

The Role of Cholesterol in Lung Surfactant

Elizabeth Eck

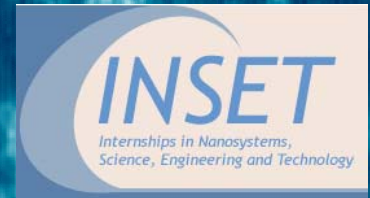
Biology

Santa Barbara City College

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Faculty Advisor: Prof. Joseph A. Zasadzinski

Department of Chemical Engineering



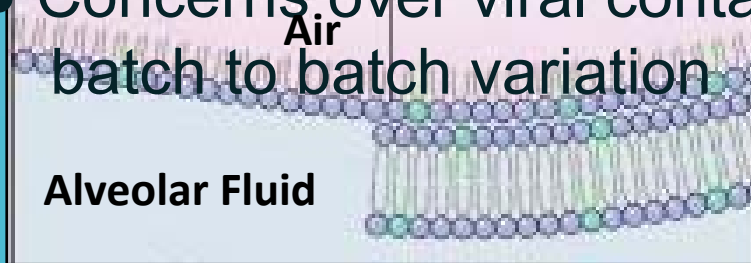
Background photo courtesy of Prajnaparamita Dhar

Lung Surfactant

Function: lowers surface tension inside the lungs

Deficiency or Dysfunction leads to disease

- Neonatal Respiratory Distress Syndrome (NRDS)
- **Animal Derived Surfactant**
 - Acute Respiratory Distress Syndrome (ARDS)
 - **Successful treating NRDS not ARDS**
 - **Concerns over viral contamination, batch to batch variation**



Air-water Interface

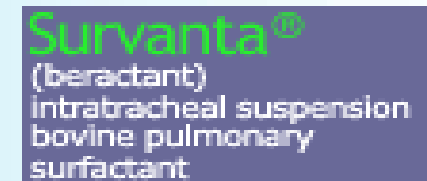
Courtesy of Zasadzinski Lab



Goal: Determine optimum composition of lung surfactant which results in the best performance

Cholesterol

- Present in native lung surfactant: 3-10% by weight
- Function is unknown
- ARDS associated with high levels of cholesterol: $\geq 20\%$ by weight
- Carefully removed from most replacement surfactants

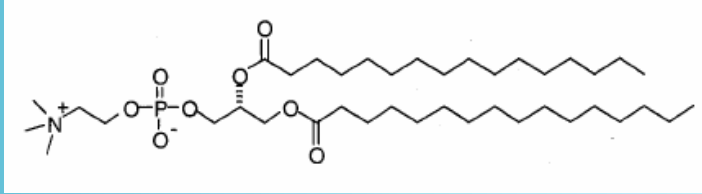


	Native Surfactant	Curosurf	Infasurf	Survanta
Cholesterol (Avg. % by weight)	5	0	5	0

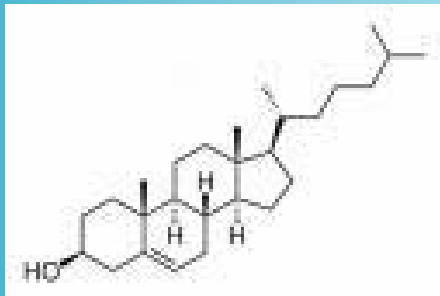
Braun et al., Biophys. J. 93 (2007)

Components of Lung Surfactant

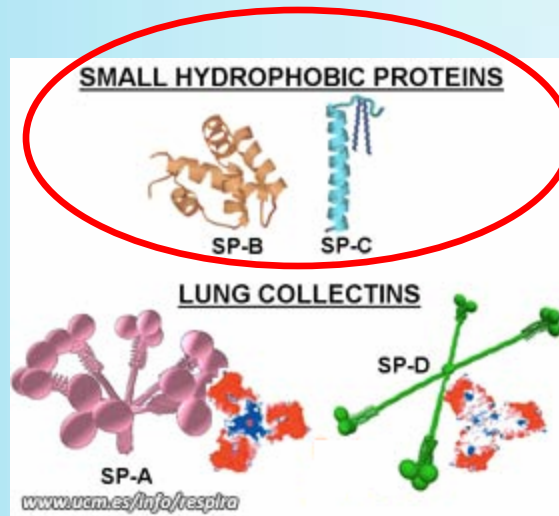
Dipalmitoylphosphatidylcholine (DPPC)



