Lower Limit PFOA Concentrations and the Effect on Lipid Membranes

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INTRODUCTION

Poly/Perfluorinated substances (PFAS) are a class of synthetic molecules that threaten the health of communities and wildland environments. PFAS consist of carbon chains, usually 4 – 10 carbons in length, with fluorine atoms attached where hydrogen atoms would be in a hydrocarbon. Known as 'forever chemicals', PFAS do not break down easily in the environment and have shown an affinity for bioaccumulating in wildlife[(1)]. They are commonly found in clothing water proofer, food packaging and firefighting foams. PFAS have also been found to cause numerous adverse health effects due to a lack of metabolic pathways in organisms. At present, the federal limit for PFOA is 4 ppt or 10^{-3} nM[(2)]. It is not widely understood how PFAS at its lower limits affect biological material. This research will examine how nanomolar concentrations of perfluorooctanoic acid (PFOA) and perfluorobutanesulfonic acid (PFBS) affect model biological membranes. It is hypothesized that PFOA is partitioning the membranes of cells and will decrease membrane stability. This research will also examine whether PFOA-PFBS mixtures exhibit a Critical Micelle Concentration (CMC) or aggregation in solution. Literature has suggested that PFAS do have a CMC, however the listed concentration varies greatly.

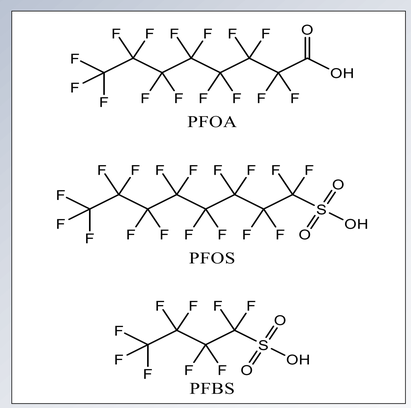


Figure 1. Common species of PFAS: perfluorooctanic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorobutanesulfonic acid (PFBS).

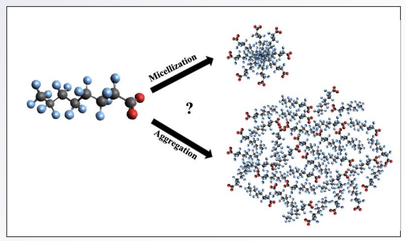


Figure 2. Does a monomer of PFOA form a micelle or aggregate when it's in solution?

METHODS

Dynamic Light Scattering

Dynamic Light scattering (DLS) measures the size of a particle via the use of Brownian motion. Data gathered from DLS displays particle sizes across different conditions, such as temperature and pH levels. Data are utilized to examine how particles behave in different environments.

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) measures the enthalpy required for a phase transition to occur in a substance. The information collected from DSC allows for an understanding of how the molecules that have partitioned into the membrane affect the gel-liquid crystalline phase transition of the membrane.

RESULTS

Dynamic Light Scattering

Utilizing DLS, Sodium Octanoate was first examined because it is a common surfactant that has a Critical Micelle Concentration (CMC). A critical Micelle Concentration is the concentration of a surfactant in which micelles will start to form. In addition to a CMC, Sodium Octanoate also has the same carbon chain length as PFOA. It is expected that they will behave similarly due to their chain length (8-carbons).

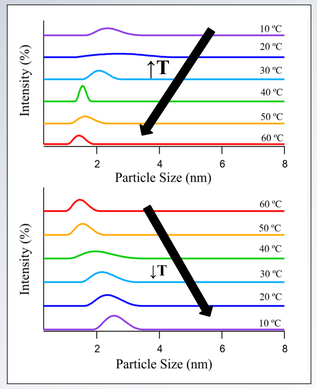


Figure 3. Sodium Octanoate (380 mM), a model surfactant. Note that the size of the micelles is < 3nm and does not change much as the system temperature changes.

