School of Chemical, Biological, and Environmental Engineering

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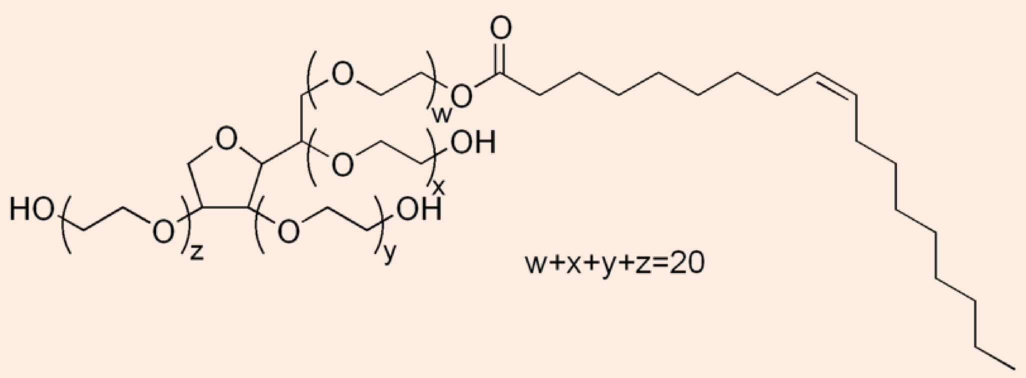
Molecular Origins of Surfactant Stabilization of a Human Recombinant Factor VIII

Understanding Surfactants

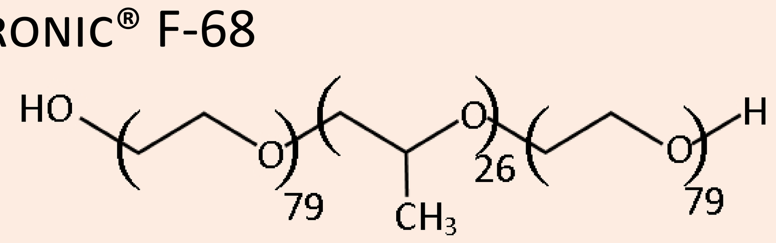
Surfactants contain hydrophilic and hydrophobic regions, depicted as a small circle and long tail, respectively. Surfactants are used to stabilize hydrophobic molecules in aqueous solutions.

Polysorbate 80



Polysorbate 80 (PS-80) is currently used in industrial production of FVIII to reduce processing losses due to surface adsorption (Figure 1).[1] PS-80 exhibits preference for air-water interface over protein adsorption, leading to protein aggregation and loss of protein activity.

Pluronic® F-68



Pluronic® F-68 is a triblock copolymer surfactant consisting of two hydrophobic PEO chains and one hydrophilic PPO chain. The PPO chain is expected to preferentially adsorb to hydrophobic regions of protein instead of the air-water interface (Figure 1), increasing FVIII stability.

