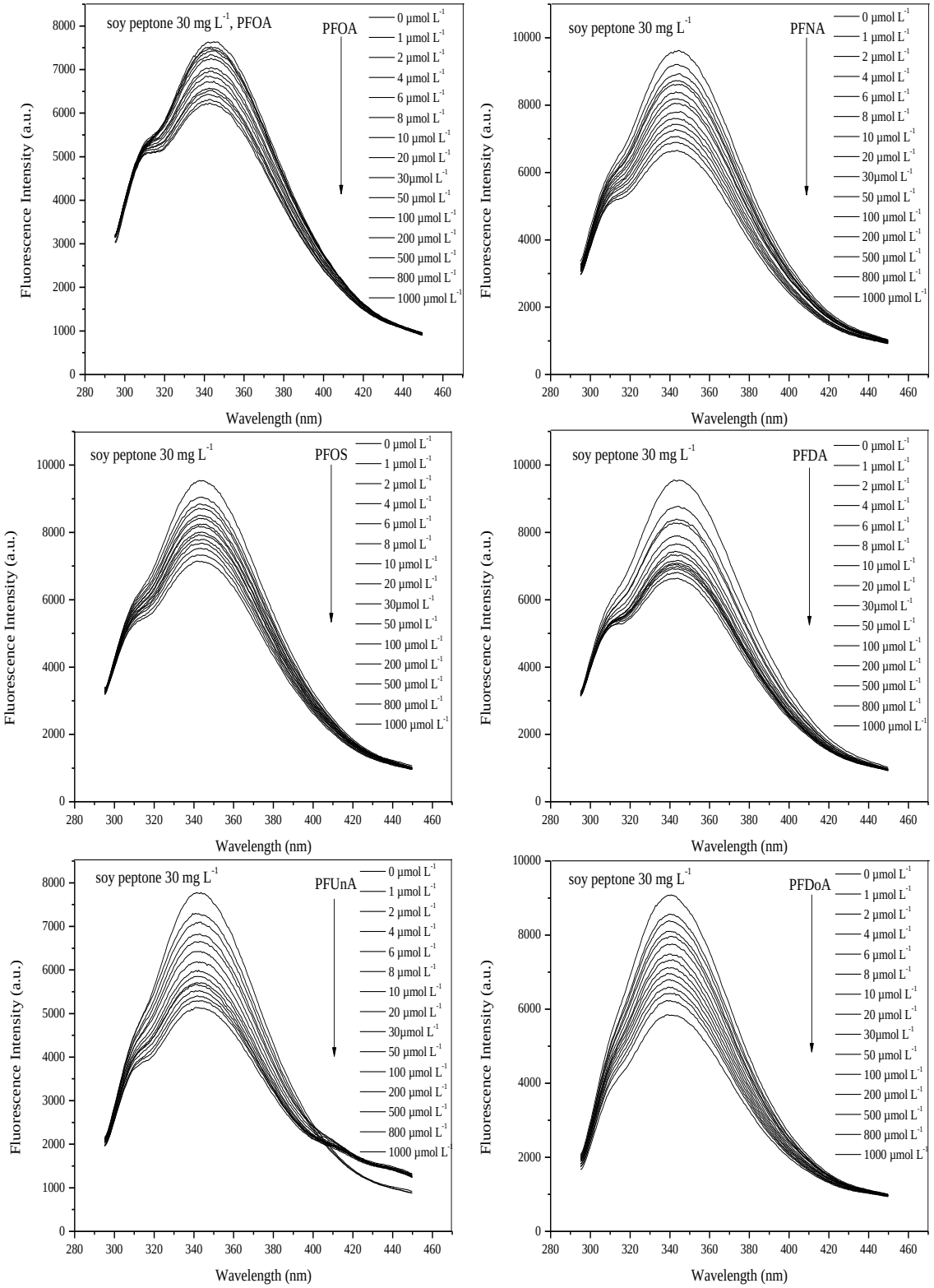

Fig. 4. Emission spectra of soy peptone in the presence of various concentrations of PFAS (λex=278 nm). c (soy peptone) = 30 mg L^{-1}

Was decreased in the range of 78~2044 and 51 ~ 1297 for (PFOA and PFDA). [12] and [13] also observed that the long-chained substances are more adsorbed than the short-chained ones.

It is apparent from (Fig. 3, 4) that bovine albumin at 20 mg L^{-1}has a strong fluorescence emission band at 340.4 nm than bovine albumin at 30 mg $L^{-1,}$ which is mainly the fluorescence emission spectrum of protein. For instance, the difference fluorescence intensity between spectrum fluorescence at the same titration of PFOS concentrations for 20 mg $L^{-1}$and 30 mg L^{-1}were decreased in the range from 84 to 1287 and 187 to 522, respectively. The result showed that an increase in the concentration of protein decreased the binding affinity between PFASs and protein and leads to changes in protein conformation resulting in lower K_{DOC} and the quantity of protein has also been shown to play a role in the bioavailability of hydrophobic organic contaminants. For example, [14] showed a twofold decrease in the K_{DOC} with increasing dissolved organic matter concentration from 1 to 20 mg C L^{-1}. It can be observed that the intrinsic fluorescence of bovine albumin in the emission wavelength range of 287 to 450 nm decreased regularly with the gradually addition of the same amount of PFAS, suggesting that PFAS could interact with bovine albumin and quench its intrinsic fluorescence. The same result was observed for soy peptone at 20 mg L^{-1} and 30 mg L^{-1} at 295 to 450nm wavelength range (Fig. 3, 4). Similar results were observed in the binding of PFOA and PFDA to serum albumin, the fluorescence emission of PFDA to serum albumin is much greater than that of PFOA-serum albumin, revealing that PFDA, which has a longer carbon chain, has a higher affinity than PFOA [15]. [16] also reported that the higher sorption of dissolved organic matter concentration at low levels of dissolved organic matter was observed. A similar observation has been made for the binding of such perfluorinated alkyl acids to thyroid hormone transport protein transthyretin [17].

3.2 FLUORESCENCE QUENCHING MECHANISM

The different mechanisms of fluorescence quenching are usually classified as either dynamic quenching, resulting from collisional encounters, or static quenching, because of the formation of a complex between the fluorophore and quencher. The two forms of fluorescence quenching can be distinguished by their different dependence upon temperature and viscosity or preferably by lifetime measurements. As can been seen in (Fig. 1 and 3), addition of six kinds of PFASs into bovine albumin and soy peptone resulted in substantial change of protein fluorescence. As PFASs concentration was increased from 0 to 1000 µmol $L^{-1,}$ fluorescence decreased gradually. At 1000 µmol $L^{-1,}$ the intensity was reduced to about 50% of the initial value, and became steady at higher concentrations.

Using Stern-Volmer equation (1-1) plot by linear regression shown in the (Fig.5), the binding constant of PFAS to bovine albumin and soy peptone are calculated to be in the order of 10^5 L mol^{-1}. Using equation (1-2), quenching rate constant k_q (L $mol^{-1}s^{-1}$) could be obtained (Table1). Generally speaking; the maximum dynamic quenching constant k_q of the various quenchers with the biopolymer is 2×10^{10} L mol^{-1} s^{-1} [18]. In (Table 1), the k_q values are much larger than 2×10^{10} L mol^{-1} s^{-1}. Therefore, we preliminarily conclude that the quenching mechanisms belong to static quenching, means that PFASs-bovine albumin and PFASs-soy peptone complex formed.

3.3 THE EFFECT OF PH AND Ca^{2+}/ Na^{+} ON BINDING OF PFASS TO BOVINE ALBUMIN AND SOY PEPTONE

The relative fluorescence intensity of bovine albumin decreased remarkably with increasing concentrations of PFAS (Fig. 1, 3). According to modified Stern-Volmer equation, the result showing that PFASs-bovine albumin complex formed and induced bovine albumin conformational changed. When performing quenching experiments, it is important to consider whether the solution change has an adverse effect on the quenching process. The effect of $Ca^{2+}$and Na^{+} on the quenching constant of the PFAS–bovine albumin system was the same as the fluorescence measurements for bovine albumin. The fluorescence intensity of soy peptone also decreased with increasing concentrations of PFAS and the concentration of Na^{+} and Ca^{2+} at 2µmol L^{-1} has a strong fluorescence emission band at 340.4 nm than at 1 µmol L^{-1} for bovine albumin (20 mg L^{-1}) respectively (Fig. 6, 7). For example, the decrease of the difference fluorescence intensity between spectrum fluorescence at the same titration of PFDA at 1 µmol L^{-1} and 2µmol L^{-1} concentrations of Ca^{2+} were in the range of 80 to 458 and 154 to 742; for PFOA from 91 to 680 and 97 to 931, respectively. Same result was observed for Na^{+} as PFOS was in the range from 77 to 1003 and 105 to 1115 at 1 µmol L^{-1} and 2µmol L^{-1} in the same amount of PFOS titration. The increasing Ca^{2+} and Na^{+} concentration increases the intrinsic interaction between PFAS and bovine albumin/soy peptone to a lesser extent. So it can be concluded that electrostatic interaction [19] and hydrophobic interaction together are playing a major role in PFAS and bovine albumin/soy peptone binding. [20] also reported that the increasing of calcium ions has been noted to increase the adsorption by binding to the sorbent and thereby increasing the electrostatic attraction to PFASs, probably because the decrease in solubility (i.e., the salting-out effect) drives the HOC molecules to the hydrophobic domains of dissolved humic material exceeding the effect on DOM structure.

