WELCOME

First Annual PhyNexus Users Group Symposium

August 27, 2014

South San Francisco, California

Why PhyNexus?

- The personal response
- The PhyNexus corporate mission
  - We apply analytical solutions to biological problems
    - Chemistry for biology and automation
    - Applied to Drug Discovery / Drug Development
  - We install and support solutions, not just products
- Solutions are enabled by technology that we develop - PhyNexus has 26 patents on various technologies and more than 16 more in prosecution.

Chromatography on a Pipette Tip

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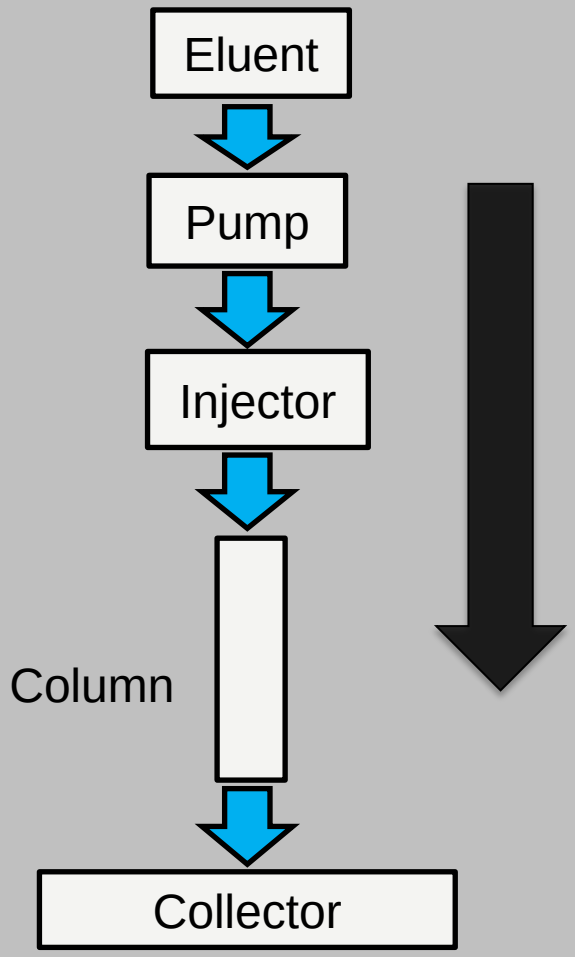
August 27, 2014

Chromatography on a Pipette Tip

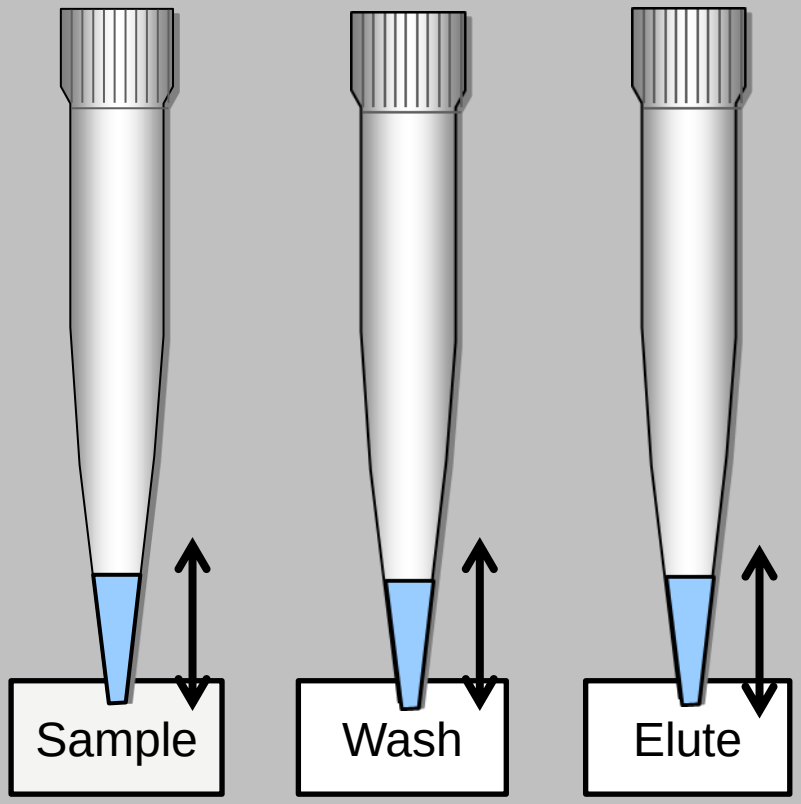
- An affinity column positioned at the end of a pipette/robotic tip
- Chromatographic concepts and principles used but in a novel manner

Conventional Chromatography



- Mobile phase is pumped down through the system with a pump in a uni-directional flow
- Sample is loaded through an injector at the top of the column
- Chromatographic separation and elution is a top down flow through the column.
- Collection of purified sample is at bottom of column.

Pipette Tip Chromatography



- Sample and mobile phase enter and exit at the column tip.
- All liquid flow is back and forth flow. This includes sample loading, chromatographic development and elution.
- This is still chromatography, just non-traditional chromatography.

