Albumin-induced inactivation of lung surfactants

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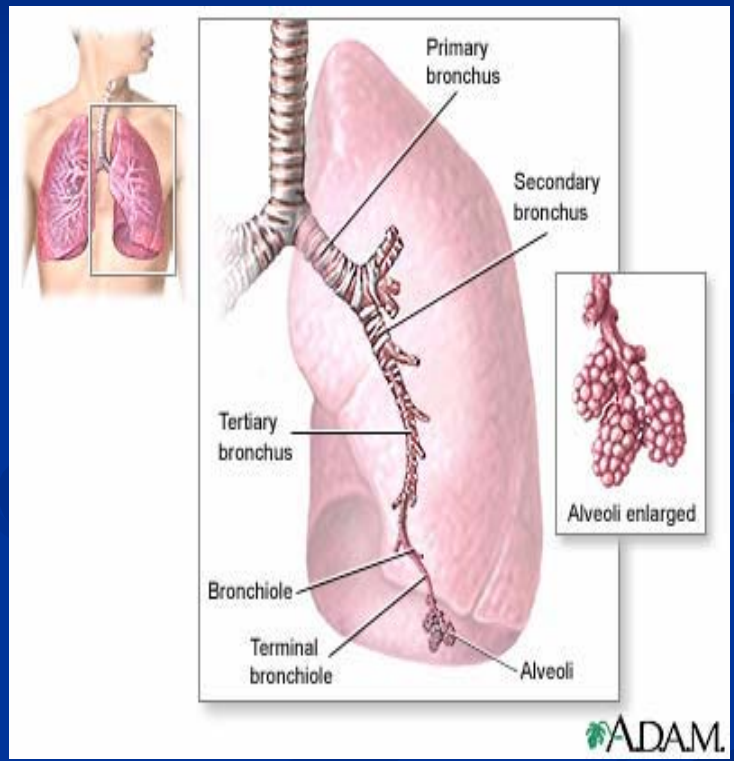
Funding: NIH

What is lung surfactant?

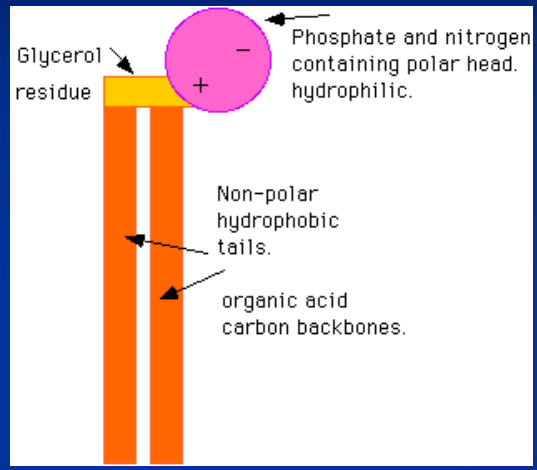
- Lipid and protein mixture lining the 600 million alveoli of the lungs
- LS modulates surface tension during breathing cycle; prevents alveolar collapse
- Reduces work of breathing
- Prevents water droplets from blocking airways

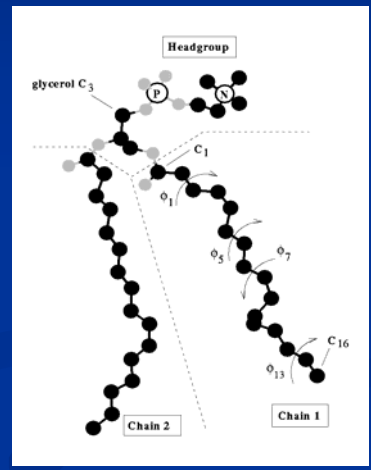


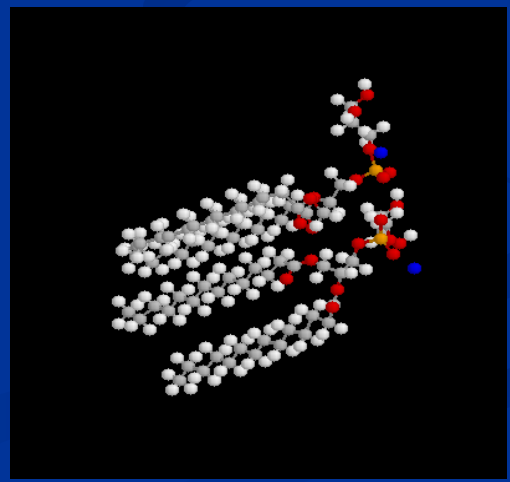
http://lungdiseases.about.com/od/generalinformation1/ss/resp_sys_tour_5.htm

Components of lung surfactant

- 35-40% dipalmitoyl phosphatidylcholine (DPPC), a phospholipid
- 30-45% other phospholipids
- 5-10% protein (SP-A, B, C, D)
- Cholesterols (neutral lipids)







http://persweb.wabash.edu/facstaff/fellers/image.html

What is surface tension?