Protein Stabilization

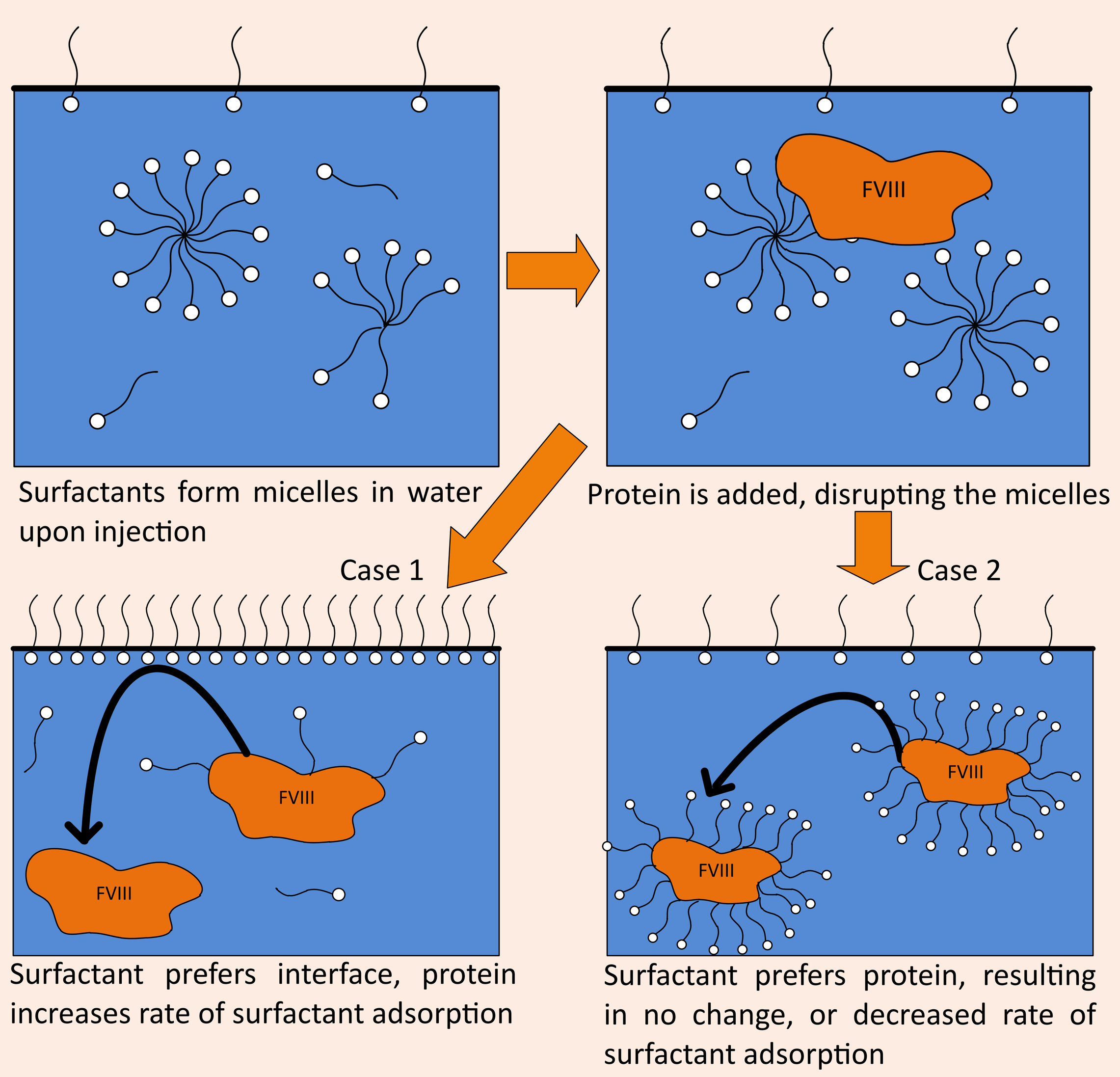


Figure 1: Two methods of surfactant stabilization of hydrophobic proteins in aqueous solutions. Case 1 (left) surfactants prevent adsorptive losses by coating hydrophobic surfaces. Case 2 (right) surfactants stabilize the protein by coating both the protein and hydrophobic surfaces, reducing hydrophobic aggregation and preventing adsorptive losses.

Project Objective

The project objective was to reduce production cost and increase yield of human recombinant Factor VIII by characterizing protein stabilization with two surfactants, Pluronic® F-68 and Polysorbate 80.

Factor VIII

Human recombinant Factor VIII (FVIII) is an essential blood clotting protein artificially manufactured for treatment of Hemophilia A. Factor VIII is a large molecule with hydrophobic regions, making it unstable in solution. Half the protein produced is lost to protein aggregation and surface adsorption, amounting to $1.2 billion of protein lost per year. The instability of FVIII limits drug supply and contributes to annual treatment costs exceeding $150,000.

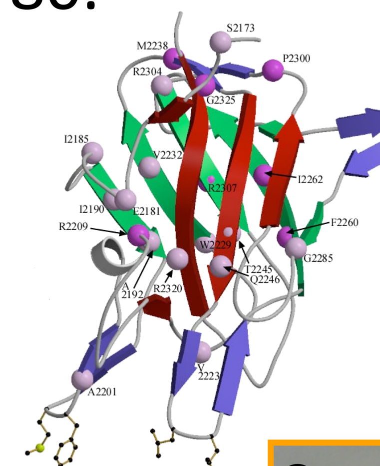


Figure 2: Molecular map of Factor VIII. The spheres indicate areas of mutation in hemophilia patients that disable the protein's function and leave those patients unable to clot naturally.[2]