Phospholipids
80-90%



Cholesterol
3-10% in native
surfactant



Proteins
5-10%

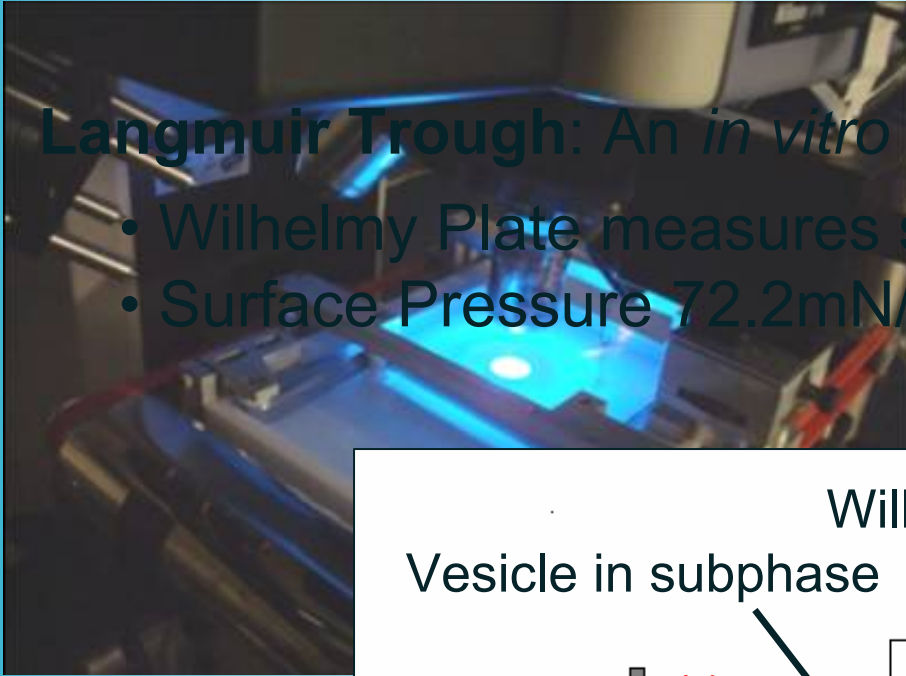


abbott.co.in

Phospholipids: <http://dl.clackamas.edu>

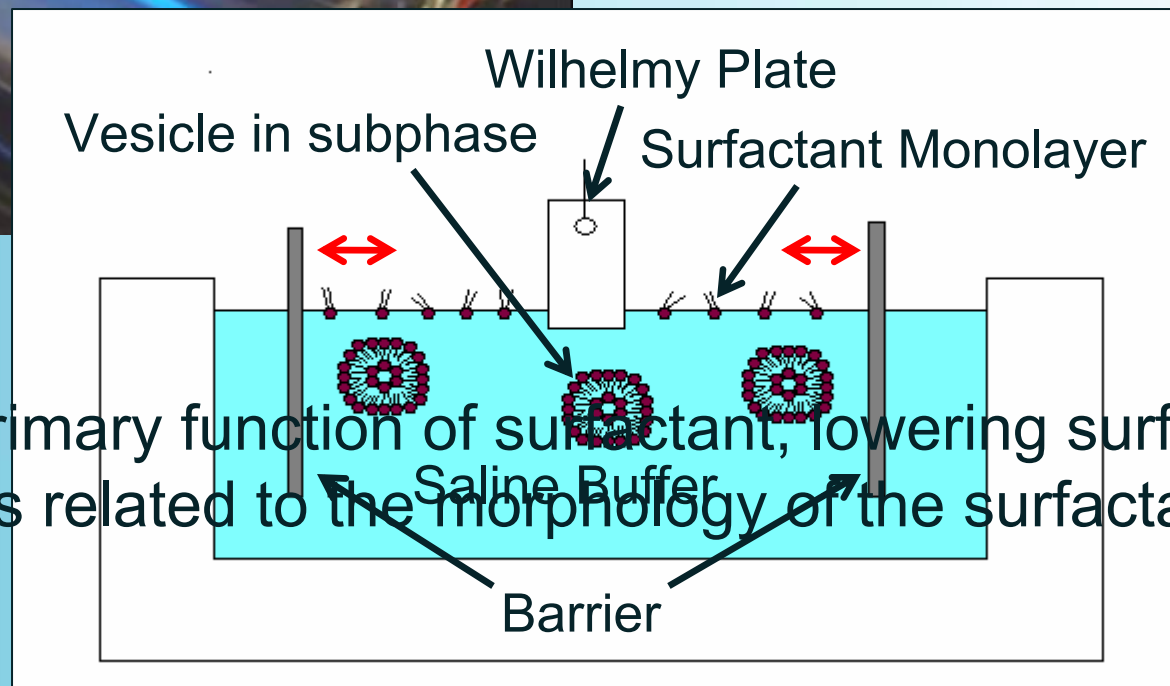
Cholesterol: <http://commons.wikimedia.org>

Langmuir Trough & Fluorescence Microscope



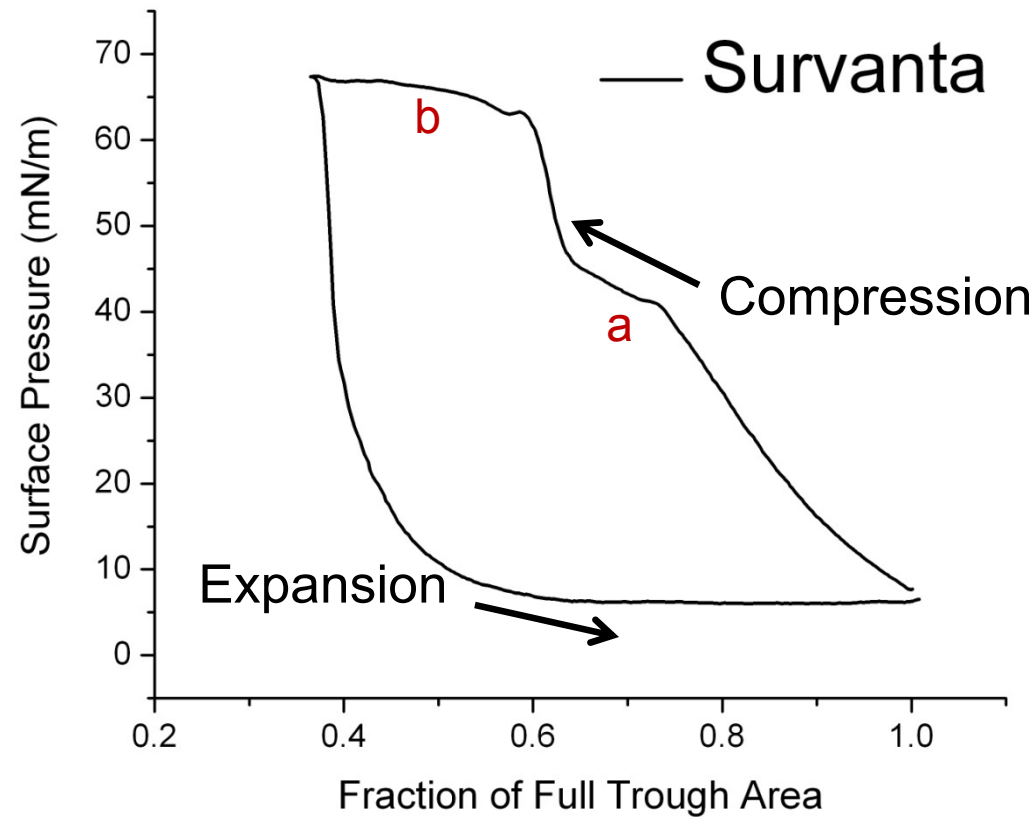
Langmuir Trough: An *in vitro* model of the breathing cycle

- Wilhelmy Plate measures surface pressure
- Surface Pressure 72.2mN/m = Surface Tension 0mN/m