- Tendency of molecules in a fluid to be pulled toward the center of the fluid
- Measured as energy/unit area (J/m^2) or force across a line (N/m)
- Surface tension of water is 72 mN/m; with LS can drop to 2mN/m or lower
- Surface pressure (Π) = amount surface tension (σ) is lowered by surfactant film ($\Pi = \sigma^\circ - \sigma$)

Notter, Robert. Lung Surfactants: basic science and clinical applications. *Lung Biology in Health and Disease* volume 149

Background

NRDS

- Premature infants lack LS in NRDS (reduced lung compliance and oxygenation)
- Replacement LS reduces infant mortality due to NRDS from 165.2/100,000 (1979) to 24.6/100,000 (2000)
- FDA-approved LS includes Survanta (bovine), Curosurf (porcine), and Infasurf (calf)

ARDS

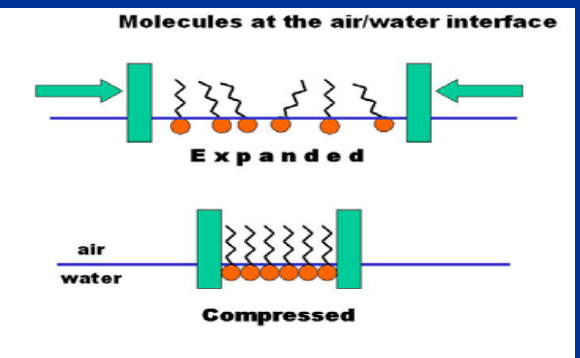
- ARDS 1.5-8.4 cases/100,000; mortality 30%
- Variety of causes results in inactivation of LS
- LS replacement unsuccessful in treating patients
- Surface-active albumin (serum protein) is elevated in alveolar fluid of ARDS patients

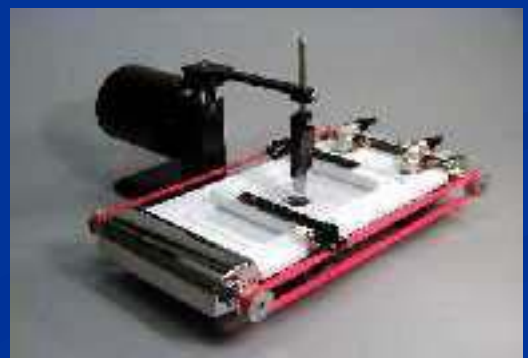
Goals of project

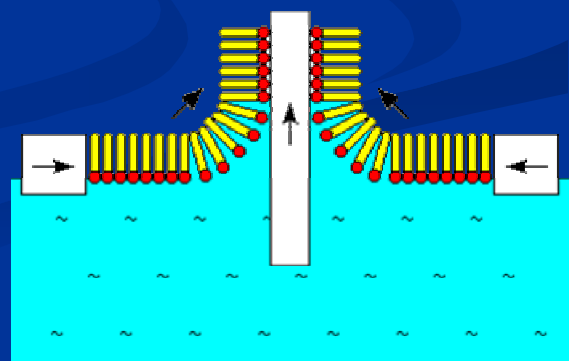
- Identify a repeatable experiment to determine effect of albumin on model LS
- Explore different aspects of adsorption to interface
- Long term goal: design synthetic replacement LS

Methods: How we study surface tension

- Langmuir-Wilhelmy force balance and Teflon trough with expandable barriers
- Isotherms – varies pressure by expanding and compressing barriers to study behavior of surfactants
- Surfactant is spread on an aqueous subphase (from solvent or from solution)







