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# True Moving Bed Chromatography (Tmbc)

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## ABSTRACT

*Archimedes is a novel new continuous purification system (1) for purification from large volumes and concentrations of product that achieve product specifications. This new system eliminates the bottleneck caused by packed columns but retains chromatographic purification resin chemistry allowing ionic, size, affinity, and hydrophobic interaction chromatography to allow achievement of product specifications. In this system resin slurry is transported continuously through a pipe with a screw driver and a peristaltic pump. During this movement, the resin is sequentially contacted by the equilibration, load, wash, elution, and strip buffers before being continuously recycled. Continuous elution is caused by adjustment of the conductivity of the elution buffer to a conductivity that enables elution of the product at controlled concentrations and in the presence of polysorbates or multiple excipients. Each buffer is adjusted to an appropriate conductivity similar to elution with a step gradient of conductivity.*

*This method results in improved manufacturing productivity (2) (3)because it continuously purifies. The system also offers improved scalability, improved resolution, faster operation, improved purity, and a smaller footprint as well as reduction of buffer utilization and resin requirements. Substantial savings in manpower, raw material, and capital costs accrue. These characteristics make it ideal for the purification of a perfused culture resulting in a fully continuous manufacturing process. Use of this system will convert conventional antiquated batch-wise manufacturing technology to modern continuous technology increasing manufacturing productivity and reducing manufacturing costs.*

**Keywords:** *Simulated Moving Bed, Chromatography, SMBC, Continuous Biopharmaceutical Manufacture, Manufacturing Cost Archimedes*

## INTRODUCTION

Proteins have been purified for decades by conventional column chromatography. Use of simulated moving bed chromatography (SMBC) is also possible (5). However SMBC is complex to maintain and is expensive to procure. When using conventional column chromatography or SMBC, the resin bed of each column must be carefully controlled and qualified by measuring HETP and asymmetry (6) to maximize the capture and resolution of proteins of the device. Although these systems are complex to operate and limited in performance, no good alternative is available for their replacement. In addition, in conventional column chromatography, the finite capacity of a resin to bind a protein in a single pass results in large requirements for resin which can be financially prohibitive case of expensive resins like protein A resins($10,000/L) (7). A

## MATERIALS AND METHODS

Pipe is clear and made of molded (not extruded) polyacrylate. It was procured from United States Plastic Corp.1390 Neubrecht Rd.Lima, OH 45801800-809-4217usp@usplastic.com Screen that retains resin particles was obtained as a nut milk bag from Amazon and cut to fit buffer inlet and outlets. Premium Fine Mesh Food Grade Nut Milk Bag 9" x 12"The detector measures A280 and conductivity. It can be procured from Reach Devices, LLC.6525 Gun Park Drive, Suite 370-179, Boulder, CO 80301.

The screw was designed using freeware software available at the Tinkercad it produces a file that can be read by a 3D printer that can be emailed to the 3D printer. The 3D printer site is operated by Sculpteo. 169 11th street, San Francisco, CA 94103. The screw is 25.4 mm ID (1 inch)

### Cost Improvements

The system described here, true moving bed chromatography(TMBC),shown in Figure 1, relies on passing a resin slurry down the longitudinal axis of a pipe and contacting the resin at several different points with buffers of

controlled conductivity. After traveling approximately three or more column diameters, the introduced buffer is pumped out and the next buffer is introduced at the next ports. In this way the resin can be loaded, washed, eluted, stripped, and re-equilibrated as it travels down the pipe in turbulent flow mode and re-equilibrated resin can be continuously recirculated to begin the process again. The use of resin slurry encourages convective mixing thus substantially increasing the rate of binding elution over a packed column which is dominated by slower diffusive mass transfer. And there is lower pressure drop across the column enabling higher velocities to be used. Finally, there are no limitations imposed on the scale of the system as in the case of column chromatography, where HETP of the resin bed is difficult and time consuming to measure.