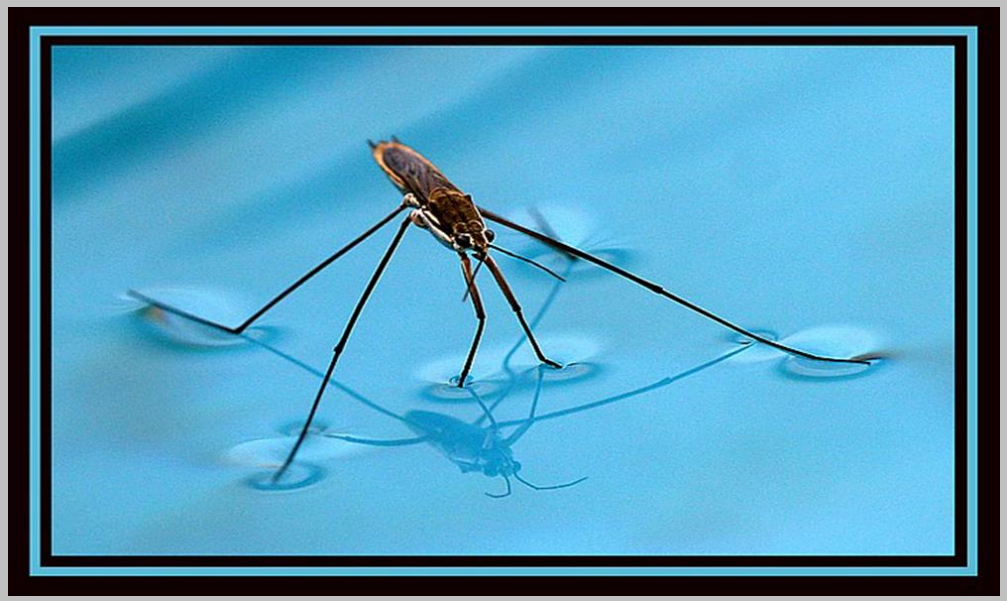
How does chromatography on a pipette tip work?

- Physical/mechanical Properties
  - Control the flow of sample and buffers
  - Control the volume of buffers needed
- Separation Chemistry Properties
  - Must operate with back and forth flow
    - Sample loading
    - Separation development
    - Sample elution/recovery

Physical/Mechanical Properties

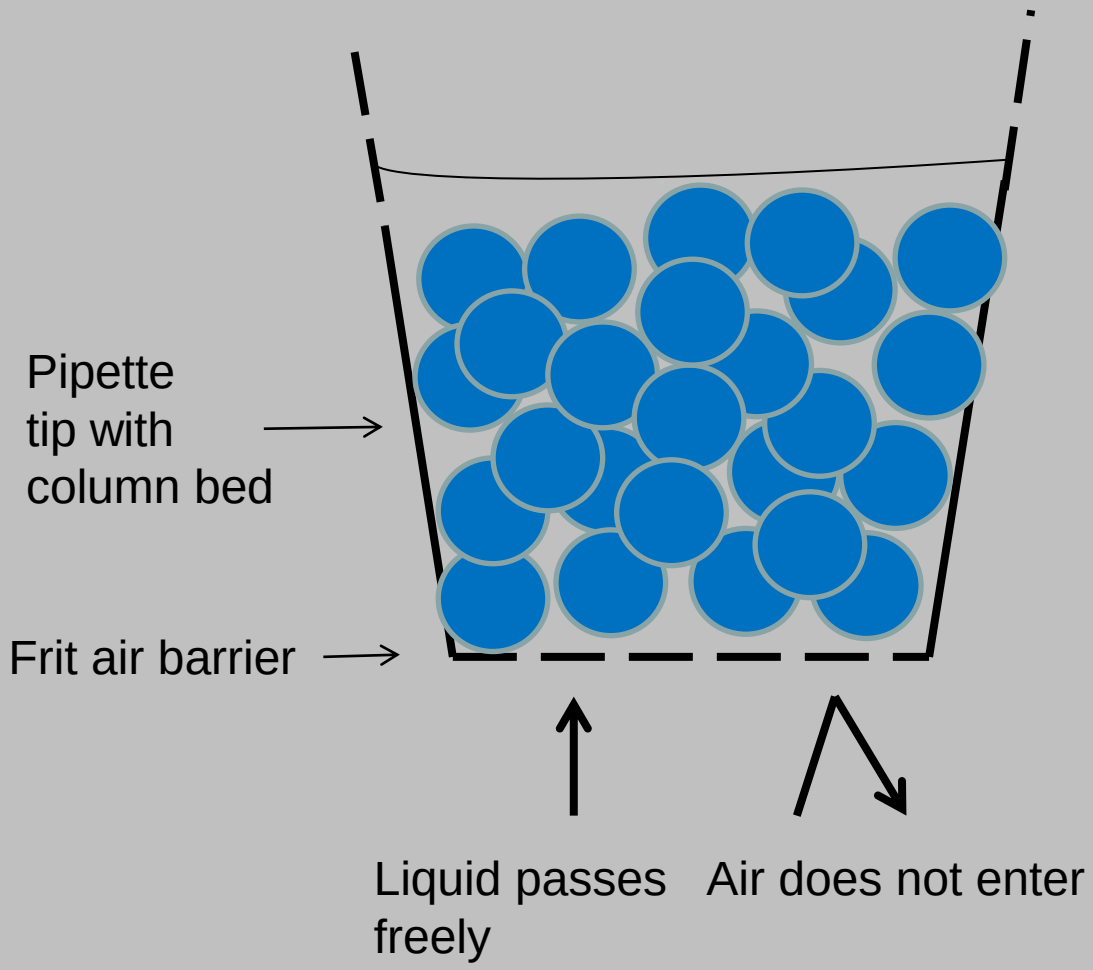
- Thin frits
  - Frits are thinner than the bead diameter
  - Virtually no column dead volume
- Hydrophilic frits
  - Will wet and draw up even a single drop of liquid to cover surface of the frit.
  - Hydrogen bonding of water to itself and to the frit.