Table 1. The quenching constants of bovine albumin/soy peptone –PFASs systems

Bovine albumin						
PFASs	**PFOA**	**PFNA**	**PFOS**	**PFDA**	**PFUnA**	**PFDoA**
K´sv (x10^5L mo^{-1})	5.64	2.79	1.97	2.91	5.73	3.835
K_q (x10^{13} L mol^{-1} s^{-1})	5.64	2.79	1.97	2.91	5.73	3.835
R^2	0.978	0.995	0.983	0.994	0.958	0.984
Soy Peptone						
K´sv (10^5)	1.84	0.44	2.53	3.83	4.55	3.19
K_q (10^{13})	1.84	0.44	2.53	3.83	4.55	3.19
R^2	0.972	0.974	0.997	0.954	0.976	0.960

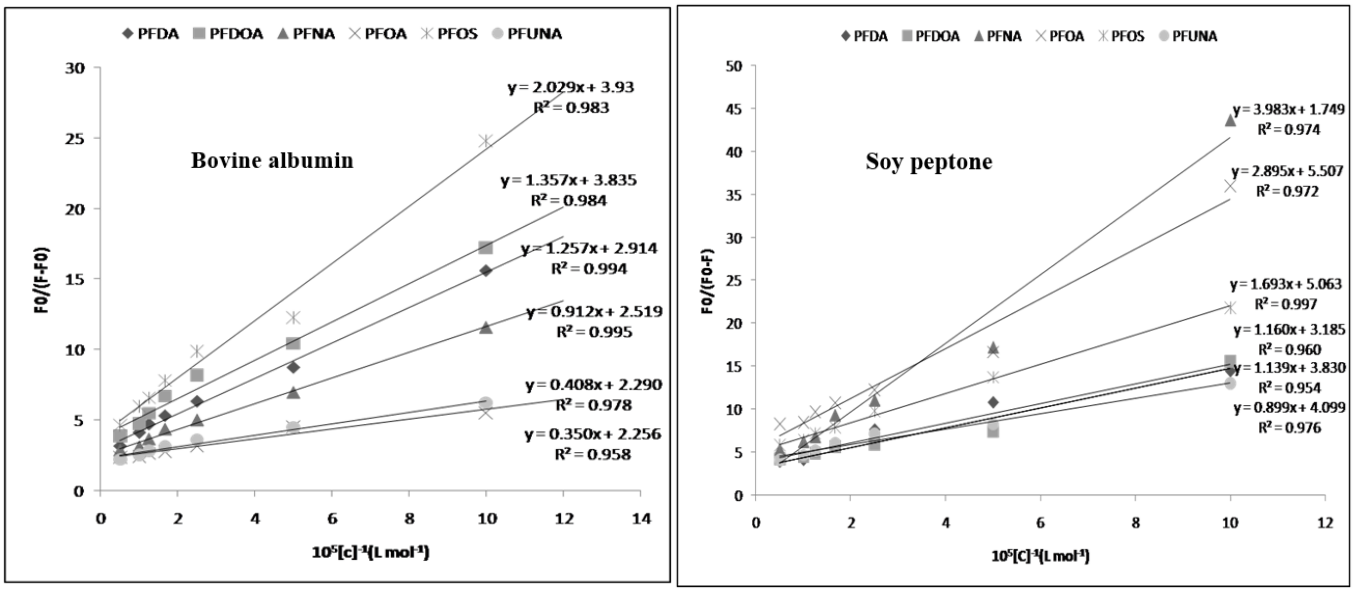

Fig. 5. Modified Stern-Volmer plots for PFASs with bovine albumin and Soy peptone

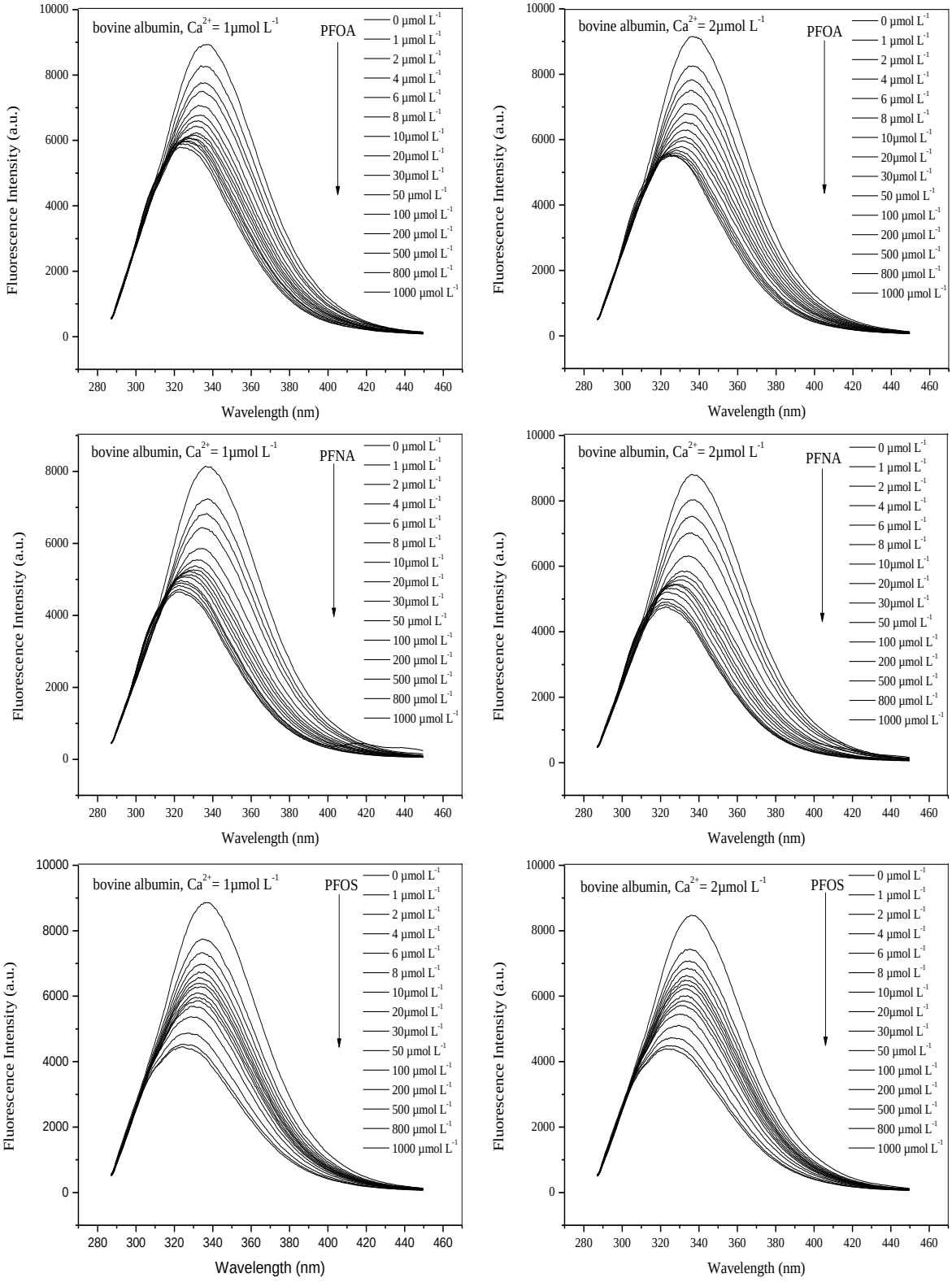

Fig. 6. a) The effect of Ca^{2+} on the fluorescence of bovine albumin (20 mg L^{-1}, λex=278nm) in the presence of difference concentration of PFASs; the pH of the system was fixed at 7.00.

