AMALGAMATED RESEARCH LLC

Find Your Unique Purification Solution

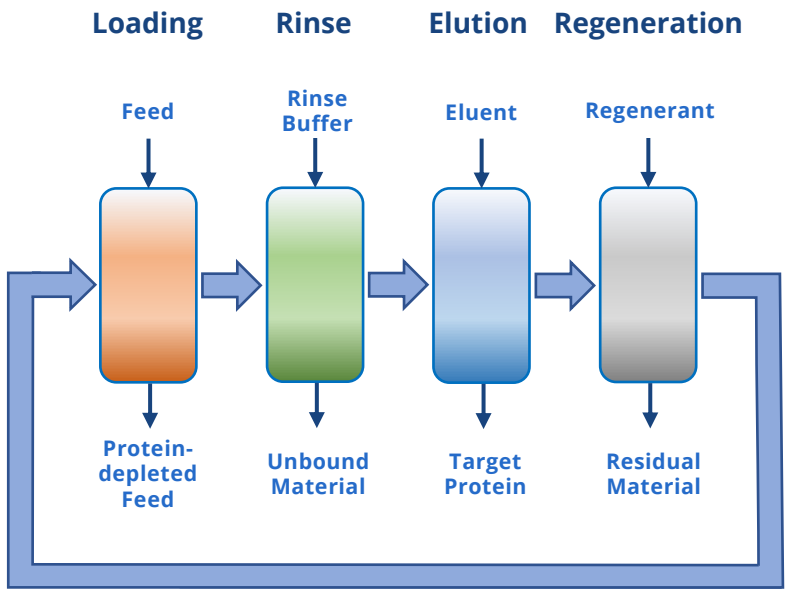
PROTEIN SEPARATION SOLUTIONS

ION EXCHANGE CHROMATOGRAPHY

Ion exchange (IEX) chromatography is a cyclic separation technique used to separate charged solutes by exploiting electrostatic interactions between the charged molecules and charged functional groups immobilized onto stationary resin beads that are packed into a column. Once the target molecule(s) have exchanged onto the resin beads, they are recovered using an eluent that effectively reverses the binding process.

IEX is one of the most common techniques used to separate charged biomolecules such as amino acids, peptides, and proteins. Using this technique, it is possible to separate biomolecules that only have a small difference in their charge properties to produce a high purity product. This makes IEX well suited for the removal of impurities or the capture of valuable proteins.

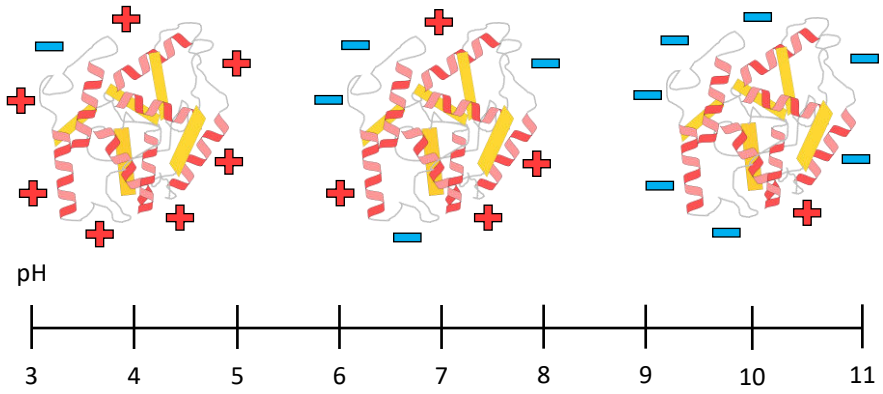
The resin beads that produce the most efficient and effective separation of proteins are often very small in order to take advantage of the improved kinetics of a fine particle size. Handling these small beads can sometimes prove to be a challenge for traditional IEX columns.



IEX OPTIMIZATION

There are many factors that influence the likelihood that a given protein molecule will bind to a functional group on a resin bead. These include the environmental pH, which changes the net charge of the protein, as well as the presence of competing charged ions (such as salts) that influence the ability of proteins to bind onto the resin bead. These factors have a major influence on whether a potential IEX separation is technically and economically successful.

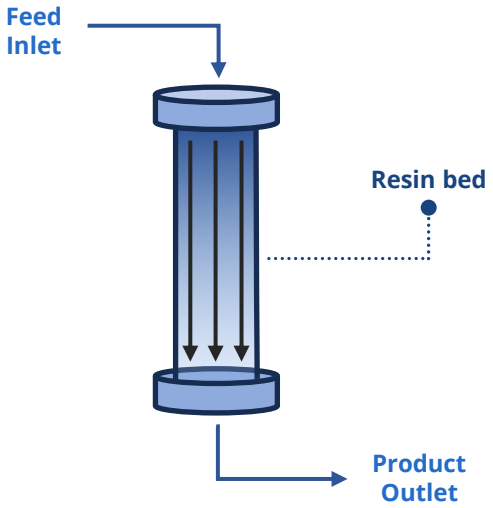
ARi has experience optimizing these factors and designing ion exchange systems in a variety of applications including protein separations for the food, dairy, and precision fermentation industries. ARi partners with each customer to develop a customized ion exchange solution that is optimized for their specific biomolecules of interest.