Hydrogen Bonding of Water



- Intra hydrogen bonding: small insects can actually walk on top of water because of a water surface “skin” produced by water hydrogen bonding
- Inter hydrogen bonding: hydrogen bonding of water with a hydrophilic frit also produces a “skin” with water. This skin is a barrier to air passing through the pipette tip column

The Pipette Tip Column Frit Air Barrier

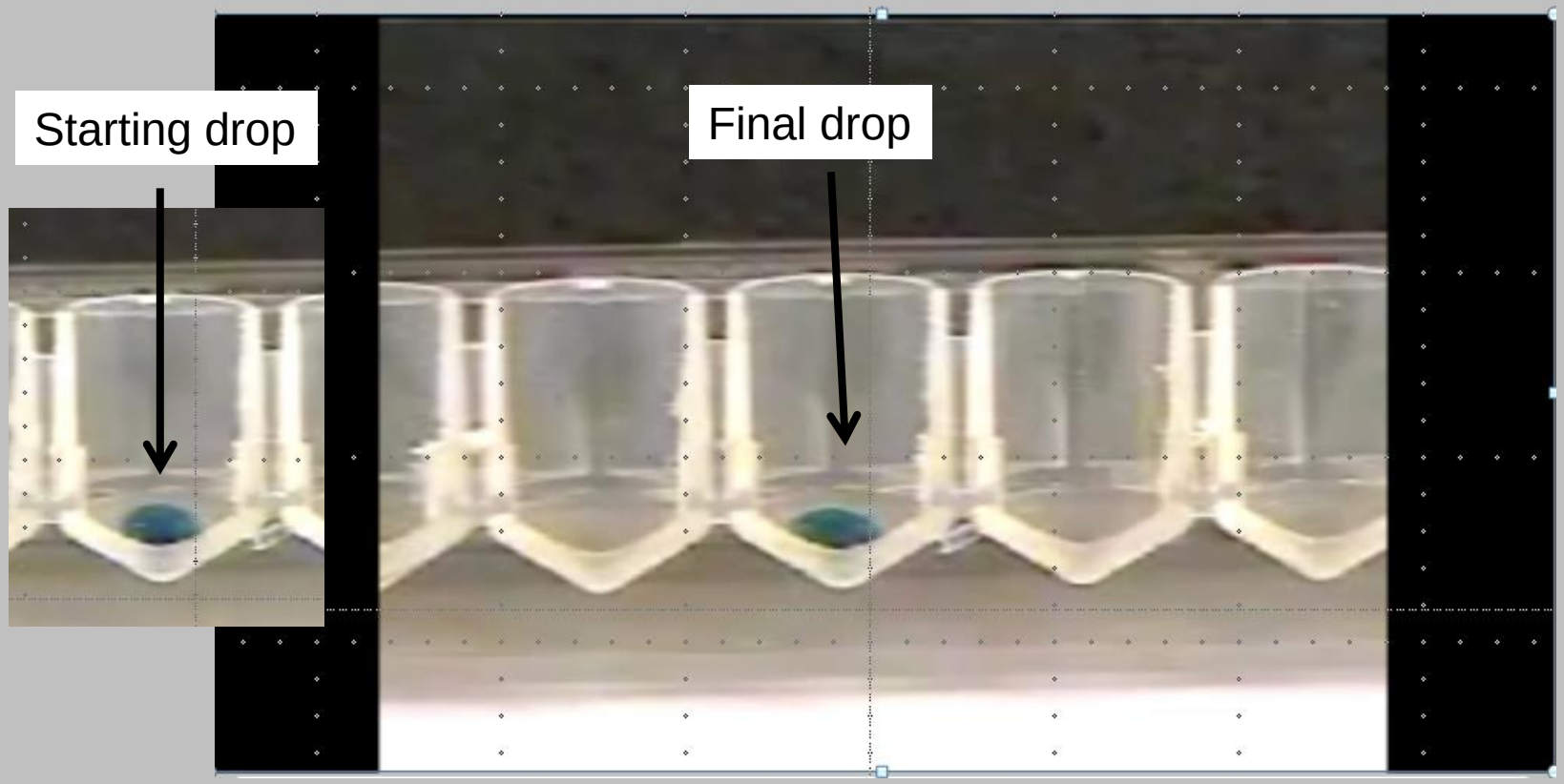


Piston Position and Fluid Flow

- Flow through the column is controlled by pressure and vacuum above the column bed.
  - Frit itself has zero backpressure
  - But flow only starts after a threshold pressure or vacuum is reached
  - Flow stops when no liquid is left in front of the frit. Even with a positive pressure or vacuum above the bed, air does not enter the column
- Software and firmware programming used to control of pipette/syringe piston position
  - Used to produce predictable vacuum and pressure above the column bed
  - Piston position and movement does not match liquid flow (it is not like pipetting liquids)