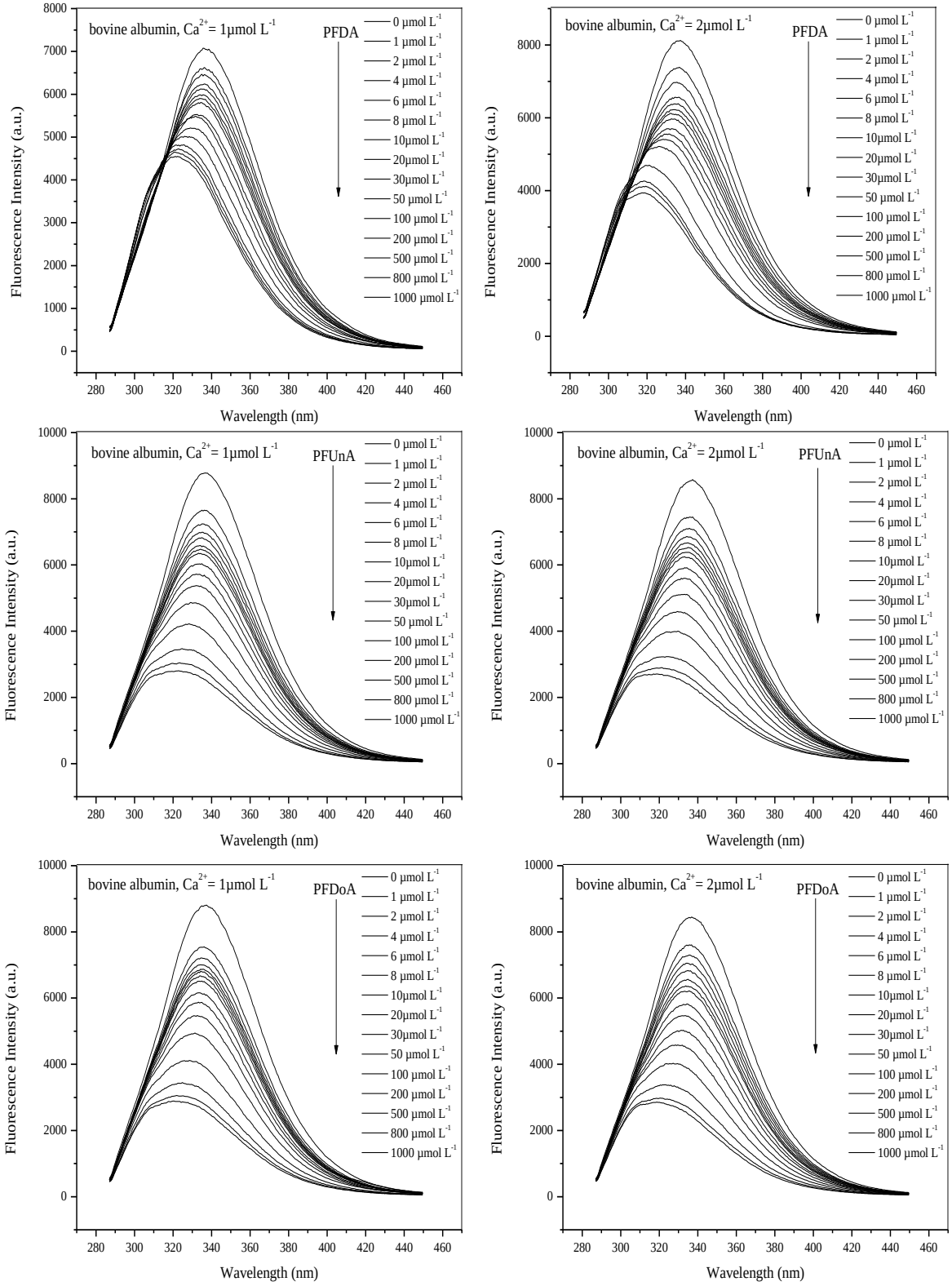

Fig. 6. b) The effect of Ca^{2+} on the fluorescence of bovine albumin (20 mg L^{-1}, λex=278nm) in the presence of difference concentration of PFASs; the pH of the system was fixed at 7.00.

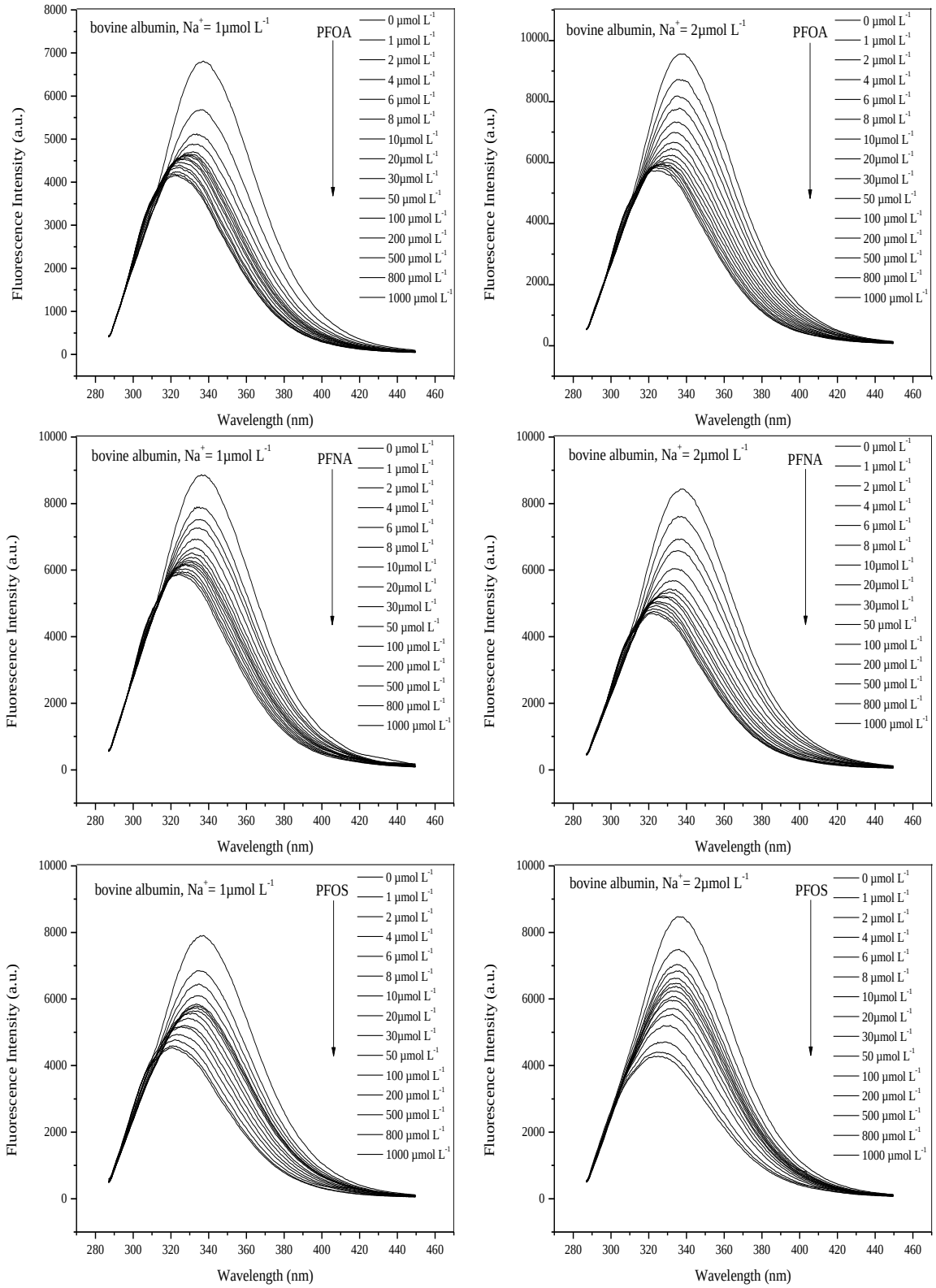

Fig. 7. a) The effect of Na^+ on the fluorescence of bovine albumin (20 mg L^{-1}, λex=278nm) in the presence of difference concentration of PFASs; the pH of the system was fixed at 7.00.

