Dual Flow Chromatography for Process Development

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Introduction

Functionalized pipette tips have been available for many years. Marketed for solid phase extraction, these pipette tips carry a solid phase contained within a polymer precipitate located at the end of the tip. In more recent development, pipette tip columns carrying conventional chromatographic media packed into beds between frits, have found use improving R&D productivity and capability in academic and industrial settings. In pipette tip columns (PhyTip Columns), sample and mobile phase enter and exit the same end. All liquid flow is bi-directional including sample loading, dilution of contaminants and elution of desired materials in a manner we call Dual Flow Chromatography (DFC).

With the exception of looks and operation, the pipette tip chromatographic column employs the same chromatographic principles and separation process which govern the conventional uni-directional column. Advantageously, in DFC, the kinetic rates of diffusion and interaction of sample and eluent with the stationary phase do not play as critical a role as in flow through chromatography. DFC columns enable comprehensive design of experiments and high throughput screening in process development to be carried out a fraction of the time.

Physical and Mechanical Properties of PhyTips

The physical and mechanical properties of the PhyTip Column are used to control the flow rate and liquid volumes flowing through the column.

Column operation

Once the pipette tip columns are engaged on a robotic liquid handler, software controls the syringe which create predictable vacuum and pressure above the column bed in the chamber of the pipette tip which in turn moves the mobile phase back and forth through the solid phase column.

Frit screens

The frit screens (60 µm thickness) which hold the chromatographic media are thinner than the average agarose based affinity bead (90 µm). This results in virtually no dead volume in comparison to the column bed volume: a frit containing a 20 µL bed volume has a dead volume of less than 0.25 µL.

Through the hydrophilic nature of the thin frit screens, a liquid layer forms and quickly coats the frit screens as soon as the tip touches the sample, allowing the pipette tip columns to process very small volumes of liquid. For example 10—15 µL of eluent for a 5 µL column

The Frit Air Barrier

Water can pass freely through the frit screen and freely through the column. When the entire sample has been aspirated by the PhyTip and there is no liquid left in the sample well, cohesive forces and surface tension at the entrance frit will stop flow through the column. Air cannot easily enter the column unless a pressure differential is applied, normally above 2—3 psi.

This is called the frit air barrier.

Equilibrium Binding

Residence time is easily controlled with DFC simply by controlling the number of back and forth flow cycles until equilibrium interaction of sample and column is achieved. This is shown in Figure 1 where 5 µL, 10 µL, 40 µL and 160 µL pipette tip columns of Protein A were used to capture an IgG. The smallest column having the fastest linear velocity of fluid flow takes the longest time to achieve complete interaction. In any case, within 3 minutes of back and forth flow interaction, the capture is complete regardless of the bed size. Stationary phase and molecular interaction is driven to completion with DFC regardless of the kinetic rate constants.

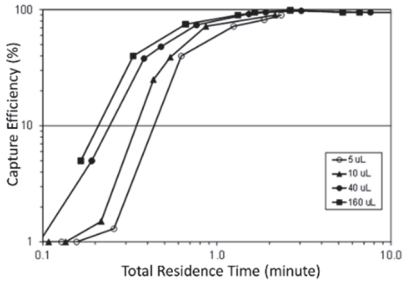

Figure 1. *Capture efficiency is increased by the residence or contact time. Capture efficiency of hIgG as a function of residence time (5, 10, 20, 40, 160 µl column IgG).*

Because the interactions are at equilibrium DFC are independent of:

1. Linear and absolute fluid flow rate through the column
2. Column bed diameter, length and geometry
3. Packing uniformity or column channeling
4. Column dead volumes
5. Amount of stationary phase in the column

PhyTips Separation is Scalable

The separation conditions developed with these small columns can be applied to all column bed sizes, including laboratory preparative columns and large scale manufacturing. PhyTip Columns mimic and predict the performance of preparative and manufacturing columns. This is because with preparative columns, the column diameter is typically large. This in turn makes the linear velocity of fluid flowing through the column very slow. Contact time is long and equilibrium interaction is achieved, just as in pipette tip columns.