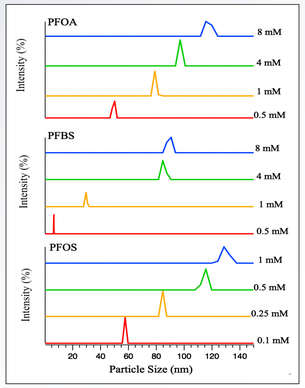


Figure 4. Three species of PFAS (PFOA, PFBS, PFOS) all show signs of forming aggregates in solution at ~mM concentrations. Aggregate size changes significantly with bulk solution concentration

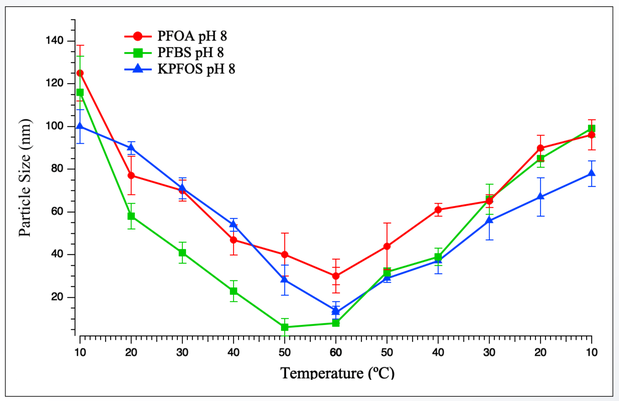
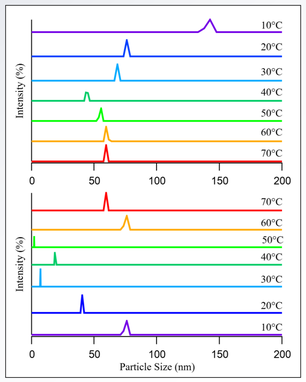


Figure 5. Temperature dependent diameters of PFOA, PFBS, and PFOS in 1 mM, pH 8.0 solutions. Solution temperature was raised from 10°C to 60°C and then cooled back to 10°C in 10°C increments. Error bars represent the 1 sigma standard deviation of nine measurements.

		Particle Size (nm)			
	Temperature (°C)	PFOA	PFBS	KPFOS	Sodium Octanoate
Heating	10	125	116	100	2.3
	20	77	58	90	2.8
	30	70	41	71	2.0
	40	47	23	54	1.5
	50	40	6	28	1.6
	60	30	8	14	1.4
Cooling	60	30	8	13	1.4
	50	44	32	29	1.5
	40	61	39	37	1.9
	30	65	66	56	2.1
	20	90	85	67	2.3
	10	96	99	78	2.5

Table 1. Size of particles as a function of temperature. It is observed that as temperature increases, the size of the particles decreases.



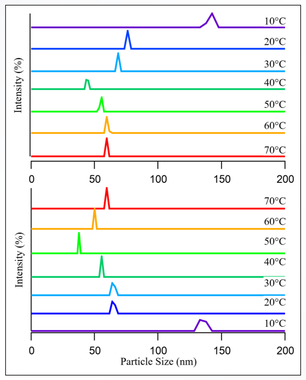


Figure 6. 20-80 PFOA-PFBS pH 8 (left)
Figure 7. 20-80 PFOA-PFBS pH 3.5 (right)
These data should be compared to those from pure PFAS in Figure 5. PFOA-PFBS mixtures were tested to discover if aggregation behavior is generalized and, if so, would aggregation would show cooperative or competitive behavior or simply reflect averaged behavior of the two PFAS surfactants acting independently. Both figures are based 0.2 mM PFOA, 0.8 mM PFBS mixtures.

Differential Scanning Calorimetry

Differential Scanning Calorimetry was used to determine the enthalpy required for a phase transition in the model biological membrane. In this experiment, DSPC, a lipid with an 18-carbon chain, was used to model a biological membrane. Lipid membranes have a gel state that melts into a liquid-crystalline state when heated. For DSPC, the gel-liquid transition occurs at 55.6 ºC[(3)]. Enthalpy is measured to observe how PFOA disrupts the 'frozen' lipids.