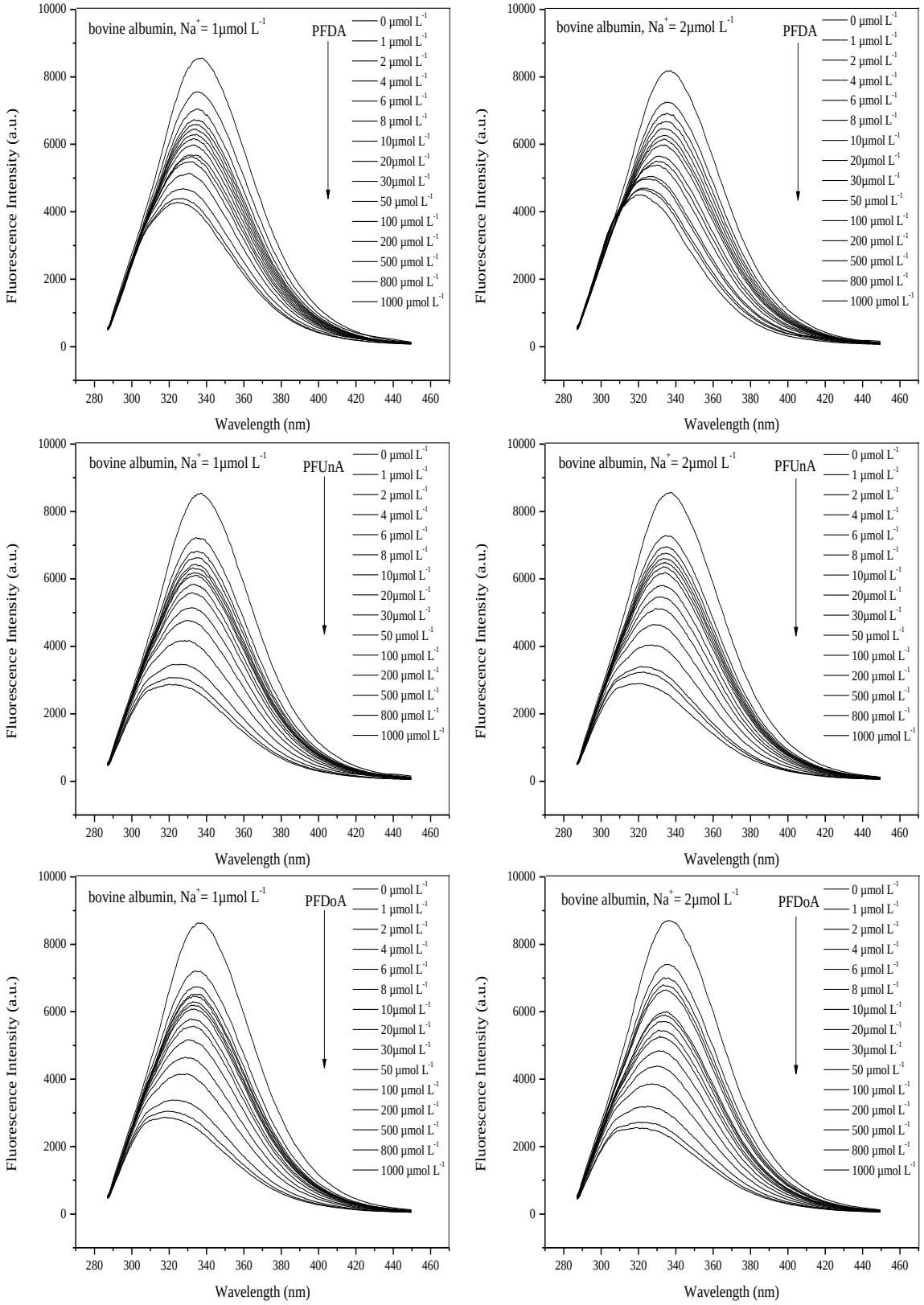

Fig. 7. b) The effect of Na^+ on the fluorescence of bovine albumin (20 mg L^{-1}, λex=278nm) in the presence of difference concentration of PFASs; the pH of the system was fixed at 7.00.

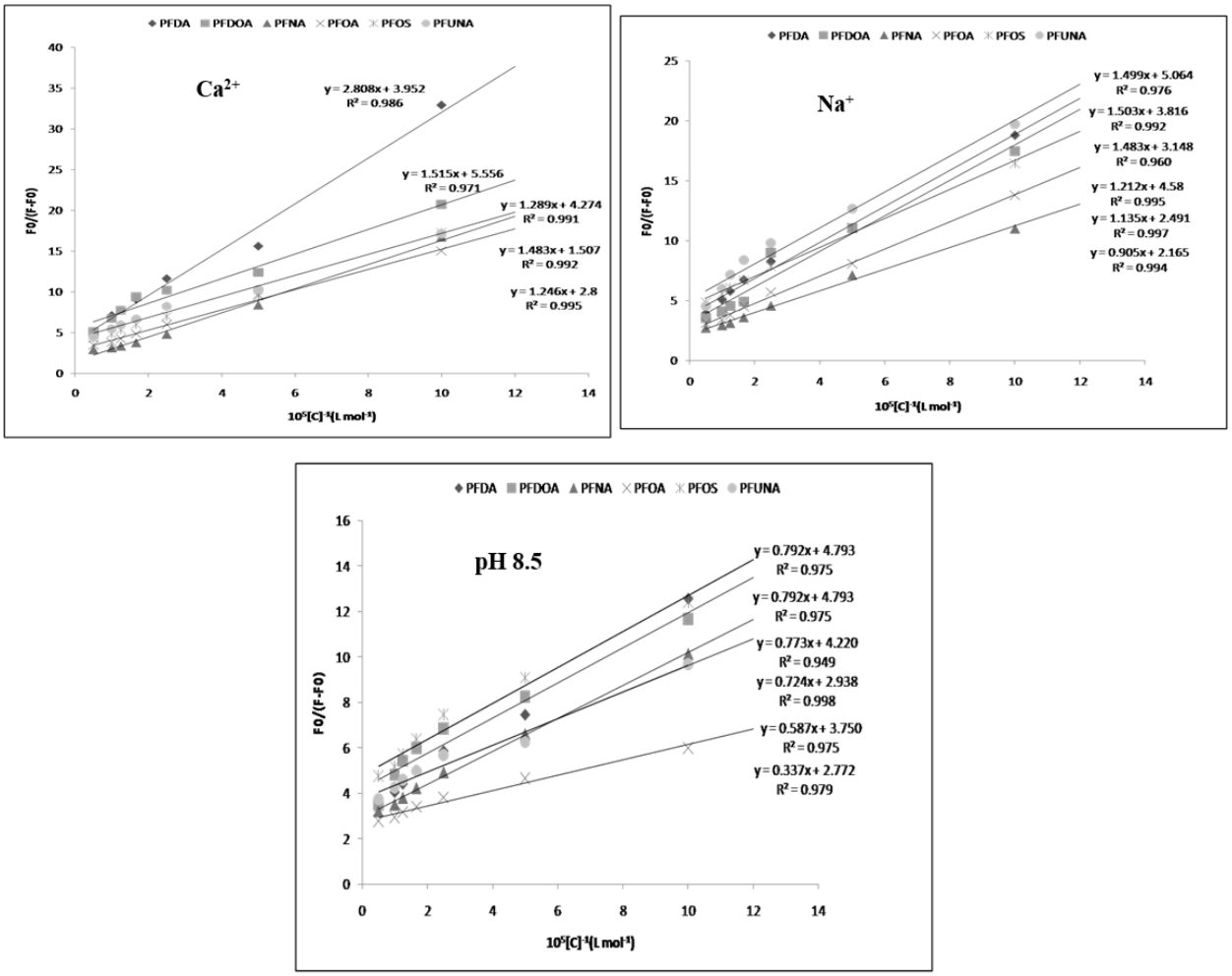

Fig. 8. Modified Stern-Volmer plots for PFASs with bovine albumin with Ca^{2+}/Na^{+} and pH 8.5