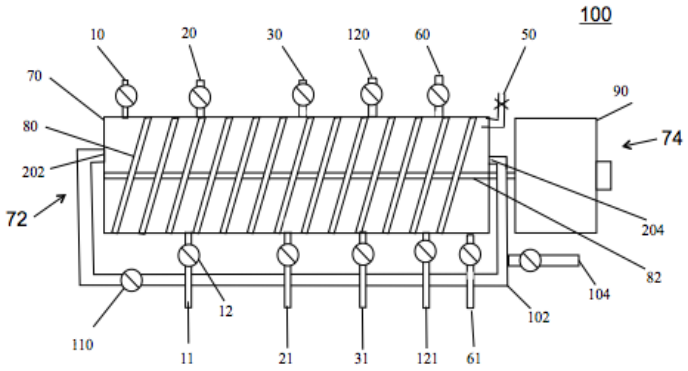


**Figure1.** *Archimedes structure and component designations Port for crude addition 10 port. Ports for buffer addition and removal: 120, 30, 120, 60, 11, 21, 31, 121, 61.Variable speed drive for spindle is 90 and screw is 80.Peristaltic pump for resin return is 110. Resin addition port is 104 and the bleed is 50.*

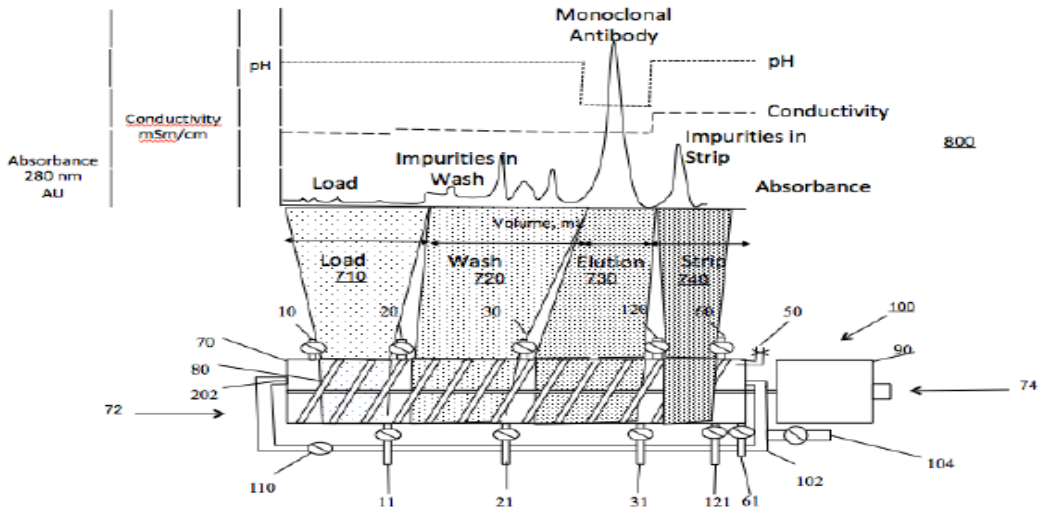


**Figure2.** *Identification of Archimedes sections that correspond to the areas of a column chromatogram from a conventional column*

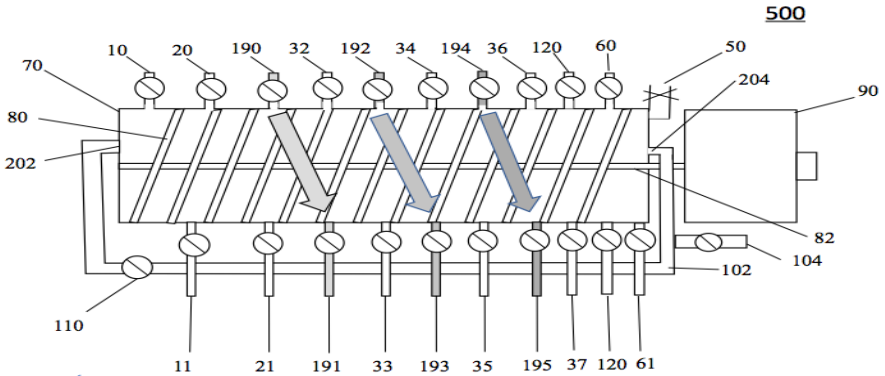


**Figure3.** *Schematic for purification of multiple blood proteins from blood plasma*

## Reduction of Resin Cost and Increased Productivity

Because conventional column chromatography is a batch wise operation, a column is scaled to process the maximum amount of load based on the dynamic capacity of the resin. The buffer flow rates are adjusted to their maximal values without exceeding the pressure that crushes the resin.

This method of process scale-up is inefficient in that it requires more resin, or multiple runs, and slower flow rates than would be necessary with a continuous operation. The Archimedes system has multiple advantages compared to conventional column chromatography. First, the system requires less resin than conventional columns because the resin is continuously striped, re-equilibrated, and recycled for reuse.