Procedures

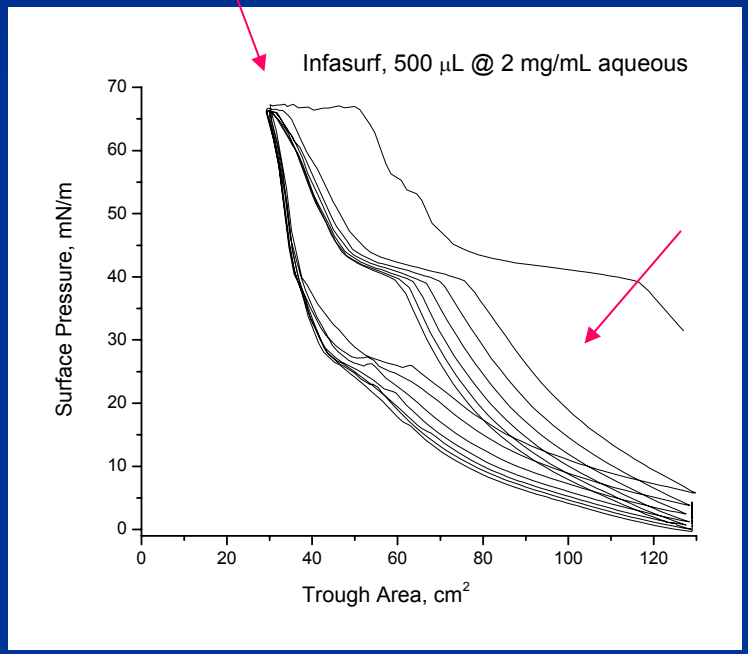
Comparison of three different LS

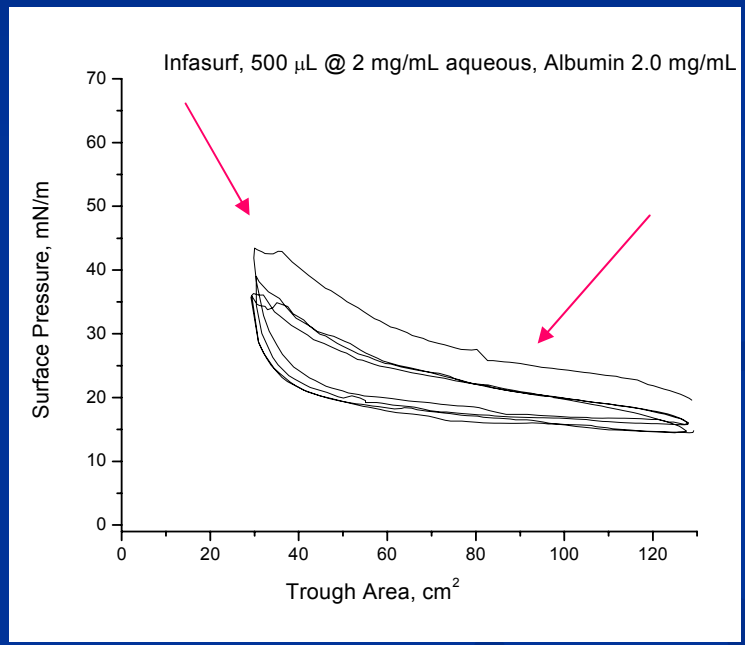
1) 500uL Infasurf suspension

2) 20uL/7.5uL Infasurf, lyophilized and reconstituted in 2:1 $CHCl_3$:MeOH
20 components (complex model including proteins)

3) 6uL simulated Infasurf (in $CHCL_3$:MeOH)
lipids only, (simple model)