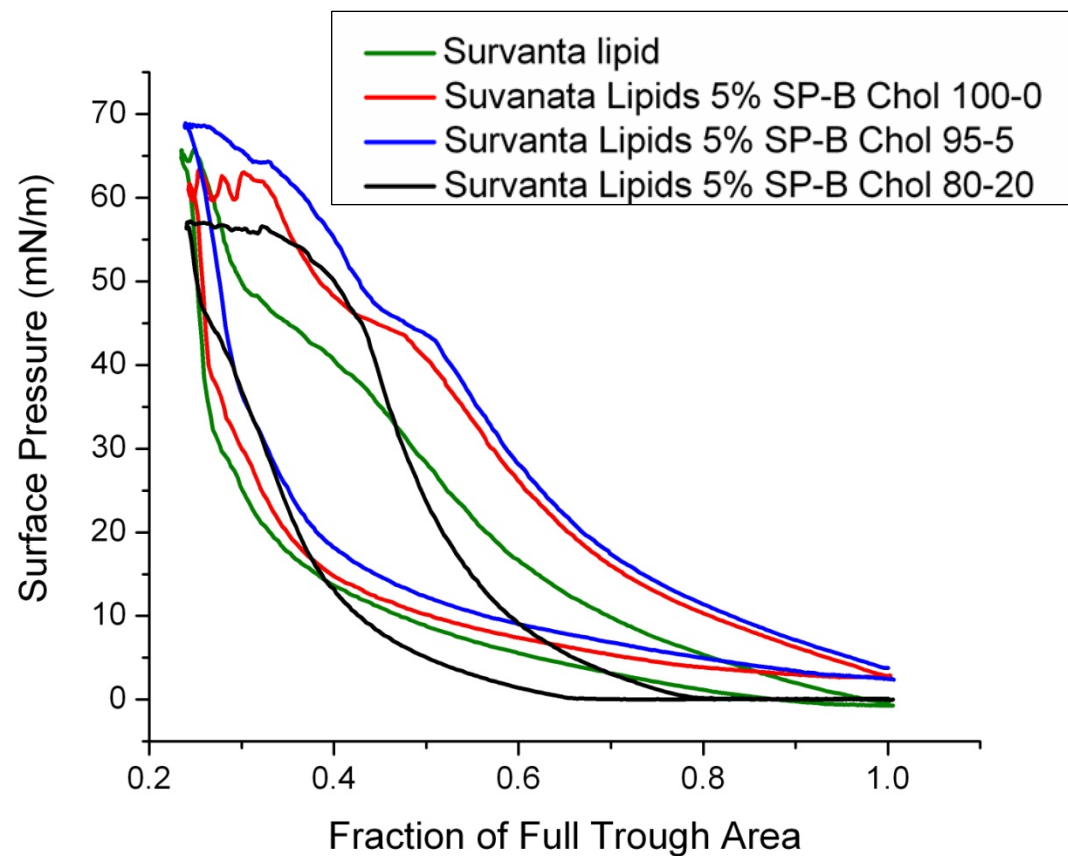
The primary function of surfactant, lowering surface tension, is related to the morphology of the surfactant film

Survanta Isotherm



Characteristics: Shoulder - **a**
Collapse plateau - **b**
High surface pressure achieved

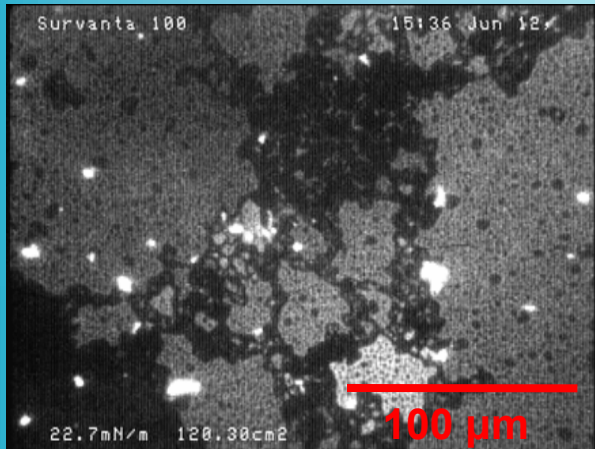
Survanta Lipids with 5% SP-B



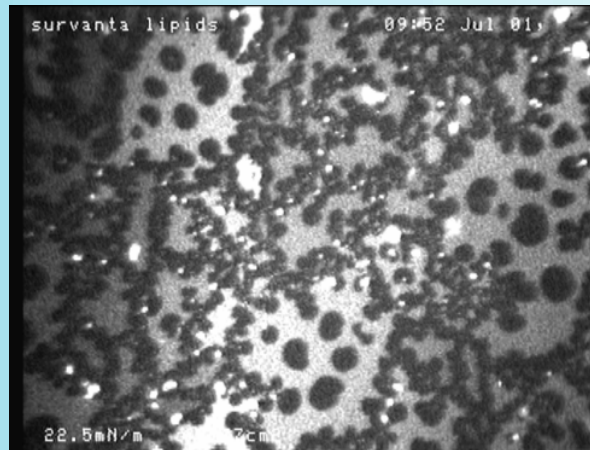
- 5% SP-B increases absorption but does not reach higher Surface Pressure
- 5% Cholesterol improves surfactant function
- 20% is detrimental

Fluorescence Microscope Images

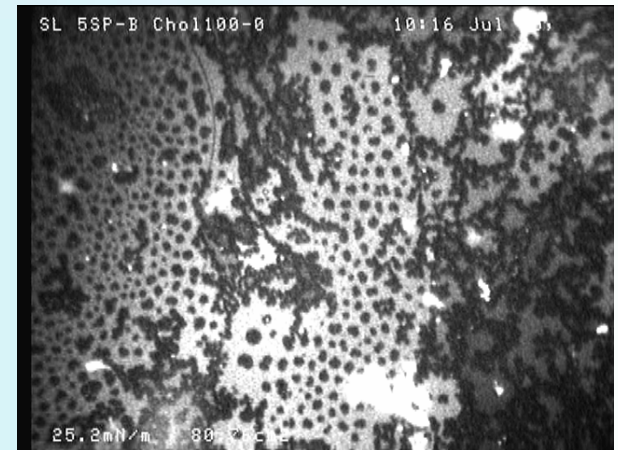
25mN/m



Survanta



Survanta Lipids (SL)