Fine control flow of fluid flow through a pipette tip column

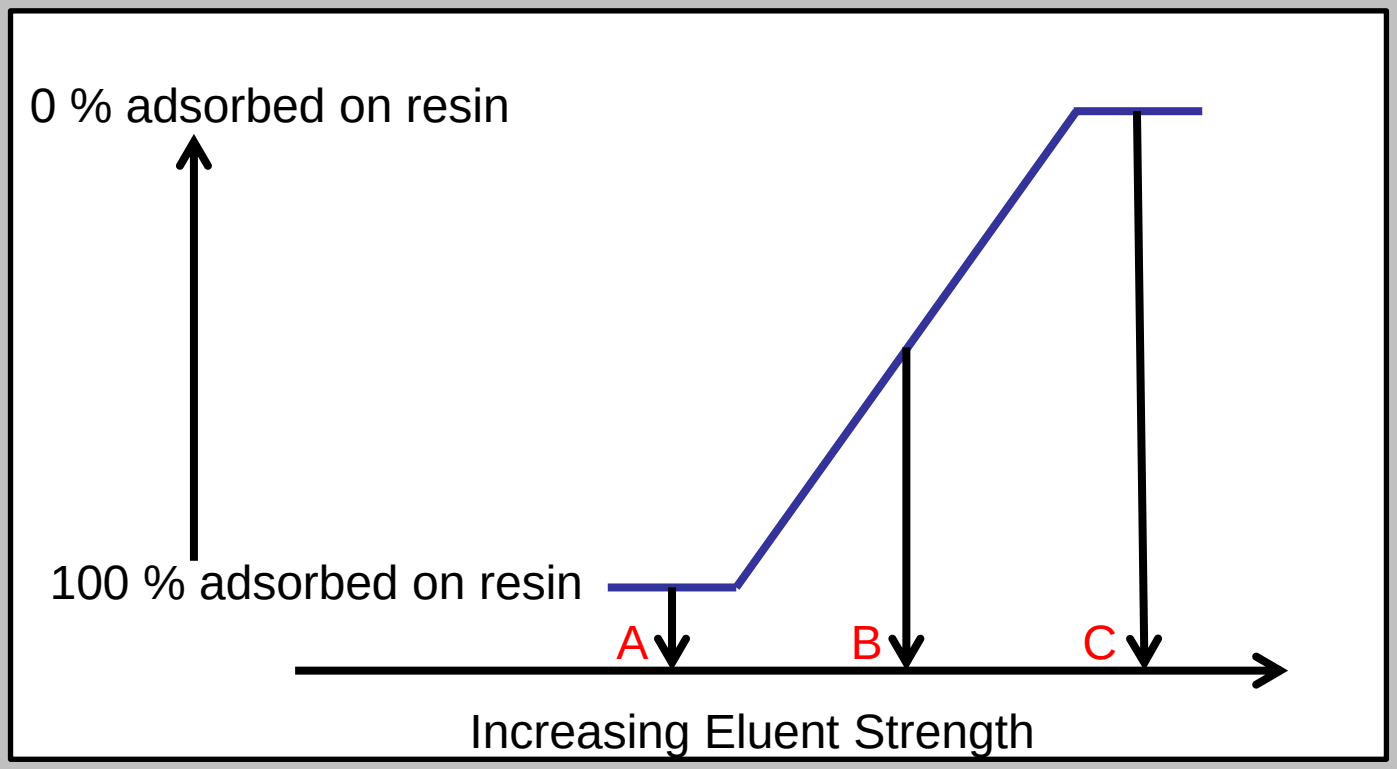
Fine control flow of fluid flow through a pipette tip column