Results: Infasurf (aq) and inactivation

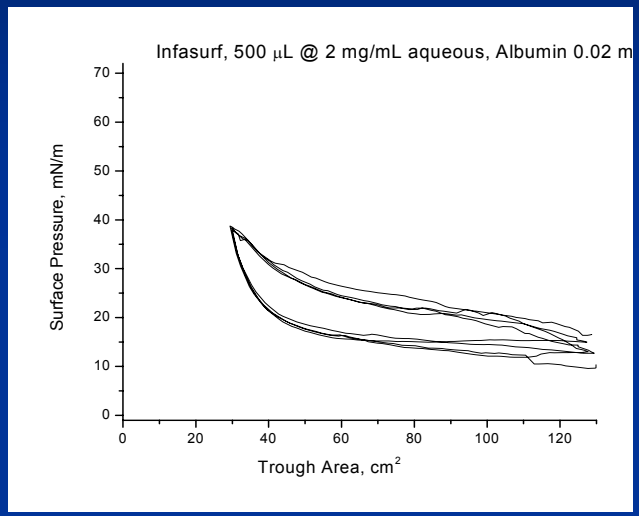


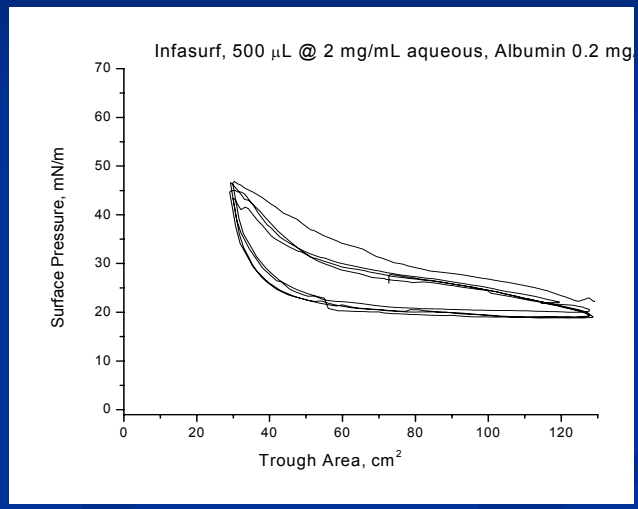


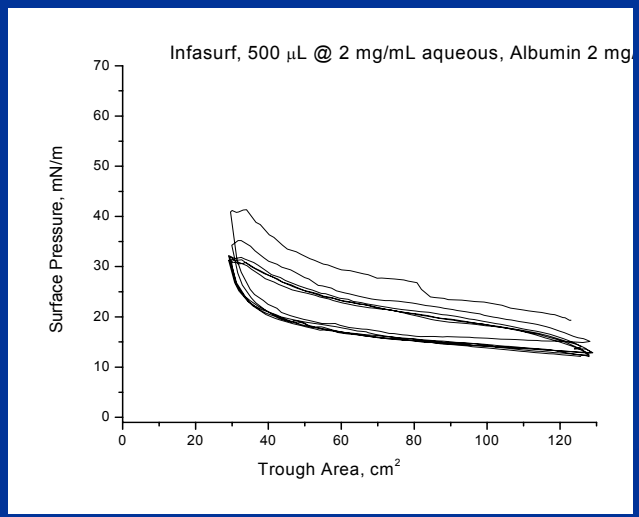
Inhibition results in: 1) lower surface pressure (inhibiting LS to lower surface tension)
2) low plateau

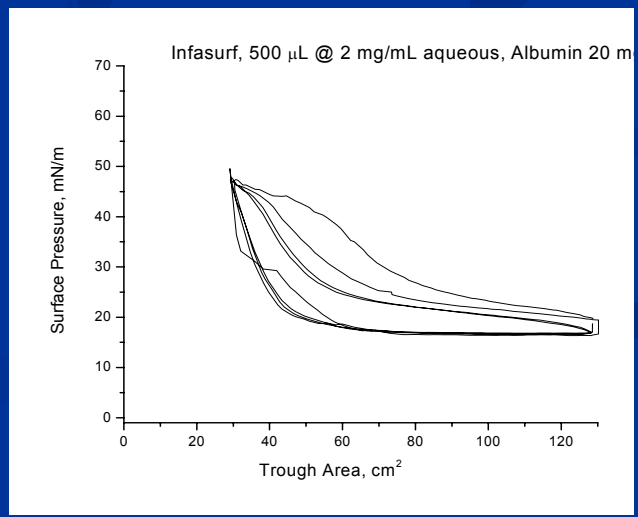
Albumin concentration study

Inconsistency resulting from suspension delivery (expect concentration dependency)