Surface Tensiometry

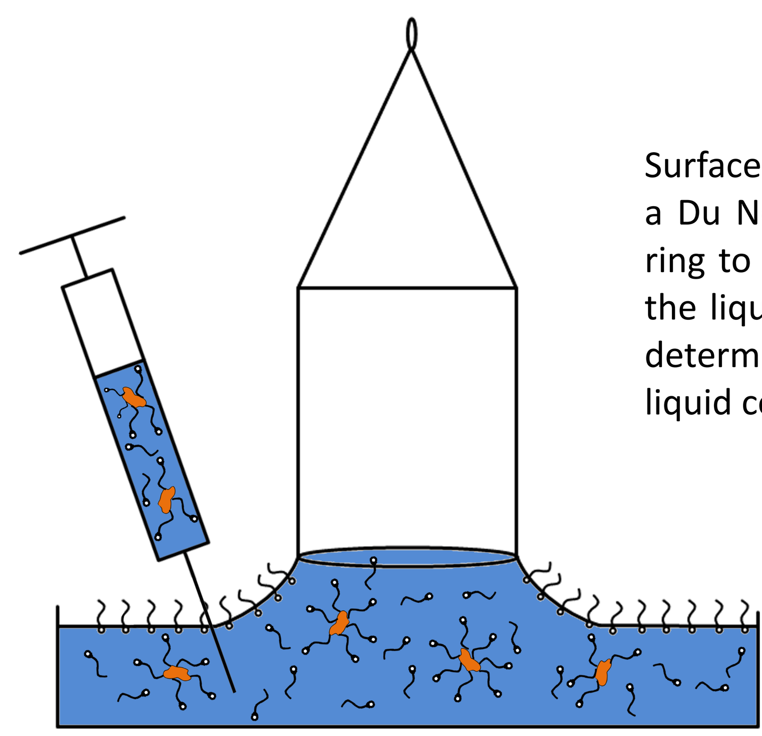


Figure 3: A representative schematic of the tensiometer with injection of surfactant and FVIII into the petri dish for surface tension measurement.

Surface tension was measured with an FTA 10 tensiometer, using a Du Nouy ring (Figures 3 & 4). The tensiometer uses the wire ring to pull up on the liquid surface and measure the weight of the liquid hanging from the ring. The solution surface tension is determined from the circumference of the ring and weight of the liquid column.

$$\gamma_c = \frac{Mg}{2L}$$

γ_c = surface tension (mN/m)
M = mass of water (grams)
g = gravity (m/s^2)
L = circumference of ring (m)