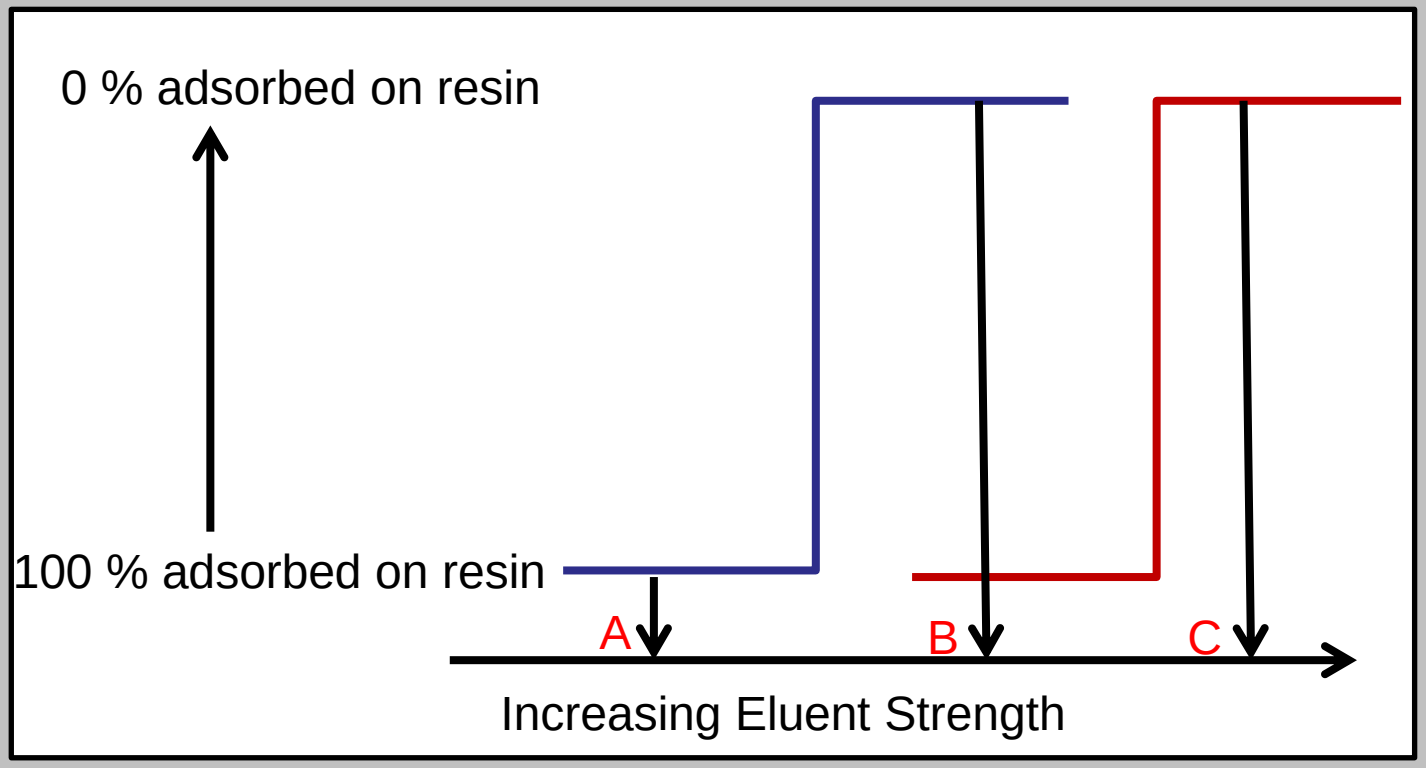
Separation Chemistry Properties

- Separation selectivity in chromatography is controlled by isotherm chemistry.
- Isotherms show the mobile phase chemical conditions
  - at which materials are adsorbed to the column media and
  - at which materials are dissolved in solution.

Gradual Isotherm Chemistry



Sharp Isotherm Chemistry



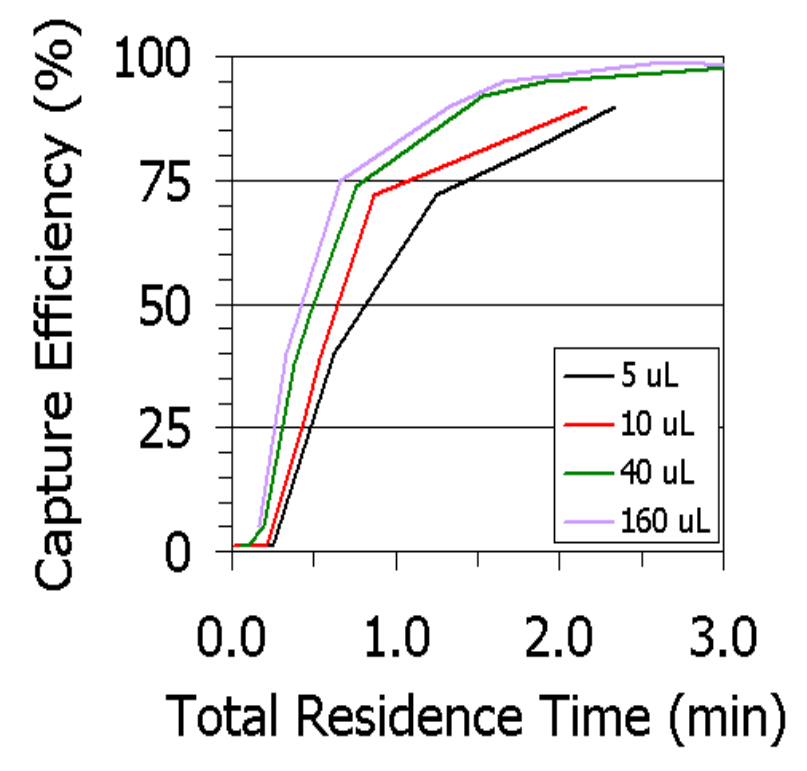
Sharp Isotherms are Difficult to Achieve

- Biomolecules interact slowly with affinity columns – slow kinetics.
- The problem is exasperated with small columns.
  - Spin columns
  - plates
- Time of interaction may be insufficient and is difficult to control.