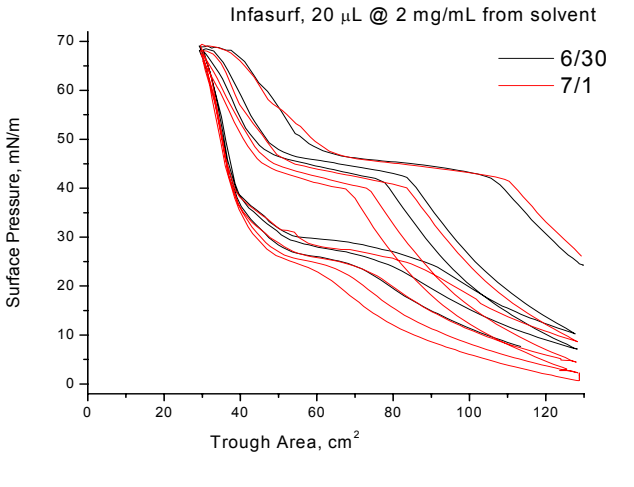


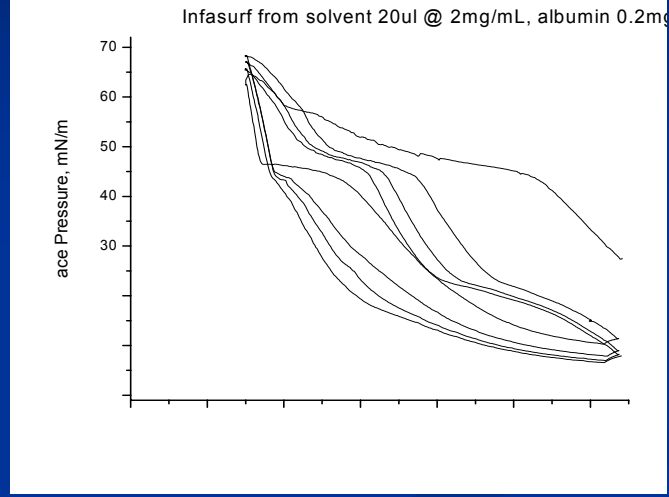


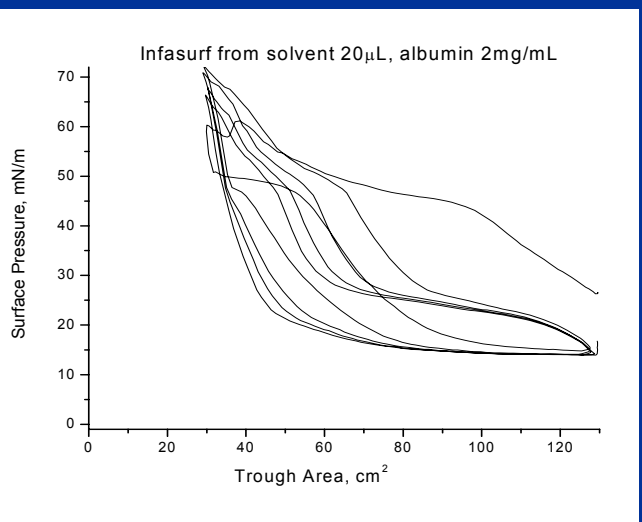


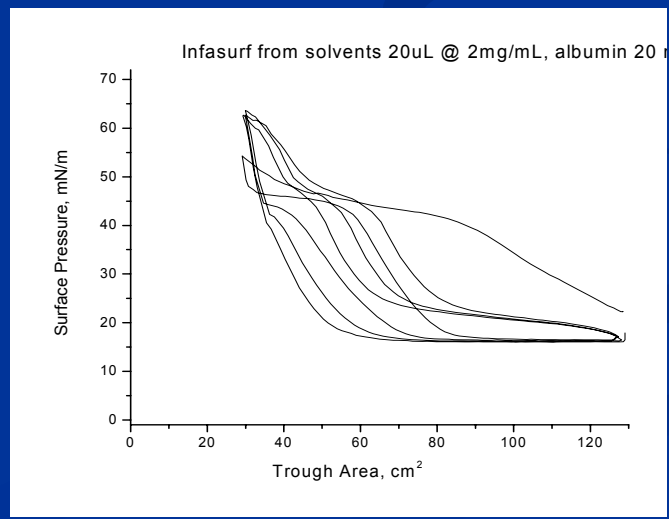


Infasurf in solvent



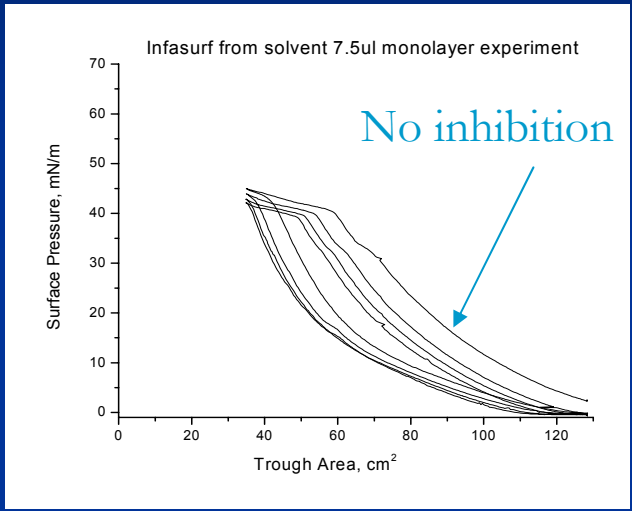


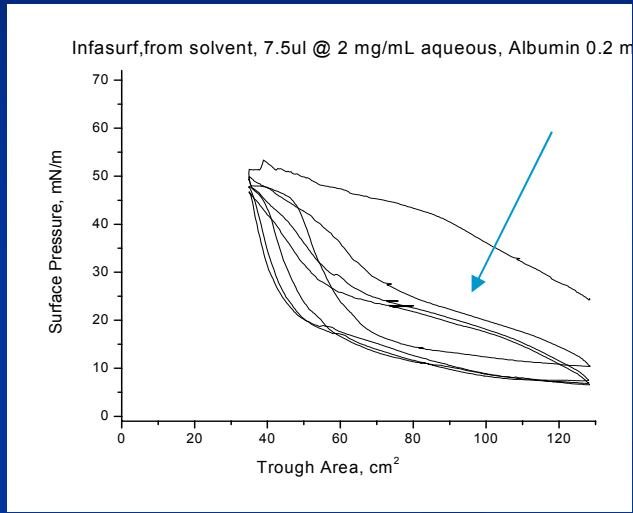