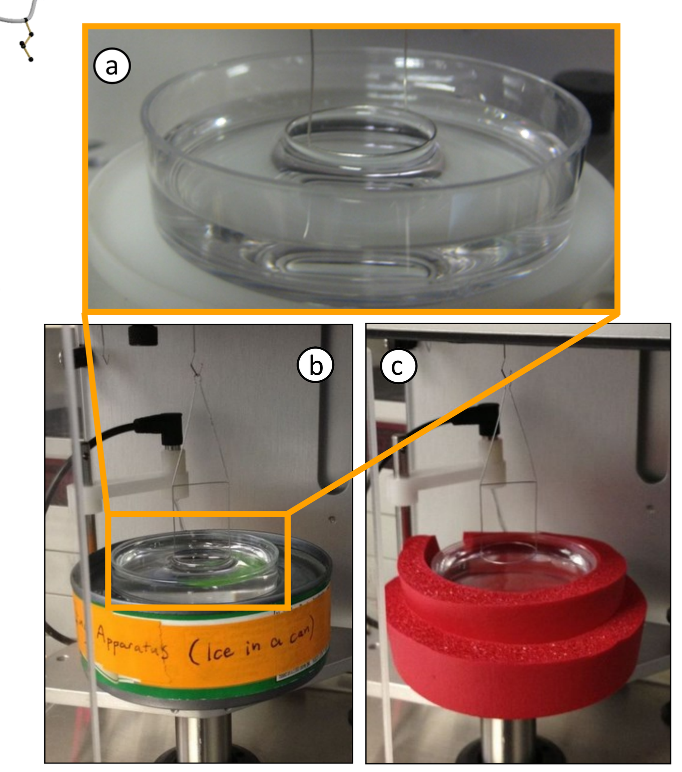
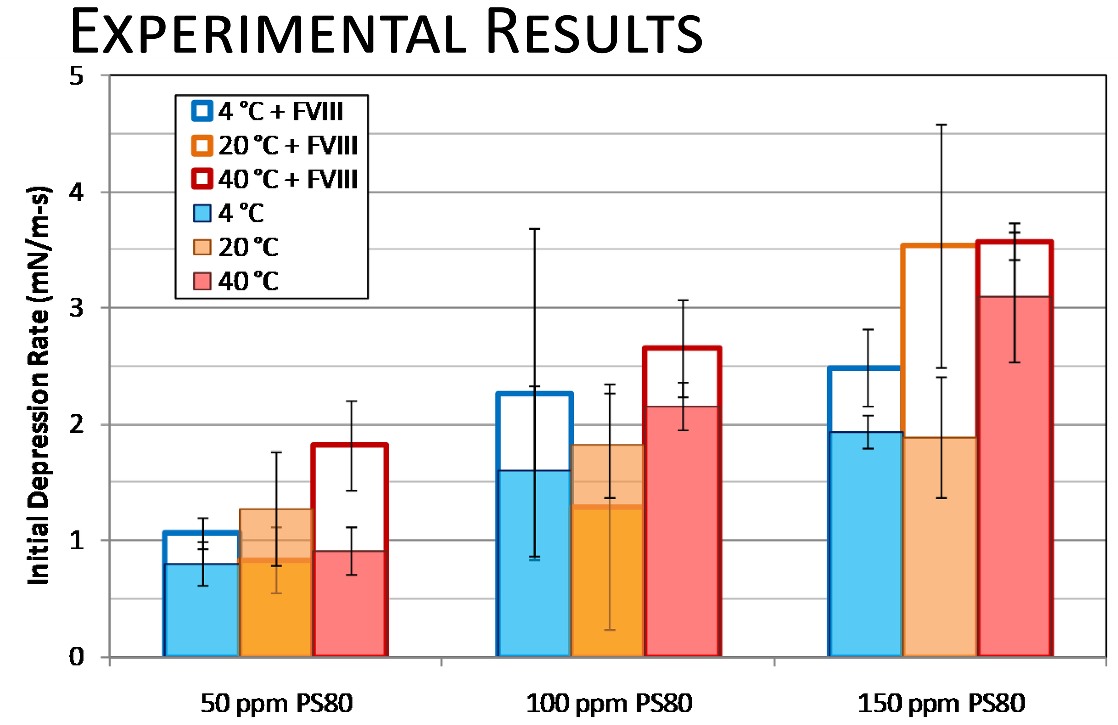


Figure 4: Photographs of the tensiometer with sample loaded for surface tension measurement, **a)** Close-up of the ring drawing up a column of liquid, **b)** The cooling apparatus used for 4°C trials and **c)** the insulated apparatus used for 40°C trials.