Chromatography on a Pipette Tip

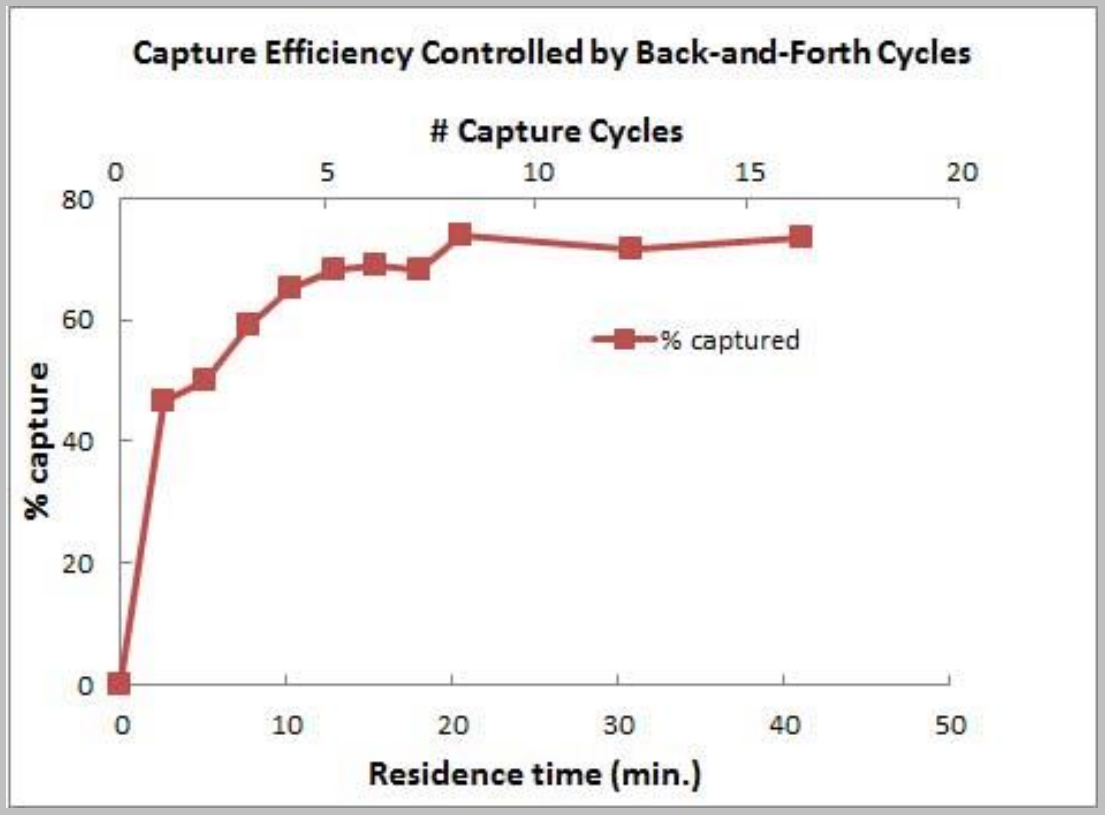
- Time of interaction is easily controlled with back and forth flow.
- Time of interaction is sufficient with several cycles
  - Active transport of sample molecules to resin functional groups
  - Multiple chances of interaction
- Sufficient time is defined as time necessary to drive the equilibrium interaction of sample and column to completion.

Capture Efficiency is Related to Residence Time



Capture efficiency of IgG as a function of residence time for 5, 10, 40, 160 µL bed Pro A columns.