SL + 5% SP-B



SL + 5% SP-B +
5% Cholesterol

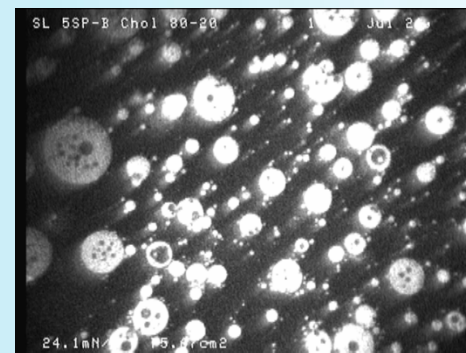
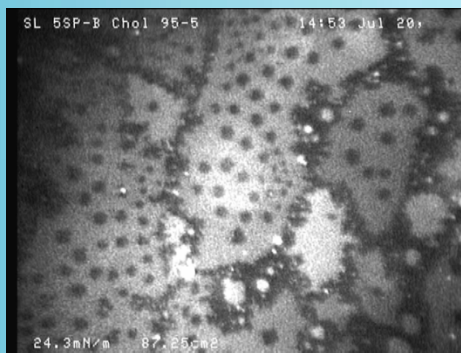
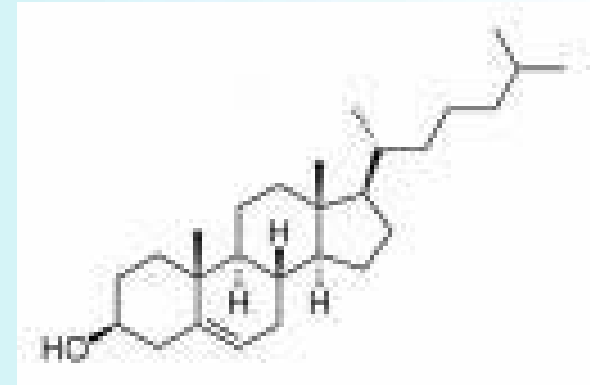


SL + 5% SP-B +
20% Cholesterol

Phase reversal:
Veatch & Keller Phys. Rev. Lett. 2002
Radhakrishnan & McConnell Biochemistry 2002
Keller et al. J. Phys. Chem. 2000

Conclusion

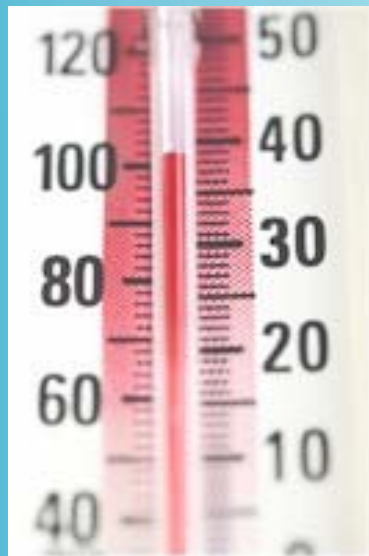
- SP-B improved absorption of a protein deficient surfactant
- 5% Cholesterol improved surfactant function
- 20% Cholesterol was detrimental to surfactant function
- Indicates optimum cholesterol concentration for most efficient function of lung surfactant exists



Images reinforce findings

Future Research

- Investigate interaction of cholesterol and SP-C
- Investigate interactions of cholesterol with SP-B and SP-C



- Additional concentrations of cholesterol
- Run experiments at physiological temperature

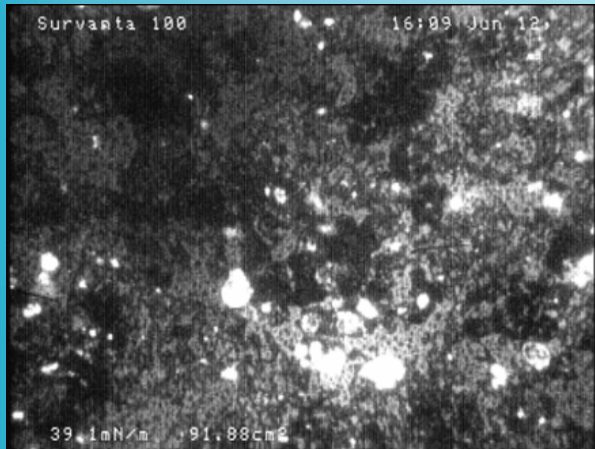
Thank You

- Prof. Zasadzinski and Zasadzinski Group members
- Dr. Prajnaparamita Dhar
- Research collaborator:
 Prof. Alan Waring, UCLA Medical Center
- INSET including:
 Nicholas Arnold, Jens-Uwe Kuhn, Olfelia Aguirre
- SBCC including:
 Profs. Bob Cummings, Blake Barron, Sally Ghizzoni
- Cottage Hospital
- Funding:

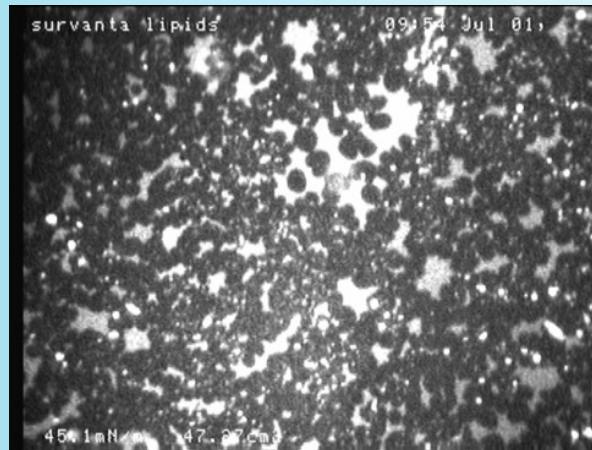


Fluorescence Microscope Images

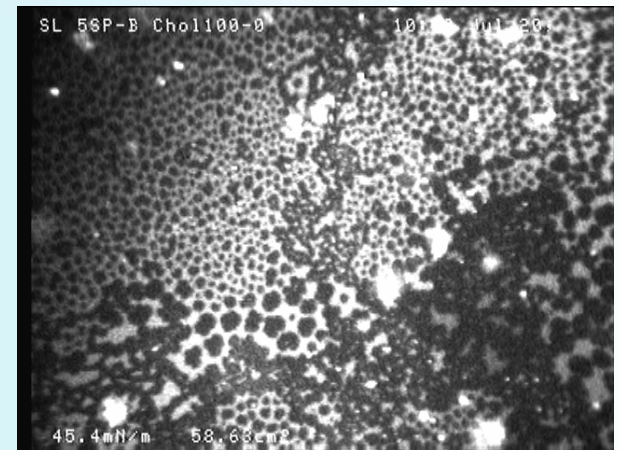
45mN/m



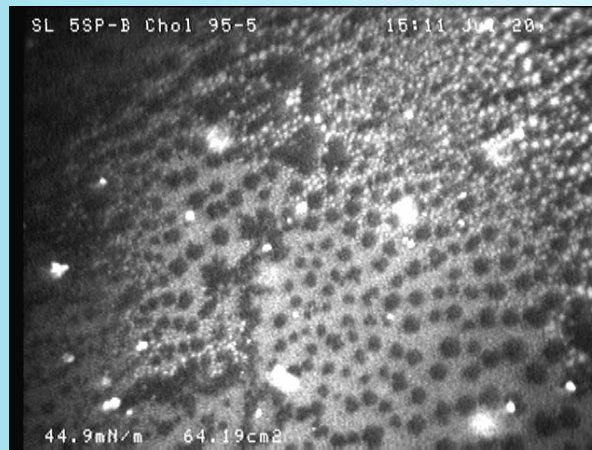
Survanta



Survanta Lipids (SL)