Experimental Results



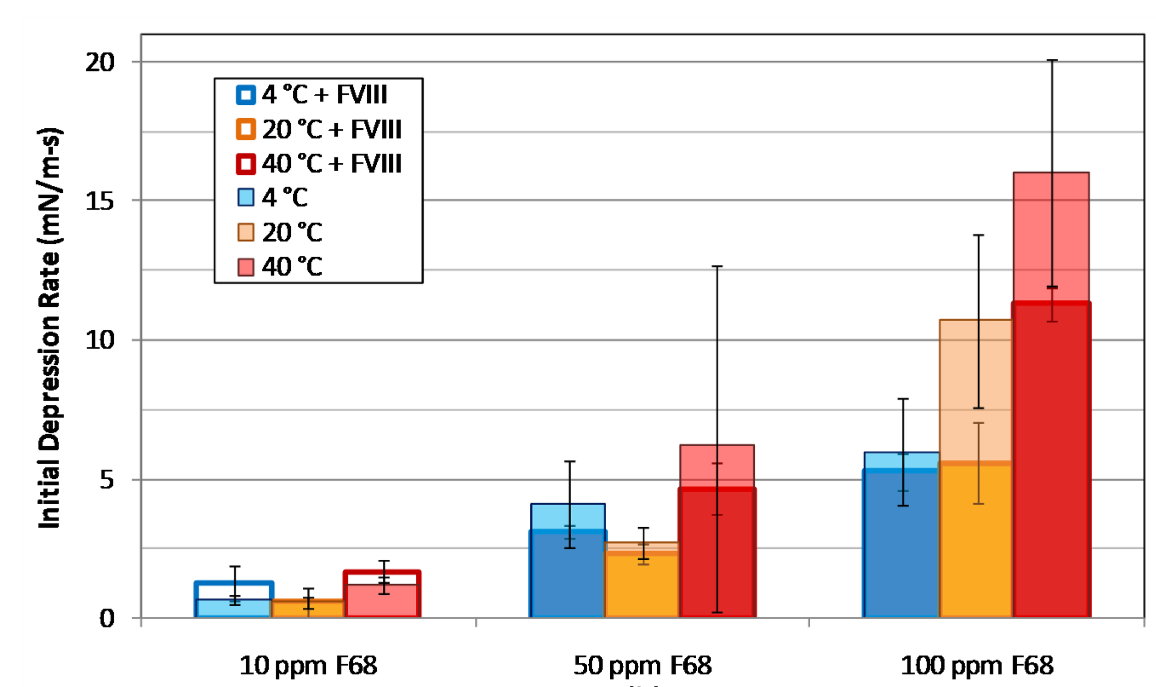


Figure 5: Initial rate of surface tension depression at various temperatures and surfactant concentrations. White space visible in the column indicates the rate of surface tension depression increased with addition of FVIII. Colored overlays indicate where the rate of surface tension depression was faster without FVIII.

a) PS80: The overall trend for PS80 was increased surface tension depression rate with addition of FVIII.

b) F-68: The overall trend for F-68 was steady or slightly reduced surface tension depression rate with addition of FVIII.

Data Analysis

Trial data, conducted in triplicate was fit with an exponential decay function to determine the initial rate of surface tension depression.

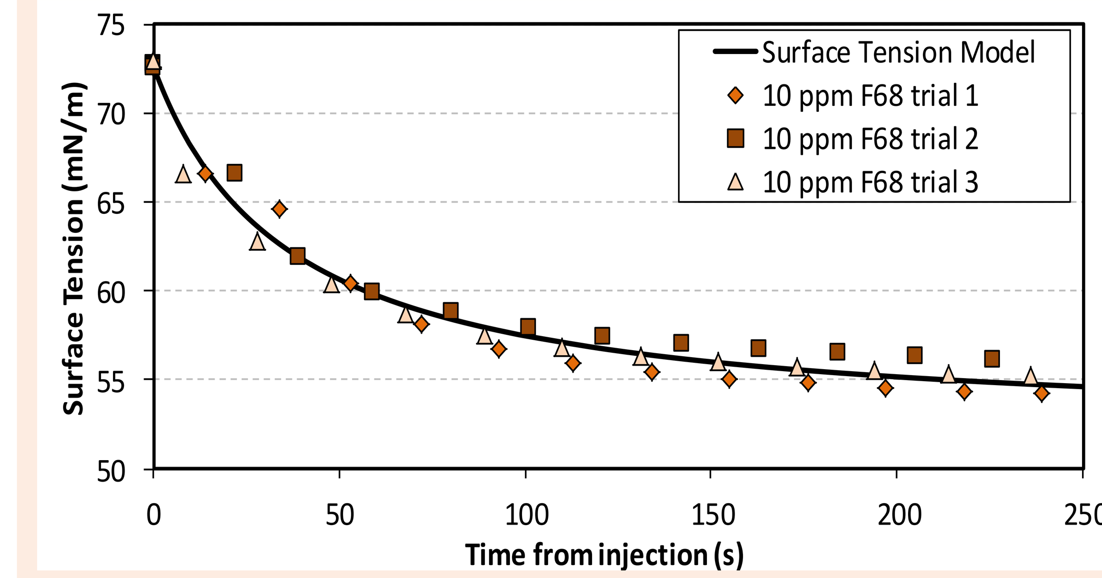


Figure 6: Surface tension depression of KG-2 buffer as a function of time after injection of 10 ppm Pluronic® F68.