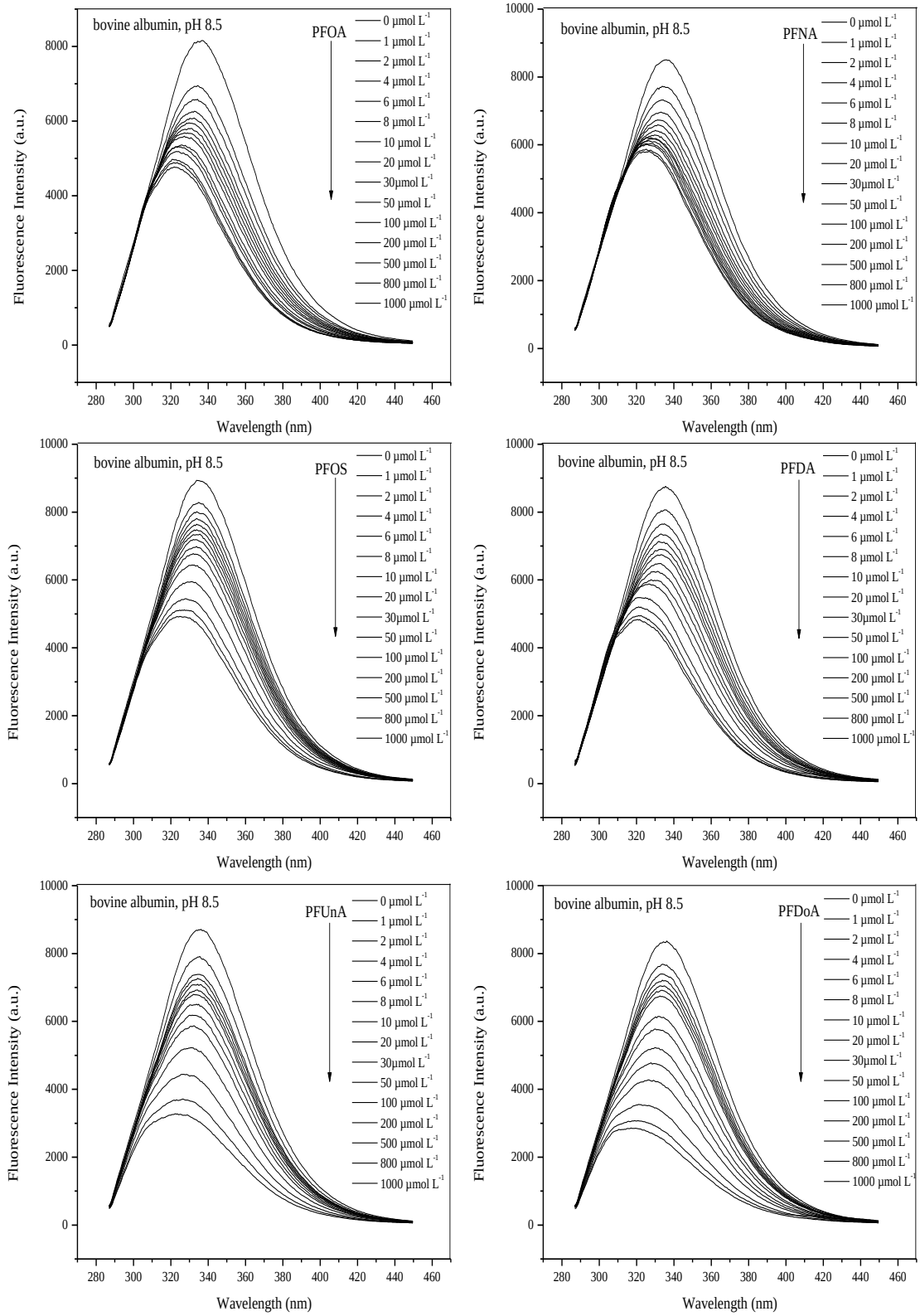

Fig. 9. The effects of pH on the fluorescence of bovine albumin (20 mg L^{-1}, λex=278nm) in the presence of different concentration of PFASs; the pH of the system was fixed at 8.5.

Table 2. The quenching constants K'_{sv} ($x10^5 L\ mo^{-1}$) of bovine albumin–PFAS systems with Ca^{2+*} /Na^{+*}, and pH 6.5, 8.5**

PFASs	PFOA	PFNA	PFOS	PFDA	PFUnA	PFDoA
bovine albumin*, 0.2 mol L^{-1} Na^{+}, 0 mol L^{-1} Ca^{2+}, pH 7 00	5.64	2.79	1.97	2.91	5.73	3.83
bovine albumin, 0.2 mol L^{-1} Na^{+}, 0.000002 mol L^{-1} Ca^{2+*}, pH 7.00	2.80	1.51	3.53	1.32	4.27	2.78
bovine albumin, 0.200002 mol L^{-1} Na^{+*}, 0 mol L^{-1} Ca^{2+}, pH 7.00	2.49	2.41	4.58	1.91	5.06	3.15
bovine albumin, 0.2 mol L^{-1} Na^{+}, 0 mol L^{-1} Ca^{2+}, pH 6.5*	1.25	2.14	1.35	1.93	4.24	2.44
bovine albumin, 0.2 mol L^{-1} Na^{+}, 0 mol L^{-1} Ca^{2+}, pH 8.5*	9.24	4.20	5.99	3.57	6.25	5.28

As shown in (Fig. 8) and Table 2, when cation was added to the quenching solution, the K'_{sv} value were decreased (except for PFOS), for example, the K'_{sv} of PFDA were decreased from 2.91 to 1.32 and 1.91, and the K'_{sv} of PFUnA were decreased from 5.73 to 4.27 and 5.06 with Ca^{2+} and Na^{+} respectively. When the salt concentration in quenching solution changed, means the ion strength changed, the hydrophobic force between quencher and protein will be affected. So, according to the results of $K'_{sv,}$ we may conclude that hydrophobic force and electrostatic interactions are occurred in the PFASs- bovine albumin complex. In addition, small K'_{sv} values typically reflect steric hindrance (retardation of inter- or intramolecular interactions) of quencher-fluorophore collisions.

Increasing pH of bovine albumin solution from 6.5, 7.0 to 8.5 had effects on the fluorescence quenching process. As shown in Table 2, when the pH of bovine albuminsolutionwas8.5 (Fig.9), the K'_{sv} values were increased and for pH 6.5 all K'_{sv} values were decreased. For example, the K'_{sv} of PFDA was increased from 2.91 to 3.57, and the K'_{sv} of PFUnA was increased from 5.73 to 6.25. When the pH condition in quenching solution changed, the electrostatic interactions between quencher and protein will be affected. So, the results of K'_{sv} suggested the occurred of electrostatic interactions in the binding of PFASs and bovine albumin.

In addition, as shown in (Fig. 10,11,12), these results indicated that there were strong interactions and energy transfer between PFASs and bovine albumin /soy peptone; both bovine albumin and soy peptone at pH 6.5 has a strong fluorescence emission band at 340.4 nm and 344.8nm than bovine albumin/ soy peptone at pH 8.5 and pH 7.00 (Fig 1,3), which is mainly the fluorescence emission spectrum of protein. For example, the difference fluorescence intensity between spectrum fluorescence of PFDA at the same PFDA titration at pH 6.5, pH 7.00 and pH 8.5 were in the range from 129 to 371, 51 to 331 and 93 to 244 for soy peptone and 245 to 1122, 172 to 770 and 138 to 697 for bovine albumin respectively. Decreasing pH from 8.5 to 6.5 had effects on the fluorescence in the presence of PFAS. By contrast, the binding of PFAS-bovine albumin/soy peptone resulted in a change of fluorescence intensity at different pH condition. Whereas the ability of PFAS for quenching in bovine albumin/soy peptone decreased in the presence of ligands as pH decreased from 8.5 to 6.5. Further we can say, as there is rational involvement of hydrogen bonds, vander Waals force and electrostatic attraction in the interaction between protein and PFAS.

The intrinsic fluorophores in bovine albumin and soy peptone shows significant advantages, because protein is highly sensitive to the local environment and also displays a substantial spectral shift. As a result, the position of the spectra maximum (λmax) depends upon the properties of the environment of the protein, and the fluorescence spectra depend upon the degree of exposure of the protein side chain to the polar aqueous solvent and its proximity to specific quenching groups [18], [21].