ARi ION-EXCHANGE TECHNOLOGIES

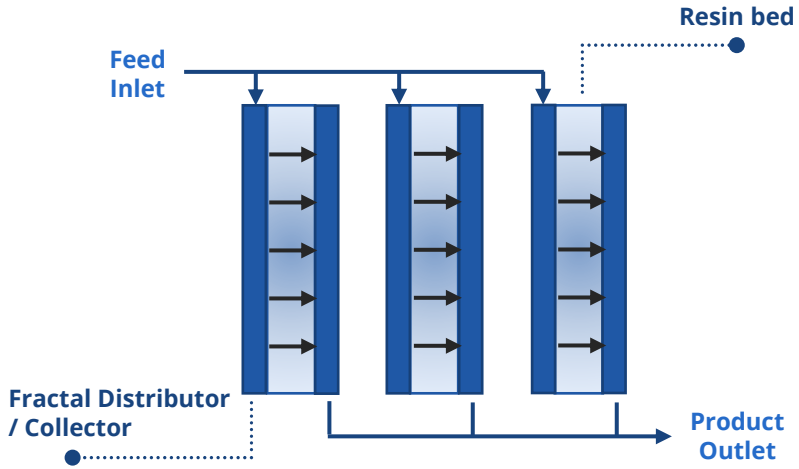
Conventional IEX Column

A conventional column design for IEX applications typically consists of a hollow cylinder that is filled with resin. During operation, the various process fluids travel vertically through the length of the column. Using a conventional ion exchange column is a simple and effective method to perform IEX separations from lab to industrial scale. A column can be easier to work with and maintain compared to more sophisticated IEX technologies. This makes them ideal for rapid R&D testing to study a wide range of operating conditions efficiently. ARi offers conventional column designs for applications where the system's pressure drop and/or binding kinetics are not major limiting factors.



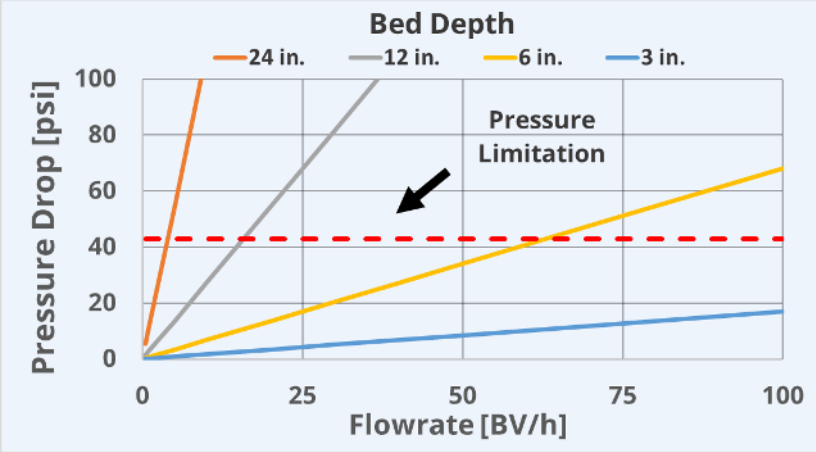
Fractal Pack IEX System

ARi's patented Fractal Pack is an efficient solution for shallow bed ion exchange and adsorption processes that use 150 µm or smaller resin beads with fast binding kinetics. A Fractal Pack system consists of shallow resin beds, typically 3 to 24 inches in depth, with a Fractal Distributor and a Fractal Collector on either side of each bed. This design allows the adsorbent volume of a large ion exchange column to be condensed into a pack of multiple shallow beds with low pressure drop. The higher flowrates achievable using a Fractal Pack enable higher resin productivity, reducing the resin requirements compared to conventional columns in certain applications.