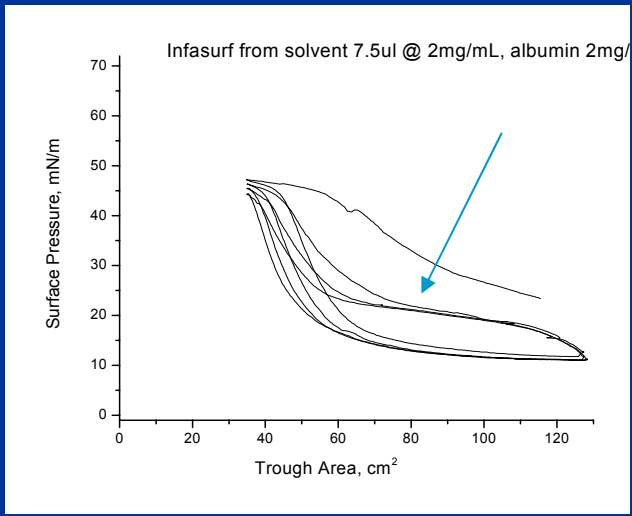


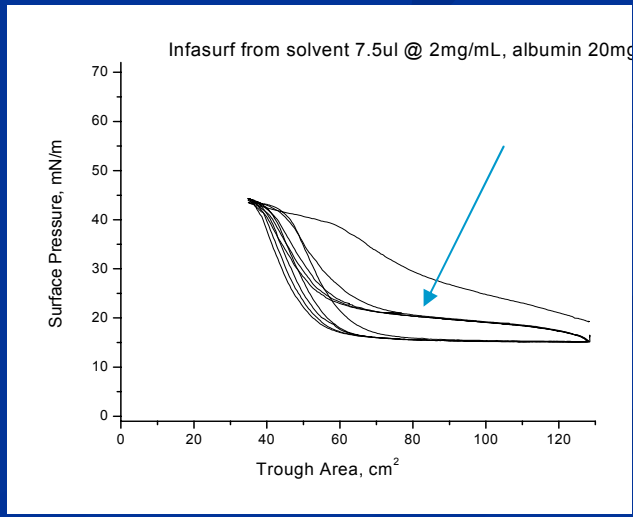
- Same effect in small doses vs. aqueous
- too much Infasurf at or near surface for albumin to interfere
- Repeatable but first cycle is hard to understand