Capture Efficiency is Related to Number of Cycles

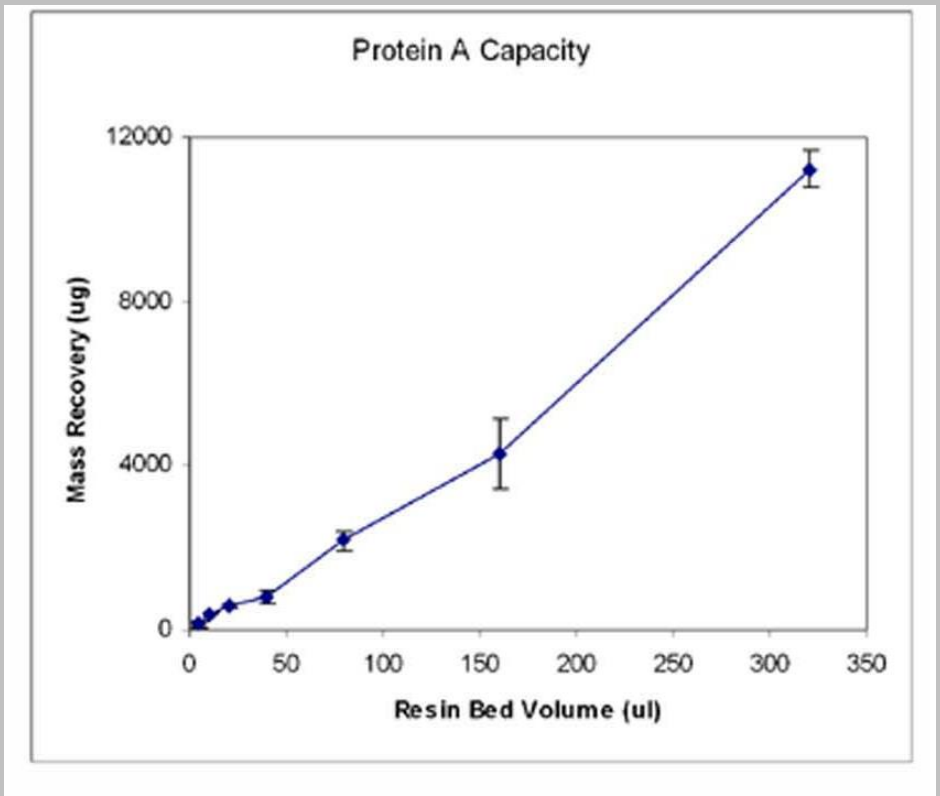


Capture of lysozyme using 200+ 5 µL bed Cation Exchange column.
Equilibrium binding is achieved with 5 cycles of capture.

Chromatography on a Pipette Tip

- Since interaction is complete to equilibrium in back and forth flow, separations are:
  - Independent of column diameter
  - Independent on packing uniformity
  - Independent on amount of resin in the column
  - Independent of flow rate
  - Only dependent on column and mobile phase chemistry.
- Result: A novel, powerful type of chromatography.
- Mimics very large diameter traditional column chromatography where the residence times are long.

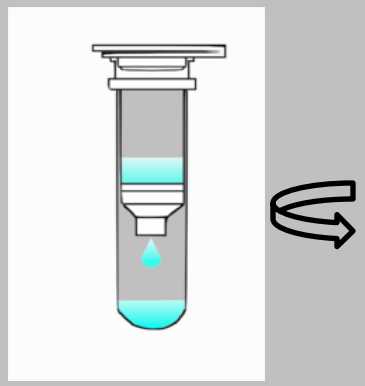
Back-and-forth flow make columns scaling predictable



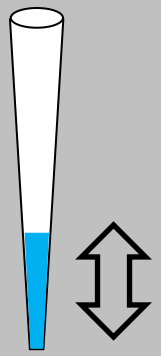
- Linear relationship between resin bed volume and recovery
- Measured dynamic binding capacity of 29μg/μL is equivalent to manufacturer's specifications
- Resin performance is not compromised by miniaturization
- Conditions developed can be applied to all column bed sizes, including large scale manufacturing

PhyTip Columns Concentrate the Sample

Compare purification of an antibody protein
with a 200 µL bed Pierce NAb spin column
and a 20 µL bed PhyTip column



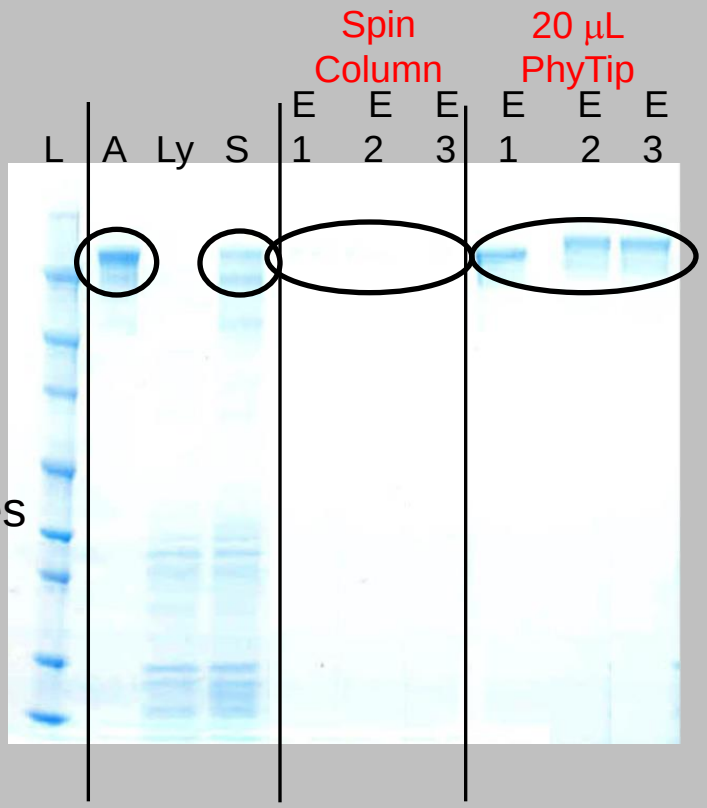
Spin column



20 µL bed column

Spin Column vs. 20 µL PhyTip 200+ ProA column

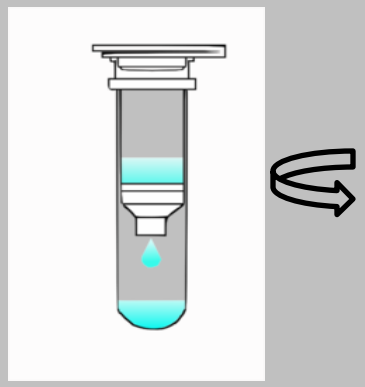
L = ladder
A = hIgG
LY = *E. coli* lysate
S = A + LY
E = elution from
Triplicate samples