The ability to use PhyTip Columns for scale-up is depicted by a linear relationship between the recovered mass and resin bed volume of PhyTip Columns as shown in Figure 2.

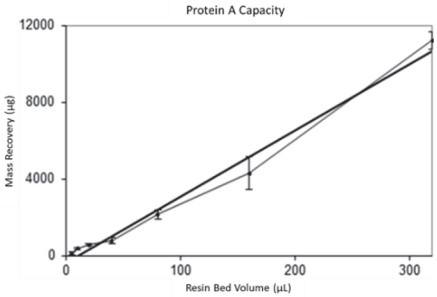

Figure 2*. Back-and-forth flow through the PhyTip Column push interaction reactions to completion making column scalingto preparative and manufacturing scale flow through column predictable.*

High Throughput Screening for Optimization of Chromatographic Conditions

Optimizing chromatographic conditions results in purer samples and increases the recovery of the product. It is imperative to have the ability to screen multiple conditions in parallel and compare results.

PhyTip Columns used together with a 12 channel pipette head enable high-throughput screening of up to 96 samples in parallel. Here, various conditions can be tested in one run by 96 PhyTip Columns. These data show one process development experiment that includes 12 types of ion exchange resins per row, 4 different loading pH conditions, 2 wash conditions and 3 elution conditions of increasing ionic strength. In total 288 conditions were tested. Emphasizing the value and importance of method development, the results show that high recovery and high activity are not correlated, helping researchers select conditions most favorable for the highest yields and activities in the products. Experiments such as these can be performed in a few hours compared to flow through column method development that may take weeks or months.

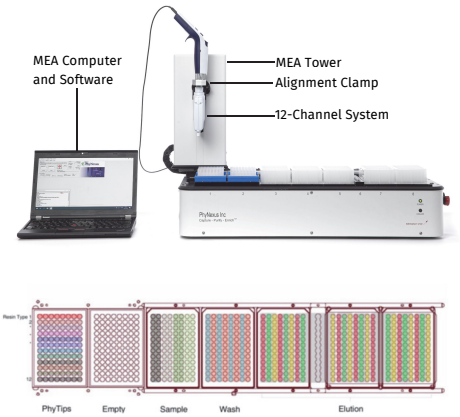

Figure 3. *The 12-channel MEA 2 Protein Purification System for parallel processing and set up for 288 condition screening.*

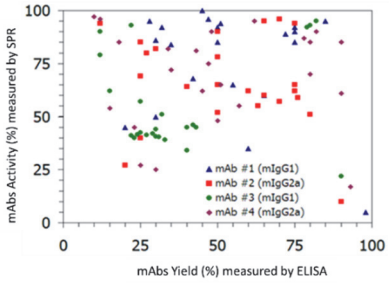

Figure 4. *Yield and activity parallel method development for purification of an antibody on Protein A pipette tip column. Multiple method conditions were tested for 4 clones.Yield was measured by ELISA and protein activity was measured by SPR.*

Summary

Dual Flow Chromatography with sample and a bi-directional mobile phase represents a new way to study and develop separations.

DFC with pipette tip columns is suitable for miniaturized, parallel and automated operation.

Separation conditions developed for small columns are scalable. Conditions developed on columns as small as 5 µL bed size can be transferred to industrial manufacturing columns.

DFC works with any column/analyte chemistry where the Langmuir adsorption isotherms are sharp. Bio molecules have sharp isotherms for most different types of chromatographic phases including mixed mode resins. Although kinetic rates of diffusion and interaction can be slow for the small bed columns, through DFC, interactions among sample, stationary and mobile phases are driven to equilibrium.

Parallel DFC chromatography can be used for method development including multi-variable condition determination, something that is difficult to perform in uni-directional chromatography.

DFC has made a large impact in the competitive pharmaceutical industry where speed is critical for method development to determine separation conditions and for high throughput parallel sample processing.

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