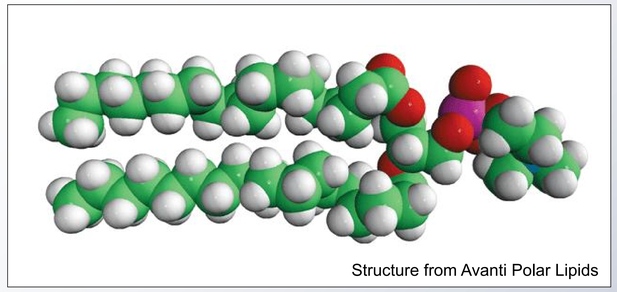


Figure 8. The model biological membrane selected for examination was 1,2-Distearoyl-sn-glycerol-3-phosphorylcholine (DSPC). This lipid is one of the most common in mammalian cell membranes[(4)].

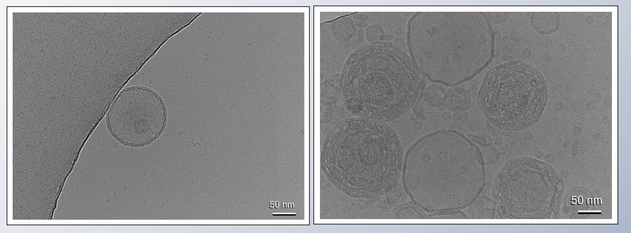


Figure 9. Cryo-EM imaging of pure DPPC after processing. Once processed, DPPC can form cell-like structures (left).
Figure 10. Cryo-EM imaging of DPPC with 100 nM PFOA (right).

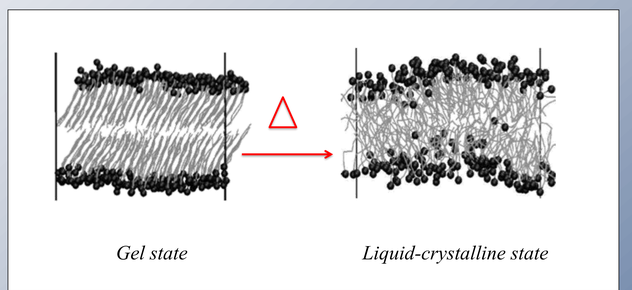


Figure 11. DSPC and DMPC behavior as the lipid transitions from a gel, or frozen, state to the liquid-crystallin state. This transition occurs at 55.6ºC in DSPC and 24ºC in DMPC.

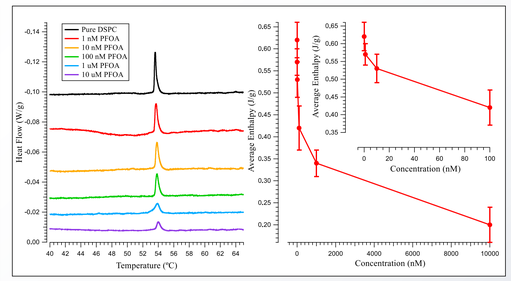


Figure 12. Enthalpy of DSPC doped with PFOA at varying concentrations. This graph demonstrates that enthalpy in samples decreased as concentration of PFOA increased. The data suggests that the membrane becomes more permeable. PFOA is disrupting frozen lipids, so when the lipid melts, there's less to melt. Transition temperature doesn't change, so it proves that PFOA is reduces the energy required to 'melt' the lipids

CONCLUSIONS

Dynamic Light Scattering

- PFAS have a similar behavior to Sodium Octanoate, but there is a much greater range in particle size; aggregates have grown up to 160nm in size.
- Upon further investigation of PFOA-PFBS mixtures, it was determined to not have a CMC and instead aggregates in solution. The particles formed change depending on bulk solution concentration and system temperature.

Differential Scanning Calorimetry

- Transition temperature of DSPC does not change as PFOA is introduced.
- DSPC's enthalpy is decreased by PFOA at all concentration levels, including sub-nM levels.
- Decreasing the transition enthalpy is characteristic of fluidizing the membrane and disruption of chain interactions
- PFOA is acting to reduce enthalpy required for the lipid to 'melt'

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ACKNOWLEDGMENTS

This material is based upon work supported in part by the National Science Foundation EPSCoR Cooperative Agreement OIA-1757351.
Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103474. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
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