SL + 5% SP-B



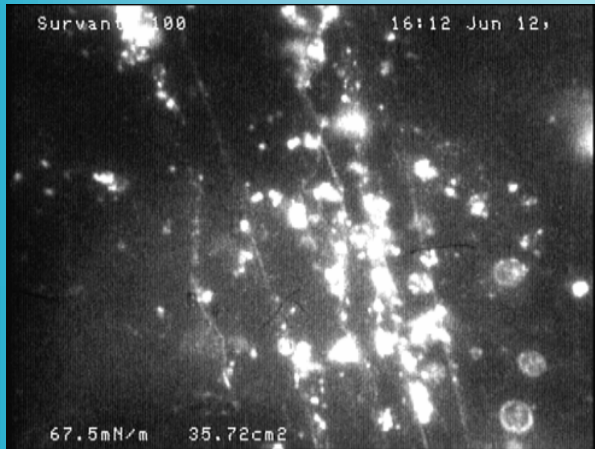
SL + 5% SP-B +
5% Cholesterol



SL + 5% SP-B +
20% Cholesterol

Fluorescence Microscope Images

Collapse



Survanta



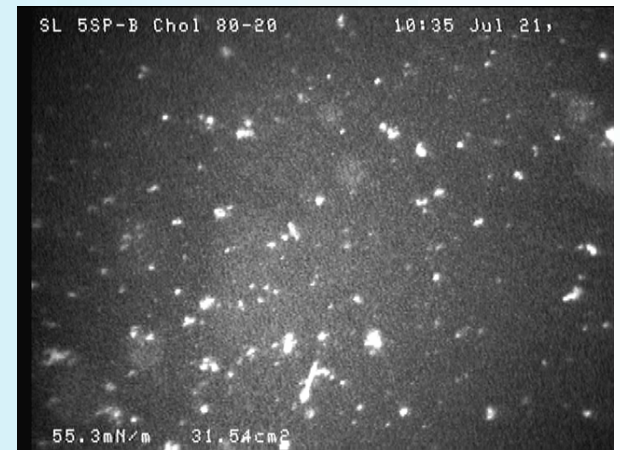
Survanta Lipids (SL)