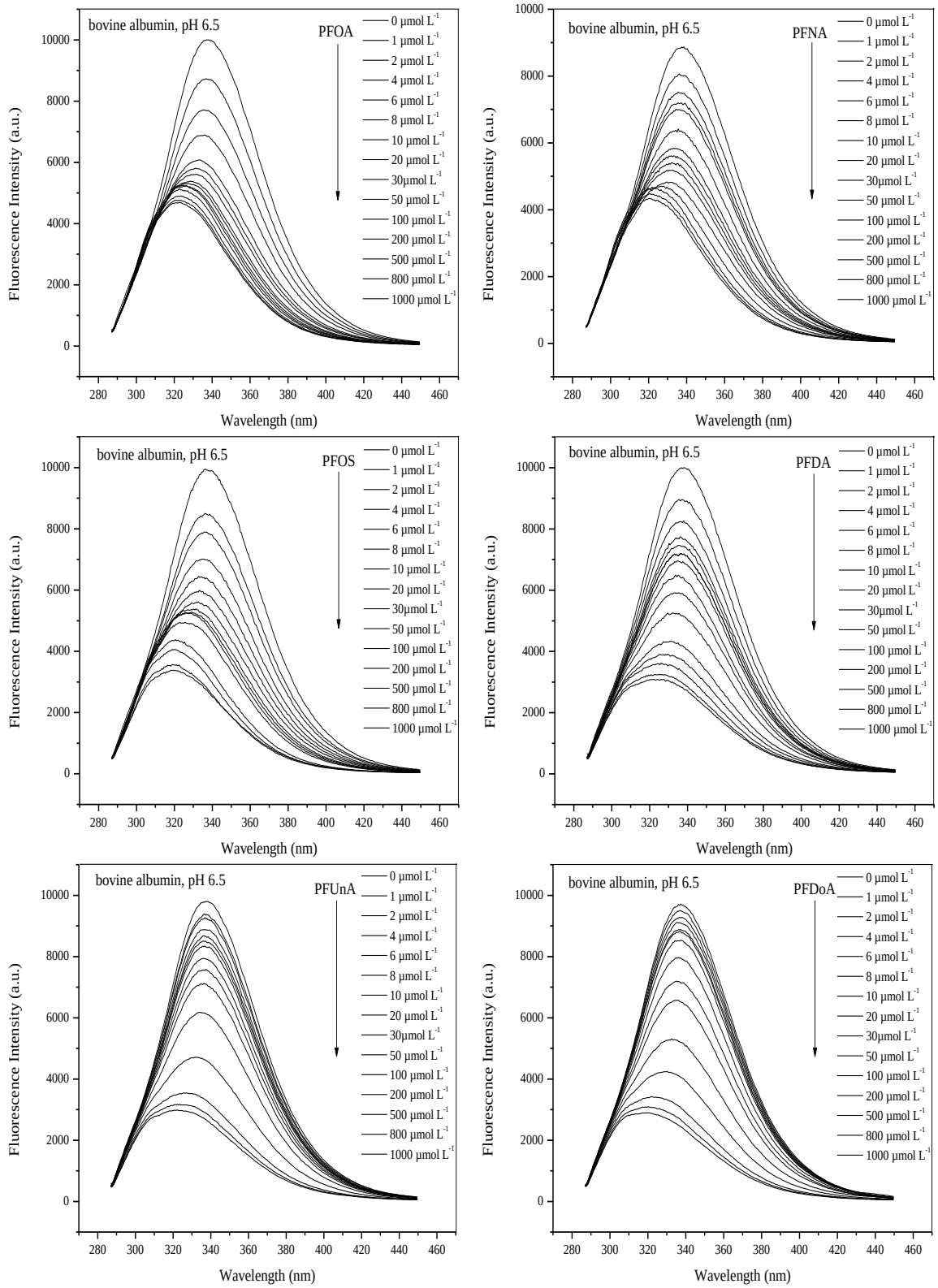

Fig. 10. The effects of pH on the fluorescence of bovine albumin (20 mg L^{-1}, λex=278nm) in the presence of different concentration of PFASs; the pH of the system was fixed at 6.5.

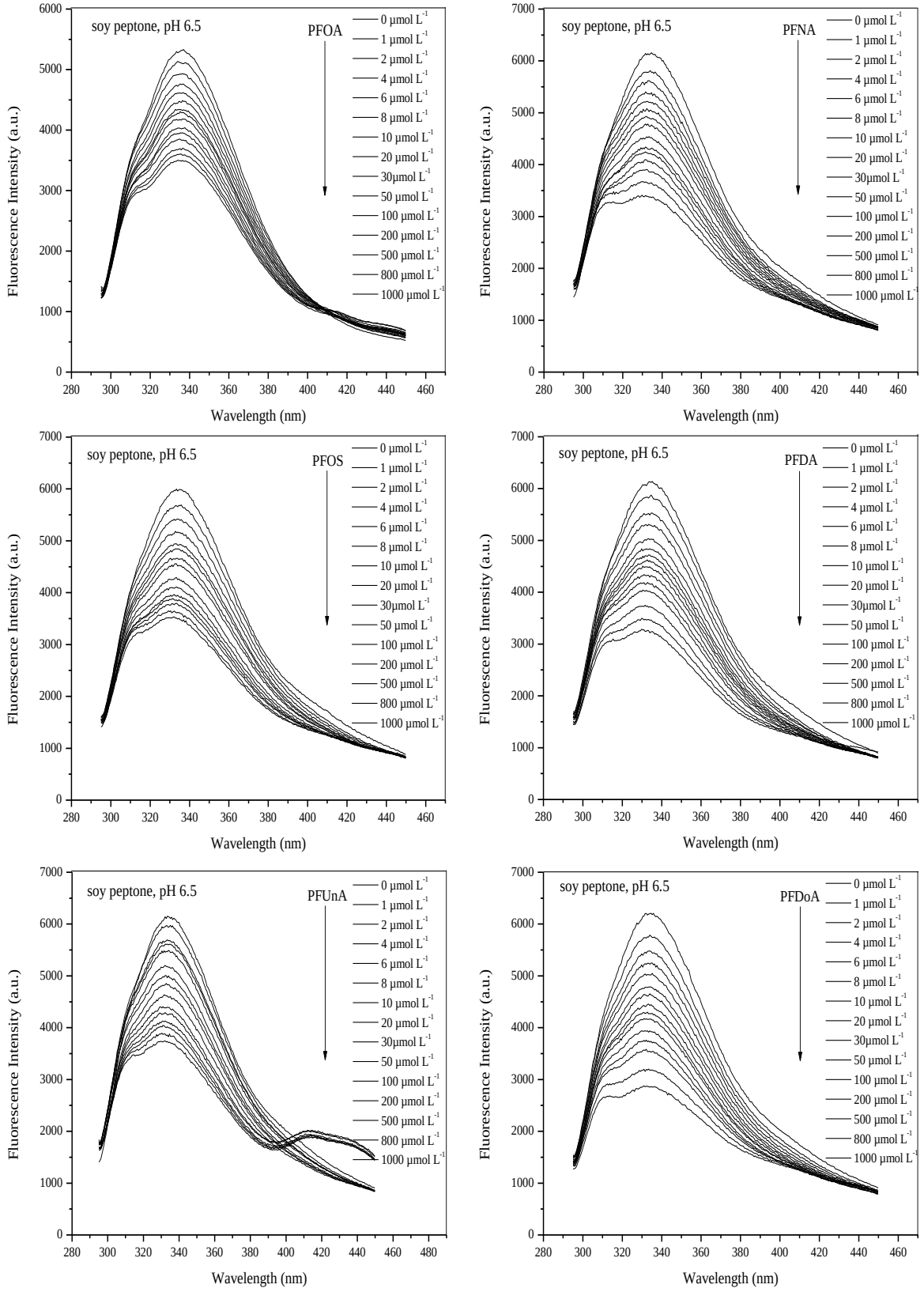

Fig. 11. The effect of pH on the fluorescence of soy peptone (20 mg L^{-1}, λex=278nm) in the presence of different concentration of PFASs; the pH of the system was fixed at 6.5.