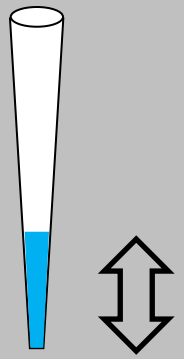
Chromatography on a Pipette Tip
Tip Concentrating Effect

- Question: What happens when you decrease the column size to 5 µL from 20 µL?
- Answer: The PhyTip Column Tip Concentrating Effect becomes stronger.
- The Tip Concentrating Effect is counter intuitive:
  - To increase the concentration of recovered protein, use a smaller column
  - 5 µL bed is a very small column bed but will give very high sample concentrations

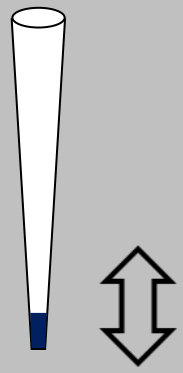
Tip Concentrating Effect increases using a 5 µL bed PhyTip column



Spin column



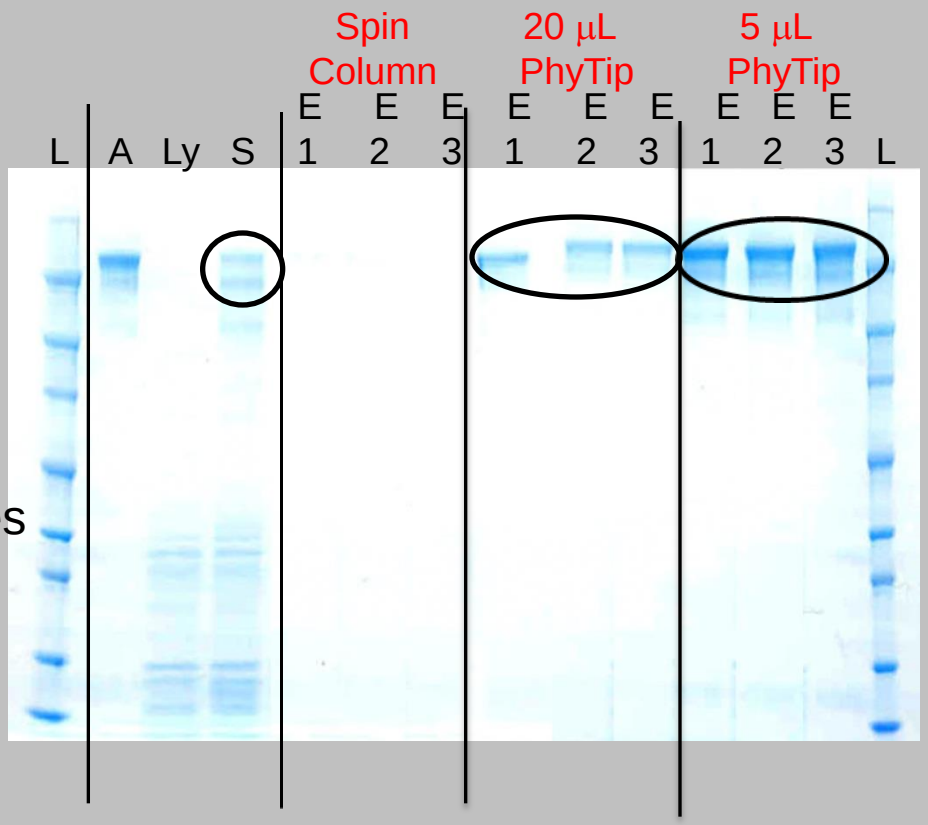
20 µL bed column



5 µL bed column

Spin Column vs. 20 µL PhyTip and 5 µL 200+ ProA column

L = ladder
A = hIgG
LY = *E. coli* lysate
S = A + LY
E = elution from
Triplicate samples



Antibody recovery from ProA Spin column and 20 µL PhyTip® column vs. 5 µL ProA PhyTip ® column HPLC data

	Vol. (uL)	mAU²	[ ] (mg/mL)	Mass (ug)	% rec.
Pierce-1	434	190.3	0.08	36	71
Pierce-2	440	170.5	0.07	30	59
Pierce-3	441	163.6	0.06	27	55
20uL-1	74	690.4	0.46	35	69
20uL-2	69	704.2	0.47	33	66
20uL-3	72	762.2	0.52	38	75
5uL-1	15	272.2	1.45	22	45
5uL-2	16	259.2	1.35	22	45
5uL-3	17	273.2	1.45	25	50

Chromatography on a Pipette Tip
Tip Concentrating Effect

- Operates with any “on/off type” high selectivity resin (sharp isotherm chemistry)
  - ProA/ProG
  - IMAC
  - Streptavidin
  - Ion exchange
  - Ion pairing / reverse phase
  - Any column/analyte chemistry where the isotherms are sharp i.e. they change rapidly and completely with changes in solvent composition
- Match bed size to sample concentration and volume to load up the bed

This Meeting

- How is PhyNexus technology used?
- What's new?
- What's on the horizon?