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

- Pipette tip with column bed
- Frit air barrier
- Liquid passes freely
- Air does not enter

Tip Concentrating Effect™
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

Starting drop

Final drop
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

0% adsorbed on resin
100% adsorbed on resin

Increasing Eluent Strength

A
B
C
Sharp Isotherm Chemistry

Increasing Eluent Strength

0 % adsorbed on resin

100 % adsorbed on resin

A

B

C
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

Tip Concentrating Effect™
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

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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?