This is important in that it can reduce the cost of expensive labile resins such as Protein a ($10,000/L). Second, a lower pressure drop is generated by operating in turbulent flow rather than laminar flow. As used in conventional columns. This results in the potential to use tenfold higher linear velocity.

Table 1 compares the parameters of a conventional chromatography column with those of an Archimedes system on two bases, 1) reduced resin utilization and 2) reduced cycle time. The basis of comparison is the purification of a 2000L bioreactor at 1 g/L (total of 2Kg).

**Table1.***Comparison of Resin Requirements of Conventional vs Archimedes technologies these estimates do not include efficiencies in mass transfer created by convection*

| | Conventional | Archimedes |
|---|---|---|
| Resin Type | SP Sepharose Fast Flow | SP Sepharose Fast Flow |
| Height (cm) | 45 cm | 18 cm |
| Diameter (cm) | 12 cm | 12 cm |
| Cross Sectional Area (cm2) | 113 | 28 |
| Vol, L | 1.3 | 0.51L |
| Particle size | 45 um | 45um |
| Linear Velocity | 700 cm/hr | 700 cm/hr |
| Operating Pressure | 2 bar | 0.5 bar |
| Flow Rate | 0.65 L/hr | |
| Vol Resin Required | 70 L | 0.5 L |
| Dynamic Resin Capacity g protein / g resin | 120mg/mL | 60mg/mL |
| Cycle Time /Batch in Hrs | 14hrs | 2hrs |

The Archimedes system utilizing 10% of the resin is capable of processing all of the product in less time (2 hours) than a conventional chromatography column (14 hours). A protein S resin would cost $50,000 for the conventional column of 5L and only $5,000 for the Archimedes system.

Note that no time has been included for column packing and qualification after sanitization of the conventional system which will reduce its productivity even further. A comparison of the processing time required by conventional and Archimedes processes using the same amount of resin is also shown. In this case the Archimedes system is five times more productive. The magnitude of the productivity increase of Archimedes is due to two factors.

First, simultaneous washes occur in the Archimedes system.Second, the Archimedes system has an increase in the rate of mass transfer due to convective mixing resulting in higher linear velocities opposed to diffusive mixing in conventional column chromatography as discussed in Section 1.Smaller particle diameters resulting in higher product capacities can also be used.

## Replacement of Uf/Df

Within the elution segment of the device, product is eluted by the conductivity of the buffer, not by the buffer flow rate. Thus, controlling flow rate at a defined conductivity will control product concentration while minimizing buffer. This characteristic of the device will minimize or eliminate the need for a separate concentration and buffer exchange step by UF/DF. The recommended flow rates in an ultra filtration device are high, typically in the turbulent flow regime to reduce concentration polarization.

This regime inhibits product denaturization and aggregate formation. Polysorbates, sometimes used to reduce the formation of aggregates, cannot be used in most ultra filtration operations because it cannot pass through most membranes. However, the Archimedes device's non-denaturing low flow rates and can be used with polysorbate in the elution buffer.

## Purification of Multiple Products (Ex Plasma Fractionation) from a Single impure Feedstock

The Archimedes system can also be used to purify multiple products simultaneously, for example, blood factors from plasma. Products

such as those shown in Table 2 are currently isolated from human plasma by a combination of ultra filtration and column chromatography.

**Table2.** *Products fractionated from human plasma – total market valuation is approximately $26 BB annually. Table 2 shows total sales of plasma products.*

| Product Type | Product | Total Annual Sales in $BB |
|---|---|---|
| Albumin | Human Serum Albumin | |
| Immunoglobulin | Intravenous Immunoglobulin | |
| | Subcutaneous Immunoglobulin | |
| Coagulation Factor Concentrates | | |
| | Factor VIII | |
| | Factor IX | |
| | Factor XIII | |
| Prothrombin Complex Concentrates | | |
| Von Willebrand Factor | | |
| Protease Inhibitors | | |
| Grand Total | | $26 BB |

A less frequently used technique is the Cohn fractionation method which requires multiple organic solvents and cryoprecipitation. The global plasma fractionation market is estimated to grow at a compound annual growth rate (CAGR) of 6.7% from 2016 to 2021 to reach USD 26.07 Billion by 2021.

Growing aging populations, rising incidences of bleeding and immune disorders, and growing use of immunoglobulin and alpha-1-antitrypsin are expected to propel the growth of this market. The high cost of recombinant blood factors limits their availability in the third world, so plasma fractionation is the principle source of those products.