SL + 5% SP-B

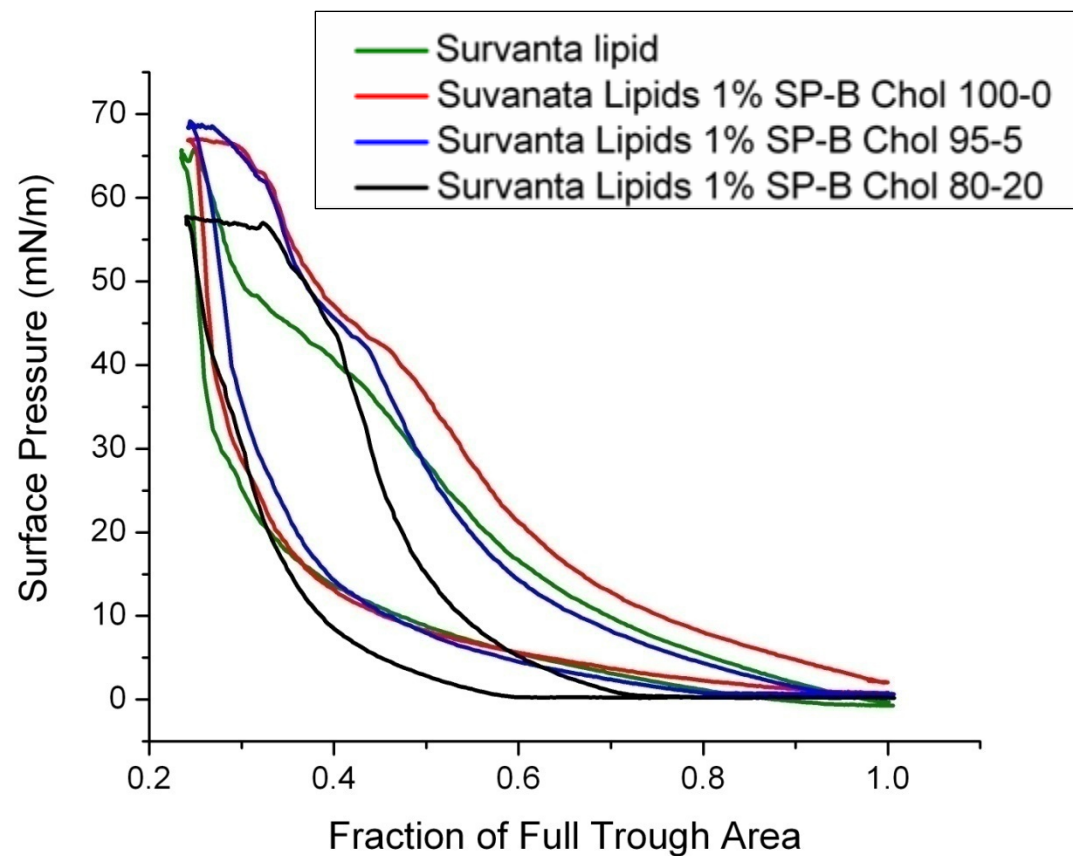


SL + 5% SP-B +
5% Cholesterol



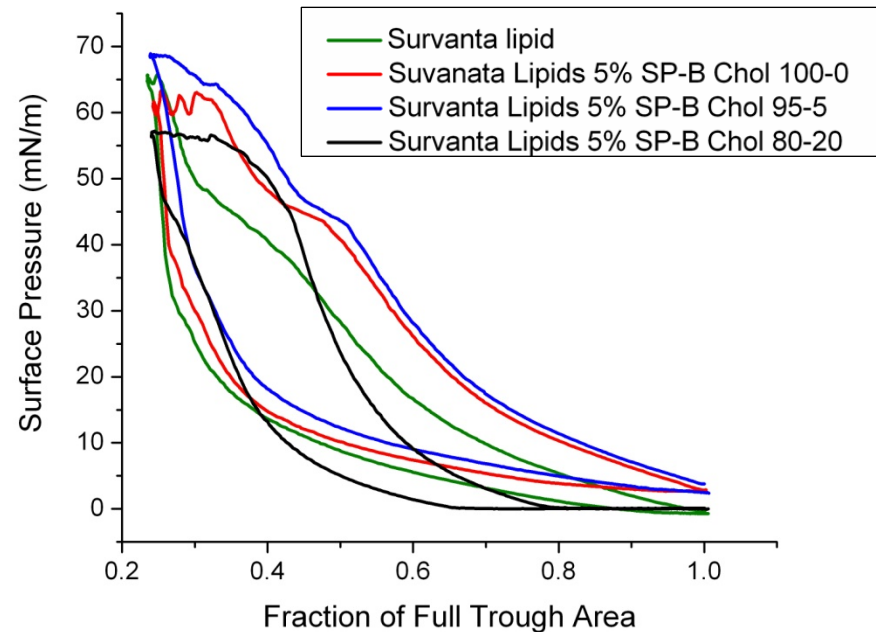
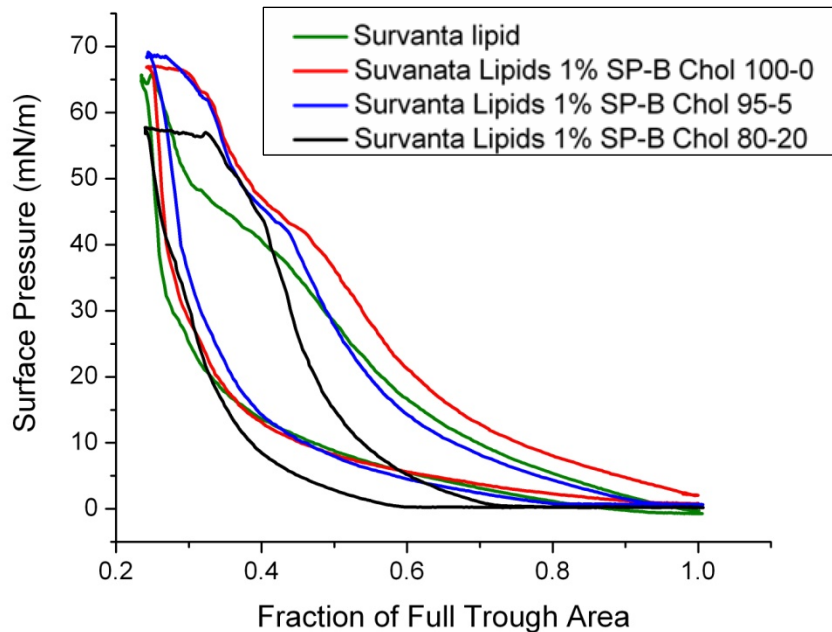
SL + 5% SP-B +
20% Cholesterol