Infasurf (solvent) monolayer and inactivation experiment





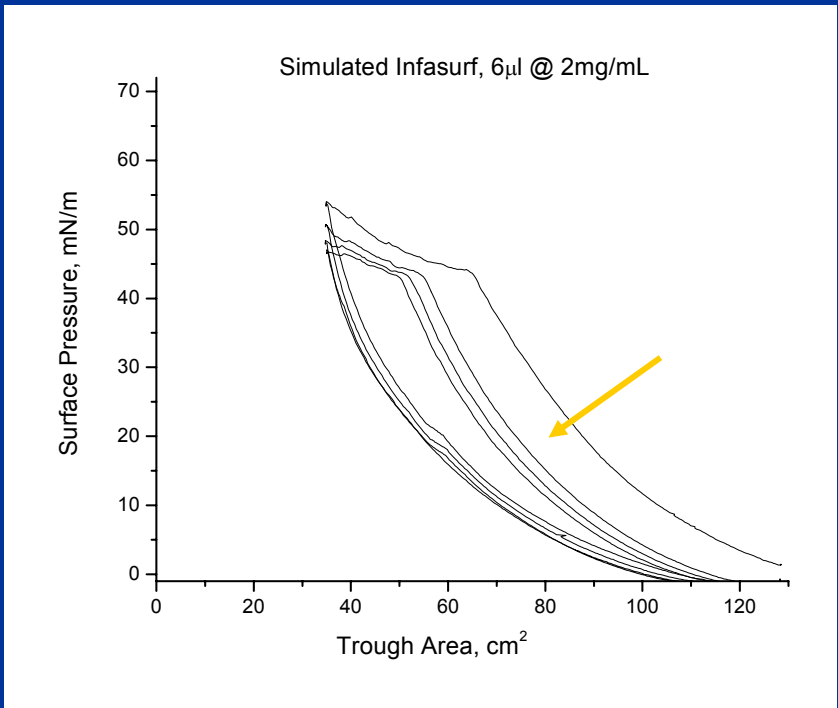


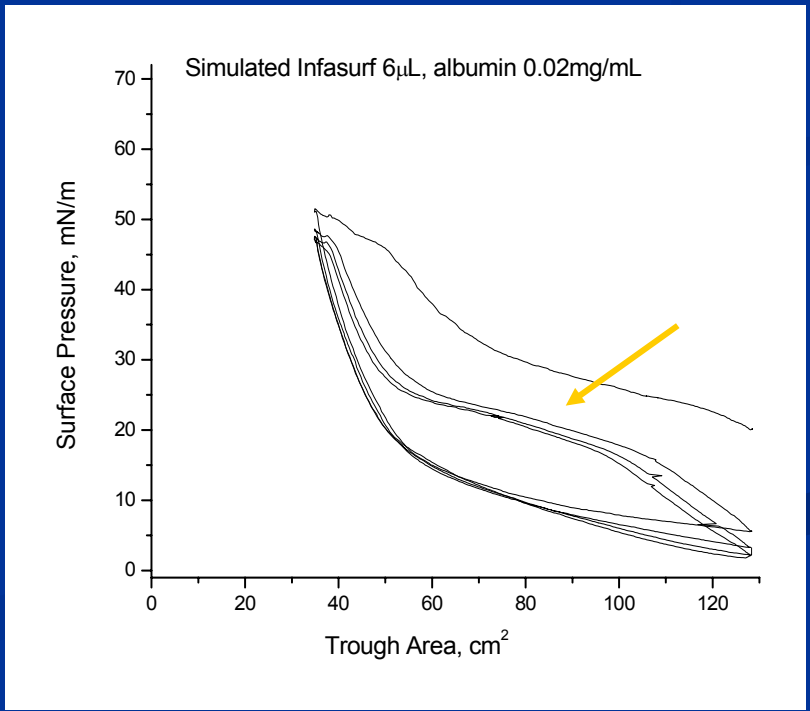


- Based on minimum molecular area (40A^2/mol), 7.5uL is a monolayer
- Lose plateau at 40mN/m
- Compare 2nd cycles, 1st cycles are odd

Simulated Infasurf and inhibition

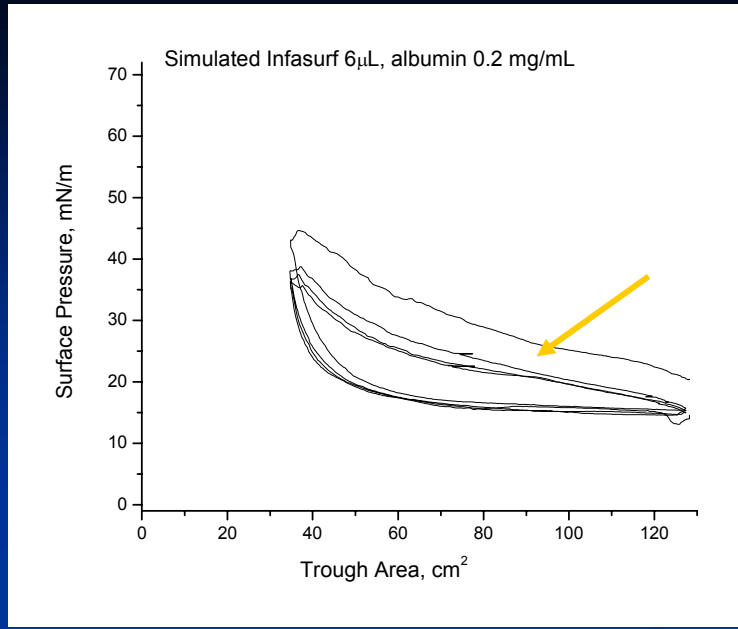
Components: 45.7% DPPC
28.6% POPC
17.1% POPG
2.9% POPE
5.7% Cholesterol