Use of the Archimedes system for purification of multiple plasma products will employ a longer times than a single product allowing multiple buffers of ever higher conductivities to contact the resin. Each protein product is eluted sequentially at a specific conductivity.

## Purification of Bulk Biochemical's

Bulk biochemical's such as amino acids, vitamins, enzymes, and organic acids are extracted from natural sources. This extraction uses a variety of unit operations including chromatographic isolation and purification. Bulk biochemical's are generally commodities and therefore rely on low manufacturing costs to compete effectively.

They are also produced at very large volumes and rely on economies of scale to reduce their manufacturing costs. The Archimedes system applied to bulk biochemical manufacture will reduce manufacturing costs significantly.

## Isolation of Enantiomers from Racemic Mixtures

Chirality, or handedness, is ubiquitous in nature, from the microscopic scale of molecules to the macroscopic scale of living organisms. At a molecular level, chiral compounds, e.g. those featuring a tetrahedral carbon atom bonded to four different functional groups; exist in two non-super imposable mirror images. Several amino acids present in living organisms are chiral, and their interactions with other chiral molecules are stereo specific. This is particularly important in the case of synthetic pharmaceuticals administered as racemates since the interaction of the Enantiomers with the biological receptors can give rise to dramatically different effects. In the simplest case, one enantiomers exhibits the intended pharmaceutical activity while the other is inert and harmless. Though the stereo specific effect of pharmaceuticals has been, it was not until the early 1990s that strict regulations to study the effect, and eventually to manufacture single enantiomers, were put in place. These new regulations have had a major impact on the pharmaceutical industry. The percentage of single enantiomers drugs in the market increased from 10% before the 1990s to about 37% in 2005, when the sale of single-enantiomers pharmaceutical products amounted to US$ 225.22 × 109 (2005 prices).

Preparative enantio selective chromatography implemented either as a single or as a multi-column process is generally regarded as a versatile and powerful enantio separation tool. In this case, the chromatographic separation is performed using a column packed with a suitable chiral stationary phase (CSP) that exhibits selectivity and resolution for the two enantiomers.

## ROI

Recombinant biotherapeutics have worldwide sales (2015) in excess of $200BB annually. Using a typical profit margin of 95%, manufacturing costs are estimated at $10BB. Of this manufacturing cost, roughly 30% is due to purification and can be substantially reduced by the use of Archimedes technology.

Archimedes can be used to enable completely continuous manufacturing processes. By operating continuously, manufacturing plants can be much more productive, thus smaller,

saving capital that can be used for development of additional products and the associated fixed costs of approved products.

**Table3.** *Sales of Biotherapeautic products*

| Product Type | Estimated Annual Sales by 2020 |
|---|---|
| Protein Biotherapeautic | $200 BB |
| Human Plasma Therapeutics | $26 BB |
| Oligonucleotides Synthesis | $18 BB |
| Peptides and Proteins Synthesis | $20 BB |
| Total | >$264 BB |

An orthogonal method of assessing market potential assumes that this device will replace protein purification columns. Thus, the estimate is based on the number of biotechnology companies and universities in the US and EU and the number of columns used by each site. Table 4 summarizes the findings in the USA and EU at universities and companies. Assuming each company requires purification systems that can be replaced with an Archimedes device.

**Table4.** *Estimated number of chromatography columns in the US and Europe.*

| Location | Type | Sites | Units/site/yr | $M/yr |
|---|---|---|---|---|
| USA | Company | 2,000 | 4 | 8 |
| | University | 3,500 | 1 | 3.5 |
| | Total | 5,500 | | 11.5 |
| Europe | Companies | 2,400 | 4 | 9.6 |
| | University | 4,000 | 1 | 4.0 |
| | Total | 6,400 | | 13.6 |

*Asia, Australia and the rest of the world (ROW) are not included but could increase sales*

## CONCLUSION

True moving bed chromatography shown in Figure 1, relies on passing a resin slurry down the longitudinal axis of a pipe and contacting the resin at several different points with buffers of controlled conductivity. Thus the resin can be loaded, washed, eluted, stripped, and re-equilibrated as it travels down the pipe in turbulence and re-equilibrated resin can be continuously recirculated to begin the process again.

The use of slurry encourages convective mixing (), thus substantially increasing the rate of binding and elution rates over a packed column which is dominated by slower diffusive mixing. And there are no limitations imposed on the scale of the system as in the case of column chromatography. The new system reduces the amount of space, the manpower, buffer and resin required, and increases the product quality by enabling the use of process analytical technologies (PAT).

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