Survanta Lipids with 1% SP-B

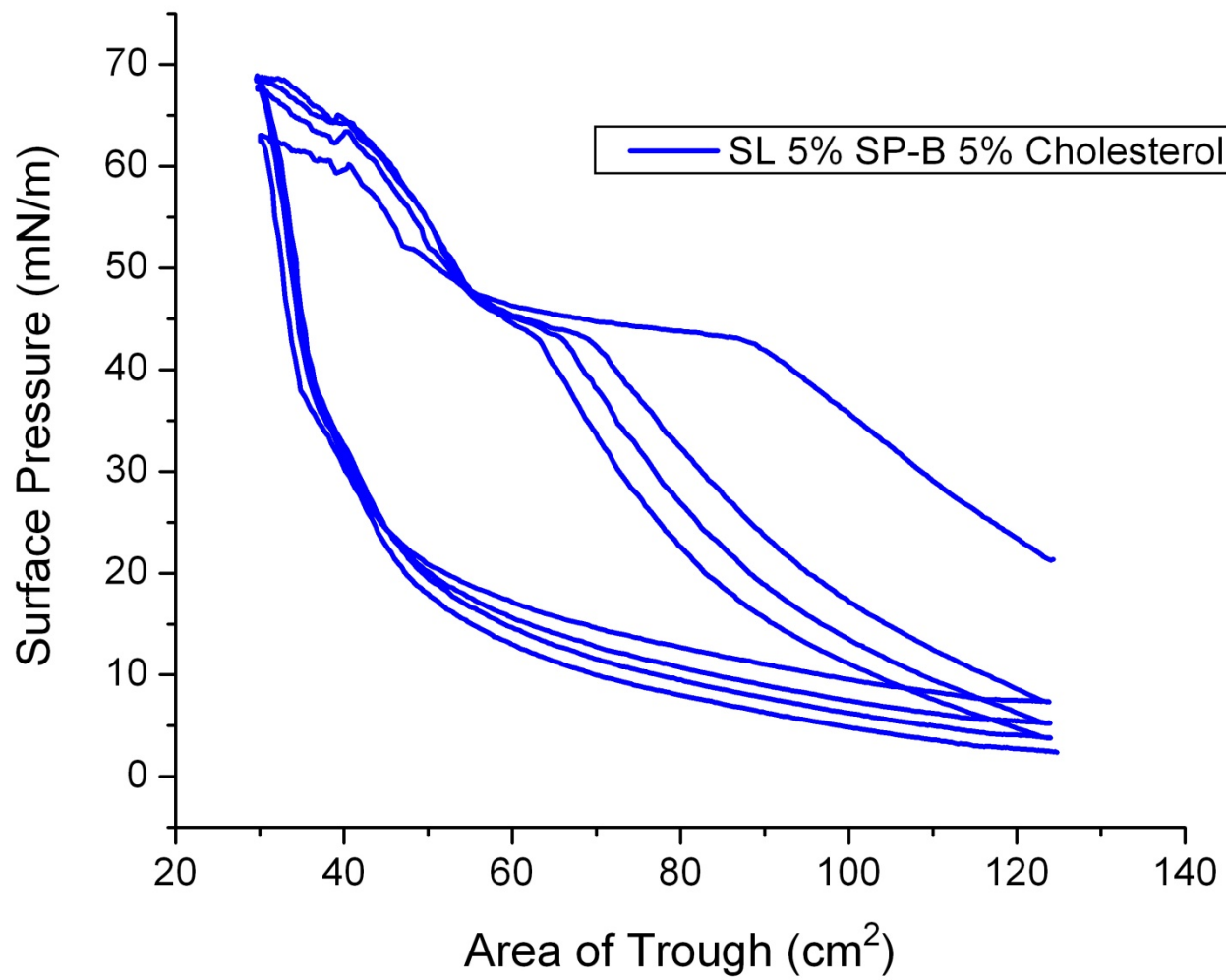


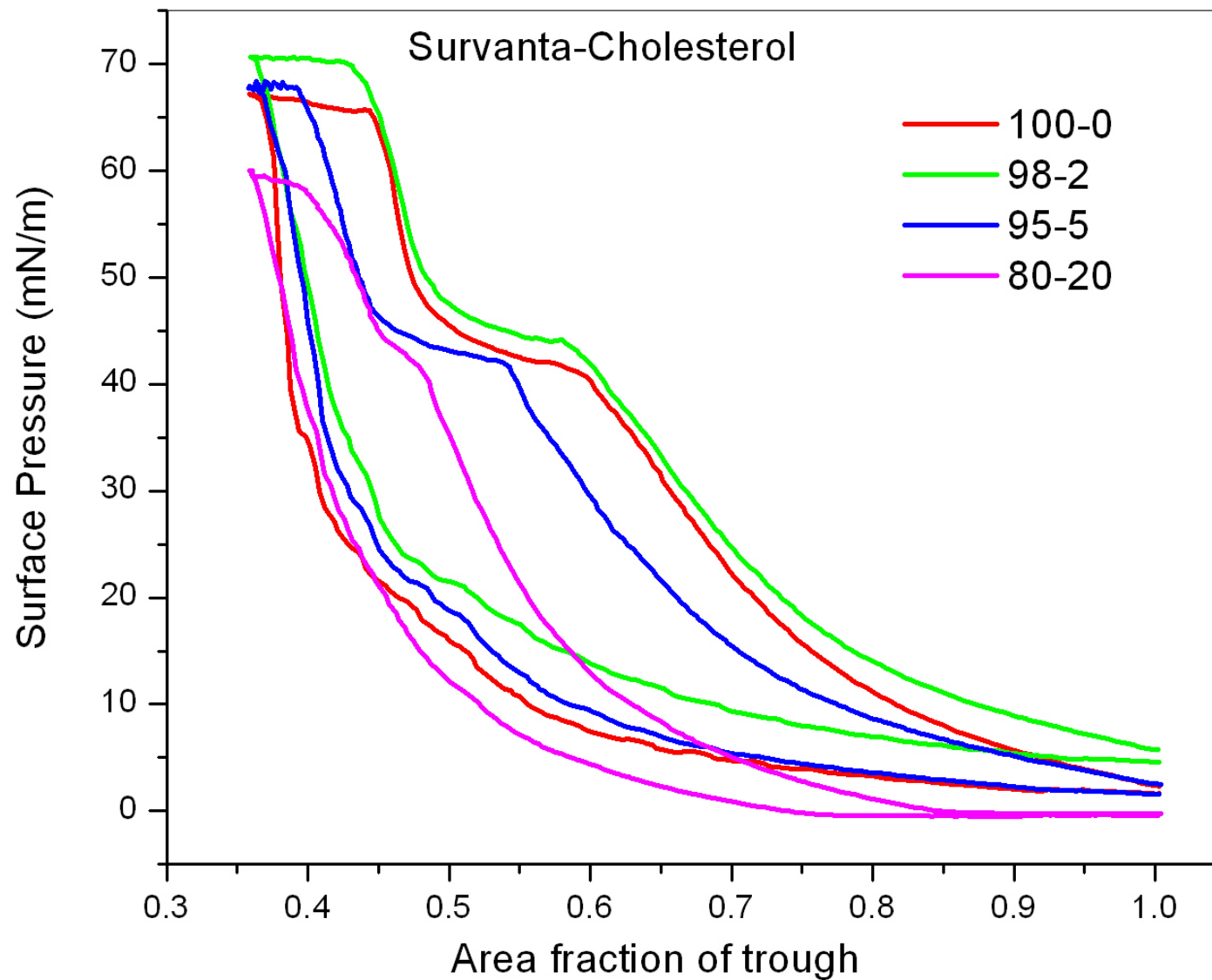
- 1% SP-B improves surfactant function
- 5% Cholesterol improves surfactant function
- 20% is detrimental