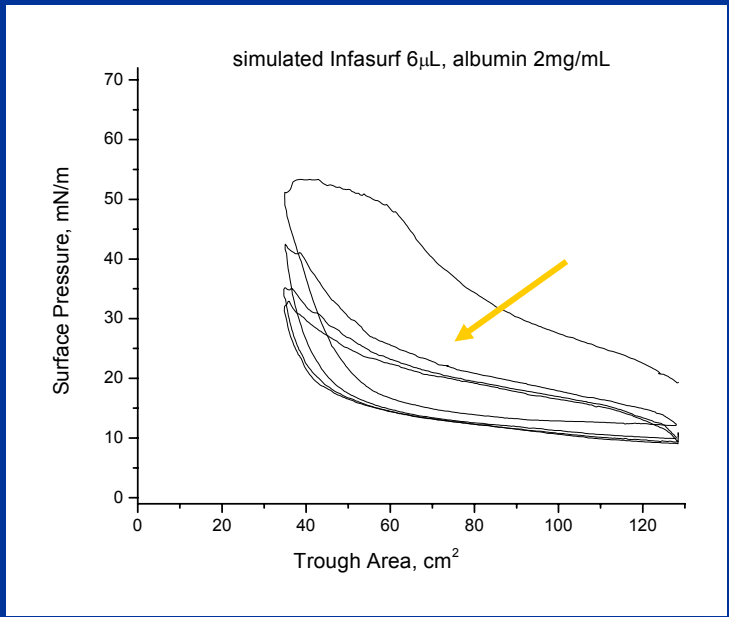


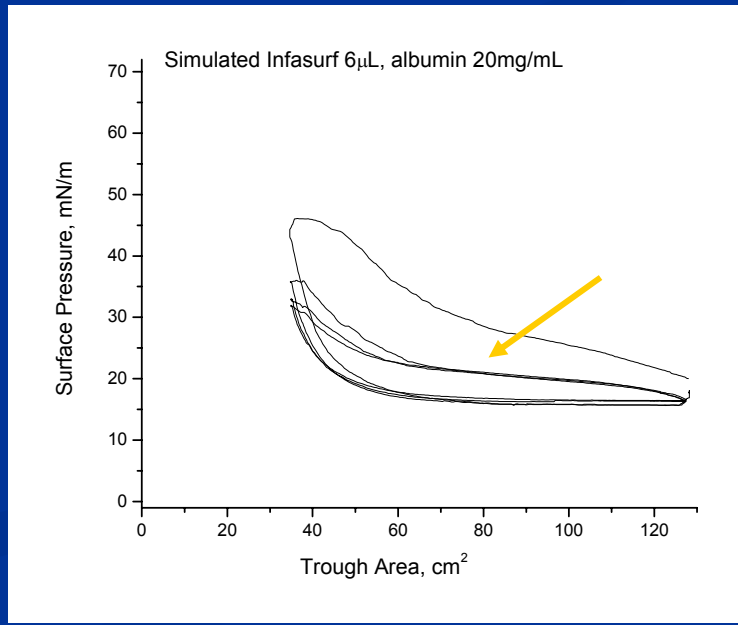


Concentration inhibition study:

- max surface pressures decrease with increasing [albumin]
- plateau is lower with increasing [albumin]







Conclusions

- Infasurf inactivated entirely when spread by aqueous - (1000 μg)
- Infasurf not inactivated when spread from solvent (large concentrations) – (40 μg)
- Infasurf somewhat inactivated when spread from solvent (small concentrations)
- Simulated Infasurf lipids much more sensitive to Albumin → proteins prevent inactivation