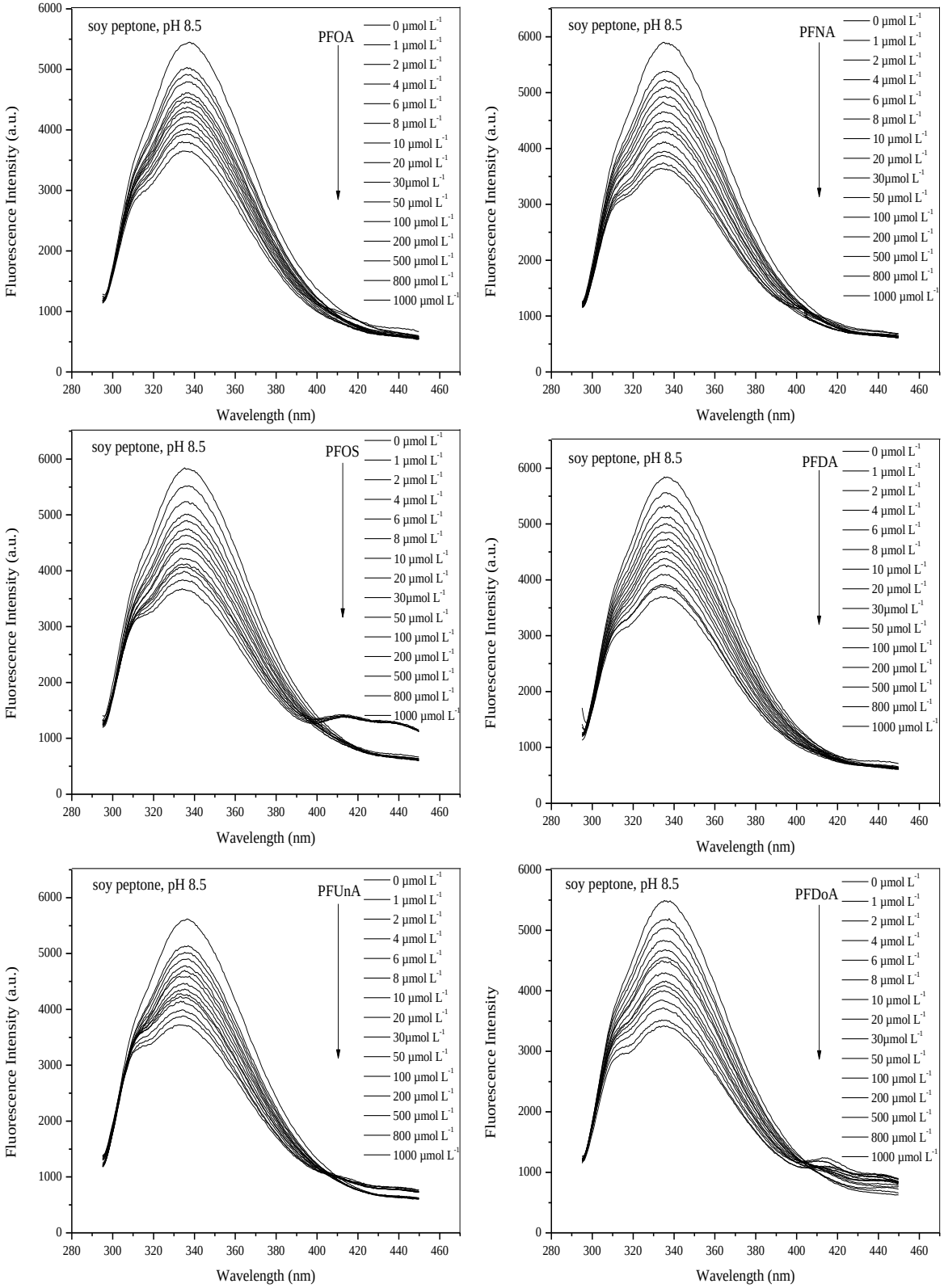

Fig. 12. The effect of pH on the fluorescence of soy peptone (20 mg L^{-1}, λex=278nm) in the presence of different concentration of PFASs; the pH of the system was fixed at 8.5.

These differences in effect of pH may be due to the structural modification of protein molecule and for this reason at a specific pH, the binding site for PFAS is more suitable or properly accommodated and at other pH values the binding sites become less convenient and less accommodating to the protein-PFAS binding. In addition we can say, as there is rational involvement of hydrogen bonds, vander Waals force and electrostatic attraction in the binding to higher affinity sites.

The water chemistry such as pH, Ca^{2+} and Na^{+} may play an important role in PFAS-protein interaction. For the spectra analysis though the absorbance and the position of peak were changed under the effect of different concentration of PFAS in all systems. The results also indicated that PFASs could decrease the intensity of the fluorescence peak of protein, and this effect may attribute to the electrostatic attraction. Furthermore, the bioavailability of PFASs still dominated by the different type of protein even in the presence of ionic strength and different pH level because of the results obtained in this study. Finally, PFASs binding to protein was correlated with the concentration of protein, ions valence and pH condition.

4 CONCLUSIONS

This study developed an approach to understand the interaction on bovine albumin and soy peptone with PFAS using fluorescence spectroscopic method. First, the interaction of bovine albumin and soy peptone with six kinds of PFAS was proven by the fluorescence quenching experiments. The results may provide basic knowledge for a better understanding of the properties of the protein involved in the interactions.

The results obtained from fluorescence spectra indicated that PFASs could quench the intrinsic fluorescence of protein through a static quenching procedure, that means the complex of PFAS-protein were formed. With increase in the ionic strength and pH, the effective quenching constant K'_{sv} of PFASs bound to bovine albumin were changed. Furthermore, with increase of pH from 6.5 to 8.5, reduction in the binding affinity of PFAS to bovine albumin and soy peptone were also observed, suggesting the occurrence of electrostatic interactions and hydrophobic force in the binding.

The experiment results showed that binding is complete within 3 min and that the fluorescence of PFASs compounds associated with protein is fully quenched in all systems.

ACKNOWLEDGMENTS

This study was supported by National Science Foundation for Distinguished Young Scholars, (No. 51325902), the National Science Foundation for Innovative Research Group (No. 51121003), National Natural Science Foundation of China (No. 51279010), and Specialized Research Fund for the Program of Higher Education (No. 20110003110030).

SUPPORTING INFORMATION

Reagents, Additional information includes the QA/QC procedures, analytical parameters of PFASs, Elemental composition of bovine albumin and soy peptone used in this study

REAGENTS

PFOA (99.9%) and PFDA (99.9%) were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA); PFUnA (95%), PFNA (97%), and PFDoA (95%) from Acros Organics (NJ, USA); PFOS (98%) from Tokyo Chemical Industries (Tokyo, Japan). A standard, purity corrected equimolar stock solution containing the PFASs was prepared in a 80: 20 (v/v) methanol/water solution with a concentration of 200 mg L^{-1} for each PFAS. Methanol of chromatography grade was purchased from J.T. Baker (Phillipsburg, NJ, USA). [1,2,3,4-$^{13}C_4$] perfluorooctanoic acid (MPFOA) (purity > 99%) and - [1,2,3,4-$^{13}C_4$] perfluorooctane sulfonate (MPFOS) used as recovery indicators were obtained from Wellington Laboratories (Guelph, Canada). Ammonium acetate (98%), Methyl-tert-butyl ether (MTBE, 99.5%), and tetrabutyl ammonium hydrogen sulfate (TBA) were purchased from Sigma -Aldrich Chemical Co. (St. Louis, MI, USA), and were used to extract PFASs from *D.magna*. Bovine albumin and soy peptone were purchased from Sigma-Aldrich (Missouri, USA) and Organotechnie (La Courneuve, France), respectively. Hydrochloric acid (HCl, 37%), sodium hydroxide (NaOH, 99.99%), Calcium chloride ($CaCl_2$) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).Anhydrous sodium hydrogen phosphate (Na_2HPO_4), Humic acid and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$) were from Fisher Chemical (Fairlawn, NJ). Dialysis bag of spectra6 7000 Da molecular weight was purchased from Sigma -Aldrich Chemical Co. (St. Louis, US).

QUALITY ASSURANCE AND QUALITY CONTROL

To achieve lower detection limits, all accessible PFASs materials on the LC-MS/MS were removed from the instruments and apparatus. Polypropylene tubes and septa were selected after a thorough checking of blanks. Solvent blanks used to monitor instrument background and calibration verification standards used to monitor the validity of the calibration during the sample run were conducted after every 10 samples; a new calibration curve was run if the quality-control standard was not measured within 80% of its theoretical value. The recoveries of PFASs, MPFOS and MPFOA, spiked to the *D. magna,* protein solution, and AFW samples, were measured.

The recoveries of PFASs and mass spectrometric isotope were conducted by spiking target analysts to the *D. magna,* protein solution, and AFW samples, in which none of target analysts were detected, and using the same extraction and purification methods similar to *D. magna* samples. For the *D. magna* samples, a total of 10 *D. magna* were placed into a clean 10 ml polypropylene (PP) tube, followed by addition of 10 ng of $^{13}C_4$-PFOS and $^{13}C_4$-PFOA as well as the target analysts with an exposure time of 3 days similar to the bioaccumulation tests. After that, 2 mL of Na_2CO_3 (0.25 mol L^{-1}), 1 mL of the ion-pairing agent TBA (0.5 mol $L^{-1,}$ adjusted to pH 10), and 2 mL of MTBE were added into each PP tube. These tubes were shaken vigorously for 10 min, and then sonicated for 10 min, followed by centrifugation to isolate the organic phase. The MTBE supernatant was collected in a separate plastic tube, and this extraction process was repeated twice. The supernatants were combined and blew to dryness under high-purity nitrogen gas, and the analytes were dissolved in 1 ml 80: 20 (v/v) methanol: water solution and filtered into PP-snap ring vial (0.25 ml) with polyethylene (PE) cap. They were then analyzed by liquid chromatography- tandem mass spectrometry (LC-MS/MS; Dionex Ultimate 3000 and Applied Biosystems API 3200). For the protein solution and AFW samples, the recovery rates of PFASs were determined using the same extraction and analysis method. A total of 10 ng PFAS standard solution was added into 5 mL bovine albumin/soy peptone solution (20 mg/L) or AFW and the results indicated that the recoveries of PFASs ranged from 86% to 108%.