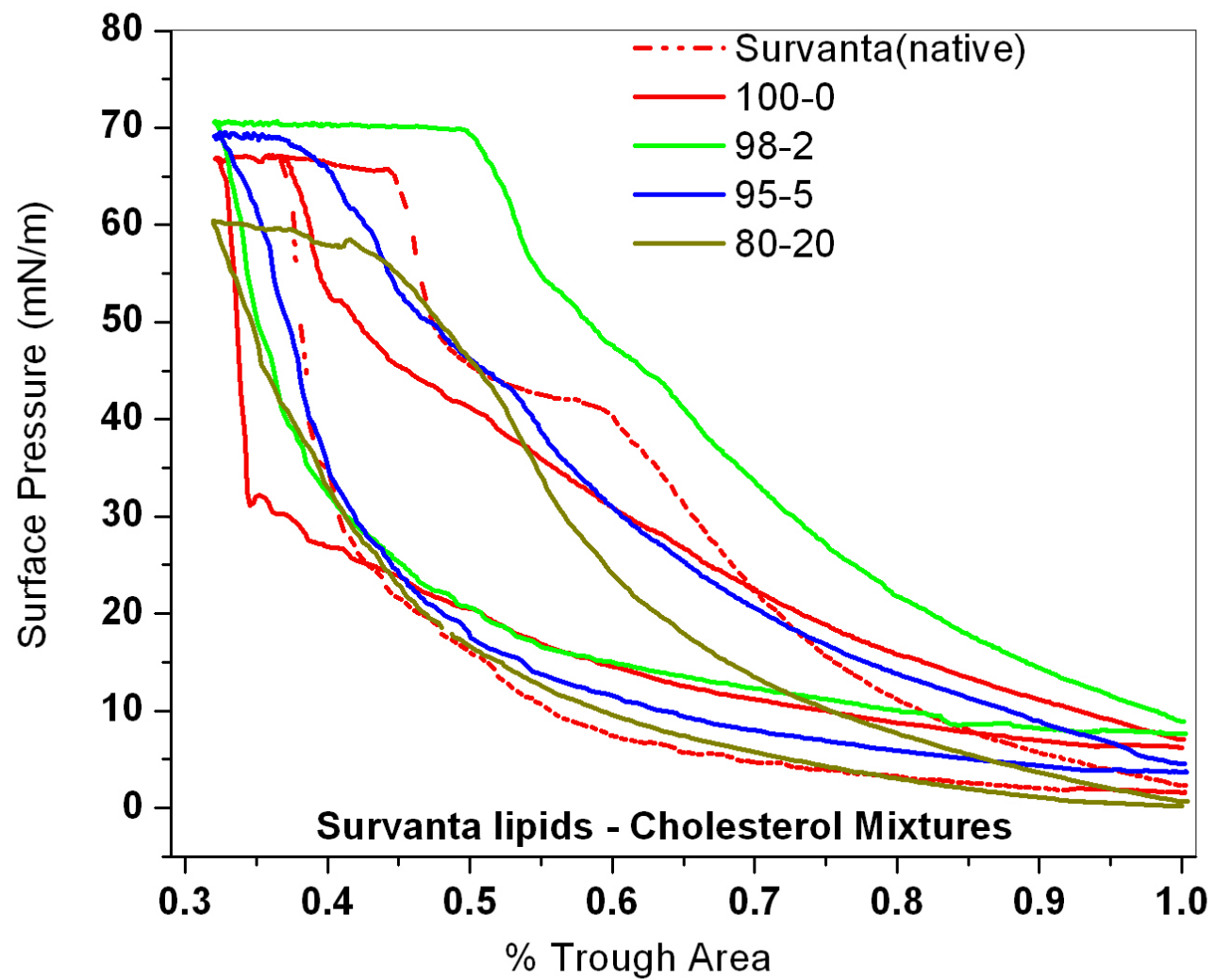
Compare



- Cholesterol trends are the same
- Reach similar high surface pressures
- 5% SP-B increases absorption even more than 1% SP-B
- All experiments were repeated to ensure reproducibility







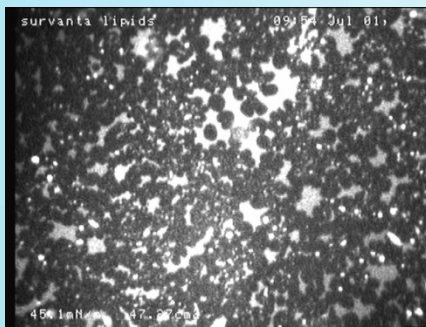
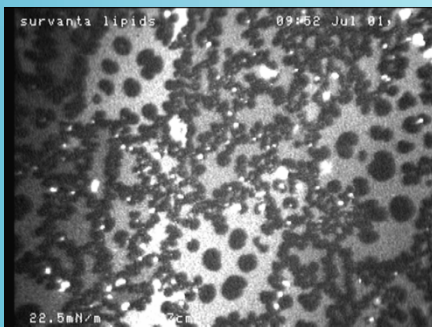
Survanta Lipids 1% SP-B + Cholesterol

25mN/m

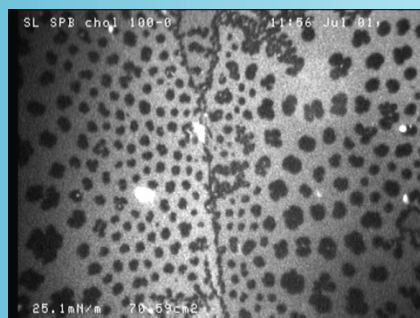
45mN/m

Collapse

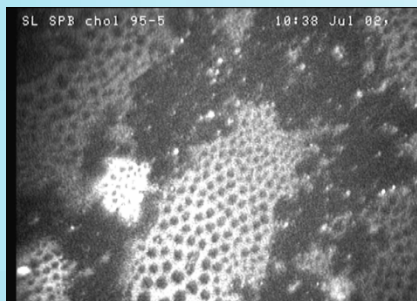
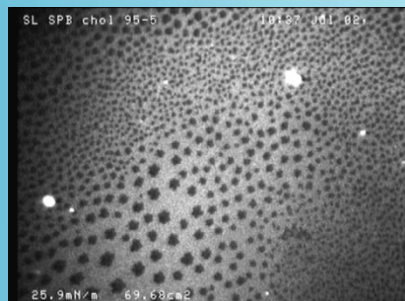
SL



100-0

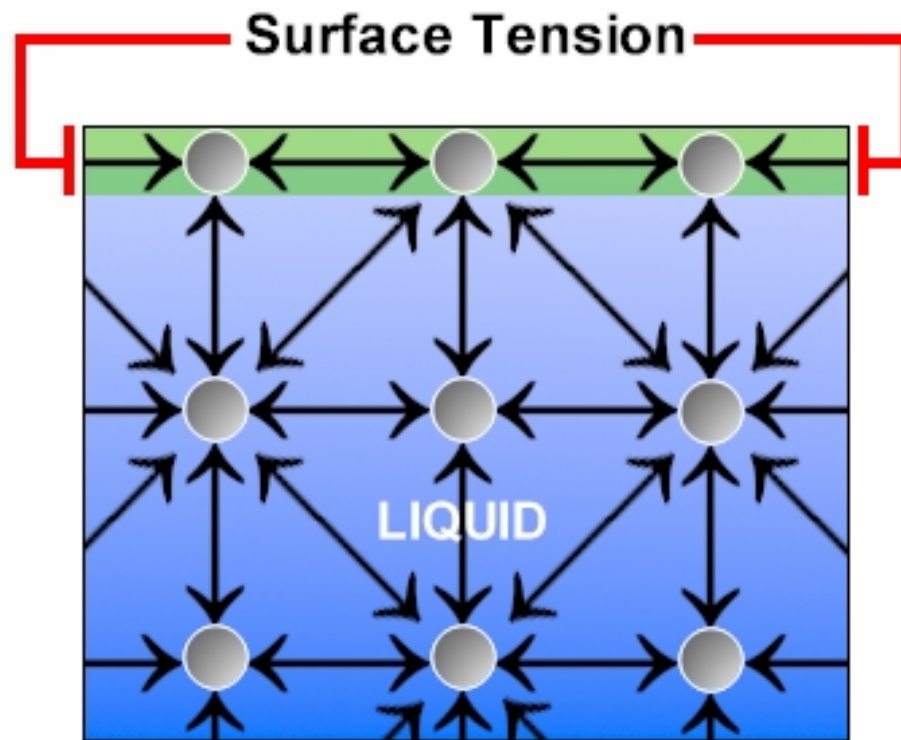


95-5



80-20





ramé-hart instrument co.