Initial rate of surface tension depression was used to solve Equation 1 for the coefficient and order of reaction.

The reaction coefficient was used to calculate ΔH of adsorption from the slope of Equation 2, as depicted in Figure 7.

$$initial\ rate = k_m \cdot C^n \quad (1)$$

$$\ln\left(\frac{k_m}{T}\right) = -\left(\frac{\Delta H}{RT}\right) + \ln\left(\kappa \frac{k_b}{h}\right) + \frac{\Delta S}{R} \quad (2)$$

k_m = rate coefficient
C = surfactant concentration (mol/L)
n = reaction order
T = temperature (K)
R = gas constant (J/mol-K)
κ = proportionality constant
k_b=Boltzmann constant (m^{2}kg/s^2-K)
h= Planck's constant (m^{2}kg/s)
ΔH = enthalpy of adsorption (J/mol)
ΔS= entropy of adsorption (J/mol-K)

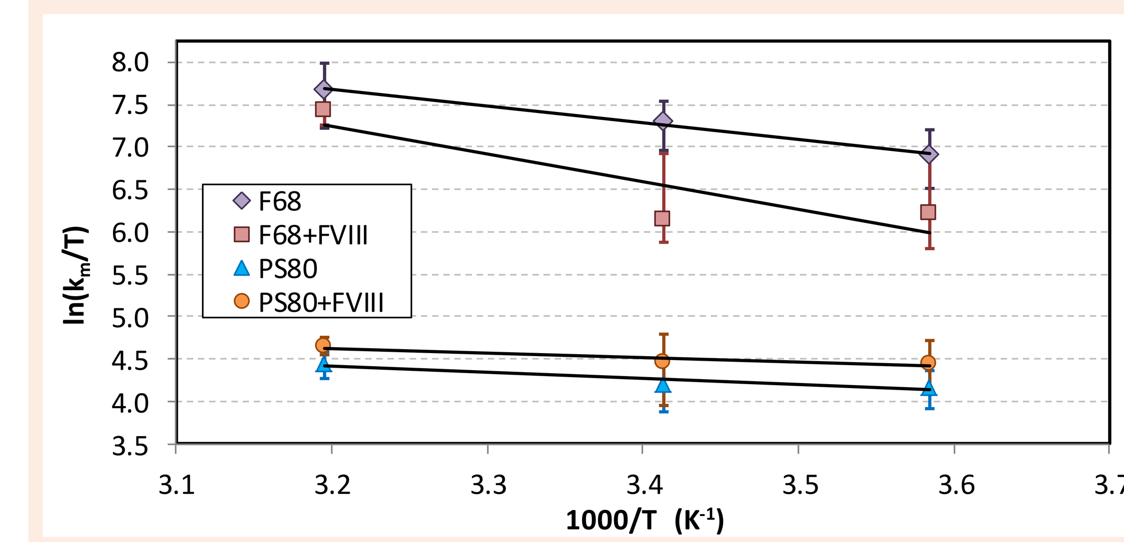


Figure 7: Linearized thermodynamic analysis of adsorption. The slope is used to calculate the enthalpy of adsorption (ΔH) for each surfactant and protein combination.

Experimental enthalpy of surfactant adsorption

ΔH_{PS80} = 5.9 ± 1.7 kJ/mol
$\Delta H_{PS80+FVIII}$ = 4.4 ± 6.0 kJ/mol

ΔH_{F68} = 16.2 ± 1.1 kJ/mol
$\Delta H_{F68+FVIII}$ = 27.0 ± 15.1 kJ/mol

Acknowledgements

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References

[1] http://en.wikipedia.org/wiki/File:Polysorbate_80.png
[2] http://depts.washington.edu/mednews/research/hemophilia.html

Conclusions

Formulation of FVIII in F-68 solution may significantly reduce processing losses by stabilizing FVIII in solution by directly adhering to the protein. Pluronic® F-68 adsorbed less quickly in the presence of FVIII and demonstrated an increased enthalpy of adsorption, corresponding to a high affinity for FVIII. Polysorbate 80 adsorbed more quickly in the presence of FVIII, corresponding to a higher affinity for the surface than protein. Protein activity must be assessed in Pluronic® F-68 solutions before changing surfactants.

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