Table S1 Analytical parameters and recoveries of PFASs

Analyte	Molecular structure	LogK_{ow}[a]	LogK_{oc}[b] (SE, n=3)	Primary and production ions (*m*/*z*)	Daphnia tissue recovery (%)	Water sample recovery (%)
PFOS	$C_8F_{17}SO_3H$	5.25	2.57 (0.13)	498.9>79.8	83± 2	86±9
PFOA	$C_7F_{15}COOH$	4.30	2.06	412.9 > 369.0	95±4	86±9
PFNA	$C_8F_{17}COOH$	4.84	2.39 (0.09)	463.0 > 418.9	81±3	93±5
PFDA	$C_9F_{19}COOH$	5.30	2.76 (0.11)	512.9 > 468.9	85±5	108±5
PFUnA	$C_{10}F_{21}COOH$	5.76	3.30 (0.11)	562.9 > 518.9	88±1	108±2
PFDoA	$C_{11}F_{23}COOH$		-	612.9 > 569.0	82±5	104±2
MPFOA	[1,2,3,4-$^{13}C_4$] perfluorooctanoic acid			416.8 > 371.8	95±2	90±5
MPFOS	[1,2,3,4-$^{13}C_4$] perfluorooctane sulfonate			503.0 > 79.9	90±2	91±6

[a] *n*-octanol/water partition coefficient, data from Arp, H.P.H., Niederer, C., Goss, K.U., 2006. Predicting the partitioning behavior of various highly fluorinated compounds. Environ. Sci. Technol. 40, 7298-7304.

[b] LogK_{oc} data from Higgins, C., Luthy, R., 2006. Sorption of Perfluorinated Surfactants on Sediments. Environ. Sci. Technol. 40, 7251-7256.

Table S2 Elemental composition of bovine albumin and soy peptone used in this study

Physico-chemical characteristics	Bovine albumin[a]	Soy peptone[b]
Molecular weight (Da)	66.5kDa	80% <7 kDa and 20% >7 kDa
Total Amino acids	607.0	494
Total nitrogen (TN) (%)	16.0	8.8 -10.1
α- amino nitrogen (AN) (%)	-	3.0 - 3.8
AN/TN x 100	-	30 – 43
Chloride (as NaCl) (%)	0.9	≤ 3.0
Sulfated ash (%)	≤2.0	≤15
pH of 1% Solution	5.2-7	-
pH (5 % solution)	-	6.6 – 7.6
Overall dimensions (Å)	40 × 140	-
Frictional ratio, f/f_0	1.30	-
Estimated α –helix (%)	54	-
Estimated β-form (%)	18	-
Residue on ignition (%)	-	≤ 18
Loss on drying (%)	-	≤ 11
Intrinsic viscosity (η)	0.0413	-
Diffusion constant, $D_{20,w} \times 10^7$	5.9	-
Fatty Acid Depleted	5.3	-
Sedimentation constant, $S_{20,w} \times 10^{13}$	4.5 (monomer), 6.7 (dimer)	-

[a] *data provided by SIGMA, 3050 Sprunce Street, Saint Louis, Missouri 63103 USA*

[b] *data provided by Organotechnie® S.A.S. Edition 2010/02 27, avenue Jean Mermoz, 93120 La Courneuve, France*

REFERENCES

[1] L. Brand, J.R. Gohlke. Fluorescence probes for structure. Annu Rev Biochem. 1972; 41: 843–868.

[2] G.Colmenarejo, In silico prediction of drug-binding strengths to human serum albumin. Med. Res. Rev. 2003; 23: 275–301.

[3] S. Sugio, A.Kashima, Mochizuki S, Noda M, Kobayashi K. Crystal structure of human serum albumin at 2.5 A resolution. Prot. Eng.1999; 12 (6): 439-46.

[4] A. Q. Gu, X.S. Zhu, Hu YY, Yu SH. A fluorescence spectroscopic study of the interaction between epristeride and bovin serum albumine and its analytical application Talanta. 2007; 73: 668-673.

[5] P. Athina, J. Green Rebecca, A Frazier Richard. Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. J Agric Food Chem. 2005; 53: 158–63.

[6] J. R. Lakowicz, Principles of fluorescence spectroscopy, 2nd edn. Kluwer Academic Publishers/Plenum Press, New York. 1999.

[7] J. Romer, M.H. Bichkel, Mehtod to estimate binding constants at variable protein concentrations. J Pharm Pharmacol. 1979; 31: 7–11.

[8] M. Bhattacharyya, V. Chauahuri, R.K. Poddar, Evidence for co operative Binding of chloropromazine with hemoglobin: equilibrium dialysis, fluorescence quenching and oxygen release study. Biochem Biophys Res Commun. 1990; 167: 1146–1153.

[9] H. Zhao, Su W, Y.H. Luo, Z.C. Li, H.F. Jiu, H. Liang, B. Chen, Q.J. Zhang, Rectification of excitation with bathochromic shift induced by intense absorption of organic ligands during emission. Spectrochim Acta A. 2006; 65: 846–851.

[10] N. Kuresbayashi, A.B. Harkins, S.M. Baylor, Use of fura red as an intracellular calcium indicator in frog skeletal muscle fibres. Biophys. J. 1993; 64: 1934–1960.

[11] Y.J. Hu, Y. Liu, R.M Zhao, J.X. Dong, S.S. Qu, Spectroscopic studies on the interaction between methylene blue and bovine serum albumin. J. Photochem. Photobiol. A–Chem. 2006; 179-324.

[12] C. Kwadijk, P. Korytar, A. Koelmans, Distribution of Perfluorinated Compounds in Aquatic Systems in The Netherlands. Environ. Sci. Technol. 2010; 44: 3746-3751.

[13] P.Labadie, M. Chevreuil, Partitioning behaviour of perfluorinated alkyl contaminants between water, sediment and fish in the Orge River (nearby Paris, France). Environmental Pollution.2010; 159: 391-397.

[14] j. Akkanen, J.V.K. Kukkonen, Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. Environ. Toxicol. Chem. 2003; 22: 518-524.
[15] P.Qin, R. Liu, X. Pan, X. Fang, Y. Mou, Impact of carbon chain length on binding of perfluoroalkyl acids to bovine serum albumin determined by spectroscopic methods. J. Agric. Food Chem. 2010; 58: 5561–5567.
[16] J. Akkanen, S. Penttinen, M. Haitzer, J.V.K. Kukkonen, Bioavailability of atrazine, pyrene and benzo [a] pyrene in European river waters. Chemosphere 2001; *45*: 453-462.
[17] J.M.Weiss, P.L. Andersson, M.H. Lamoree, P.E.G. Leonards, S.P.J. van Leeuwen, T. Hamers, Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol. Sci. 2009; 109: 206-216.
[18] Y.Z. Zhang, B. Zhou, X.P. Zhang, P. Huang, C.H. Li, Y. Liu, Interaction of malachite green with bovine serum albumin: Determination of the binding mechanism and binding site by spectroscopic methods. J. Hazard. Mater. 2009; 163 (2-3): 1345–1352.
[19] N.N. Afifi, Using difference spectrophotometry to study the influence of different ions and buffer systems on drug protein binding. Drug Dev. Ind. Pharm. 1999; 25: 735-743.
[20] C.Y. Tang, Fu.Q Shiang, D. Gao, C.S. Criddle, J.O. Leckie, Effect of solution chemistry on the adsorption of perfluorooctane sulfonate onto mineral surfaces. Water Res. 2010; 44 (8): 2654-2662.
[21] H. Lin, J. Lan, M. Guan, F. Sheng, H. Zhang, Spectroscopic investigation of interaction between mangiferin and bovine serum albumin. Spectrochim. Acta, Part A. 2009; 73 (5): 936–941.