Spectrophotometric detection of PFAS in water using bovine serum albumin and tetraphenylporphyrintetrasulfonate



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Introduction

Perfluoroalkyl substances (PFAS), known as "forever chemicals", are ubiquitous environmental contaminants whose remarkable persistence poses significant risks to human health and ecosystems.¹ Thus, it is important to develop analytical assays to determine PFAS concentrations based on widely accessible instrumentation, such as UV-VIS spectrophotometry. Tetrasodium tetraphenylporphyrintetrasulfonate (TPPS) is a watersoluble porphyrin with spectrophotometric property in water.² Interestingly, TPPS is also known to bind to the protein bovine serum albumin (BSA).³ We found that the addition of PFAS salts, sodium perfluorohexanoate (**PFHxA**) and potassium two perfluorobutanesulfonate (**PFBS**), to homogeneous BSA-TPPS mixtures appreciably alter TPPS absorption spectra. Our results show that these mixtures can be used to produce calibration curves relevant to the determination of PFAS concentrations in water.



Results and Discussion

Effect of BSA on TPPS absorption spectrum

Visible absorption of TPPS at pH 4.5 is maximum at $\lambda_{max} = 645$ nm.⁴ Introducing BSA to TPPS-water binary system induces BSA-TPPS precipitation and decreases absorption significantly at low BSA:TPPS. At BSA:TPPS > 2, mixtures become homogeneous but exhibit a different color compared to BSA-free samples (see Fig. 1A). Representative absorption spectra of TPPS-BSA-water are shown in *Fig. 1B*.



Figure 1. (A) TPPS 80 μ M in the absence of BSA (left), in the presence of BSA 5 μ M (center), and 300 µM (right). Precipitation is observed for low BSA:TPPS (center). (B) Absorption spectra of TPPS 80 μ M at pH 4.5 in the absence of BSA (•) and in the presence of BSA 5 μ M (•) and 300 μ M (•).

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Model for BSA-TPPS precipitation

A theoretical model was developed to explain BSA-TPPS precipitation at low BSA concentration and solubilization at high BSA concentration.



Figure 2. Absorbance ratio, A/A_0 , as a function of BSA: TPPS molar ratio, C_P^0 / C_L^0 , where A_0 is A at $C_{\rm P}^0 / C_{\rm L}^0 = 0$, $C_{\rm P}^0$ and $C_{\rm L}^0$ correspond to the total concentrations of BSA and TPPS. Solid curve is fit through data using BSA-TPPS precipitation model. P, L, and PL_i respectively denote protein (BSA), ligand (TPPS), and protein with j number of bound ligands, with n being the maximum value of j. β_i is the equilibrium constant for BSA-TPPS complex formation, and A(s) denotes the insoluble aggregates with solubility S_A . From model and data, we determined $\beta_1 = 9.9 \times 10^6 \text{ M}^{-1}$ and n = 15.7.

PFAS-BSA-TPPS absorption spectra

Introducing PFHxA to homogeneous BSA-TPPS mixtures induces wavelength shift in TPPS spectra due to competitive binding between PFHxA and TPPS to BSA (Fig. 3). Isosbestic points indicate the presence of two TPPS absorbing species: free and bound to BSA.



Figure 3. Absorption spectra of TPPS 80 μ M in the presence of BSA 300 μ M with increasing concentration of PFHxA. PFHxA:BSA molar ratios are designated to their respective spectrum.



A₅₁₈ /A₅₇₃ as a function of PFAS: TPPS molar ratio

 A_{518}/A_{573} absorbance ratios obtained from PFAS-BSA-TPPS spectra (Fig. 3) linearly increases with PFAS:TPPS molar ratio at low PFAS concentration. Difference in steepness of curves may be attributed to the binding affinities of specific PFAS species to BSA. Fig. 4 supports our hypothesis that absorbance ratios may be used to produce calibration curves for determination of PFAS concentrations in water.



- BSA:TPPS molar ratio.
- concentration in water.



Figure 4. A₅₁₈ /A₅₇₃ absorbance ratio as a function of PFAS: TPPS molar ratio for solutions with TPPS 80 μ M, BSA 300 μ M, and increasing concentrations of PFHxA (\bullet) or PFBS (\bullet).

Conclusion

BSA-TPPS binding produces insoluble aggregates at low BSA:TPPS molar ratio but soluble complexes at relatively high BSA:TPPS molar ratio. A model that explains this behavior was successfully developed.

To circumvent precipitation, PFAS experiments were conducted at a relatively high

PFHxA (PFBS) competitively binds to BSA in the presence of TPPS.

Our results indicate that BSA-TPPS absorption could be used to determine PFAS

These studies will be extended to other types of PFAS to characterize binding affinities due to differences in surfactant chain length and hydrophilic group.

References

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