SHALLOW BED LIMITATIONS

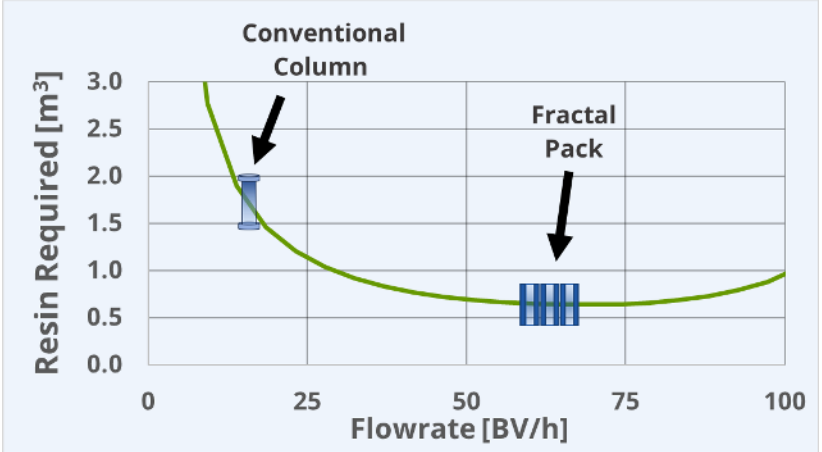
Many protein separations require the use of fragile resins with fine particle sizes to improve binding kinetics. For these separations, a shallow resin bed must be used to minimize pressure drop and avoid damaging the expensive resin. In certain cases, a conventional column design cannot practically be condensed to a small-enough bed depth to reduce the pressure drop to an acceptable level while maintaining an optimal flowrate. Thus, the flowrate must be reduced from the optimal value, leading to inefficient operation and reduced throughput.



To demonstrate the limitation of shallow bed conventional columns, consider a case study of separating proteins using a 100 µm particle size resin. Here, the system loading is maximized at a flowrate of 60 BV/h. However, a typical industrial IEX column has a minimum bed depth of about 12 inches and the pressure drop with this bed depth limits the flowrate to values much lower than 60 BV/h. Because of this, the conventional column system must be **2.7x larger** than an optimal system and incur **2.7x the resin cost**.

FRACTAL PACK ADVANTAGES

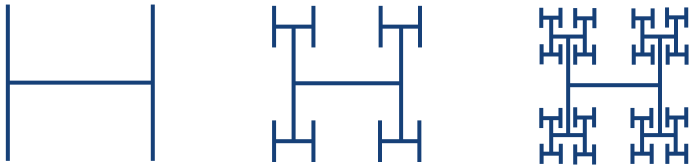
Because the Fractal Pack is a modular system consisting of multiple shallow resin beds, it has a wider range of possible bed depths and operating flowrates than a conventional column. For many protein separations, the Fractal Pack's larger range of flowrates enables it to operate at a higher overall throughput and productivity, minimizing the system size and total resin inventory required for the application, yielding significant cost savings



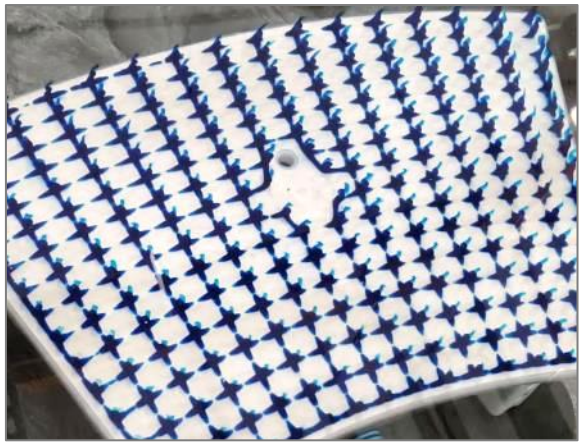
Smaller resin beads	Low capital cost
Low pressure drop	Modular for easy expansion
High resin loading	Small footprint
Simple and rapid resin removal	Easily scaled to any size
Sanitary design	Minimal peripheral equipment

FRACTAL FLUID DISTRIBUTION

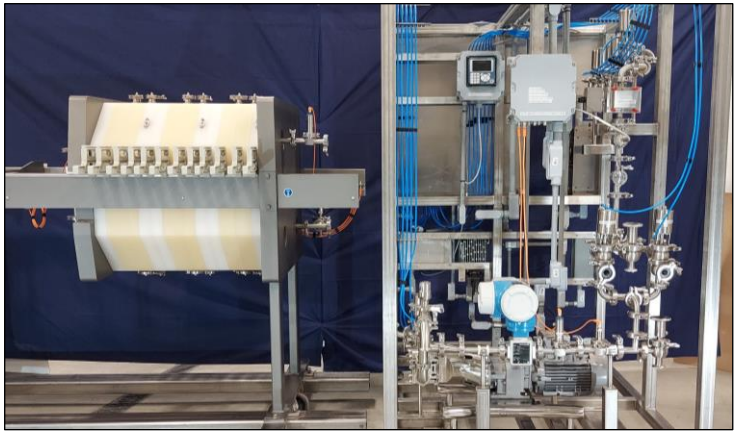
Fractals are self-similar structures, exhibiting a repeated geometric pattern at many scales. The “H” pattern below is an example of a fractal, as the pattern is repeated through three iterations and decreases in size with each repetition.



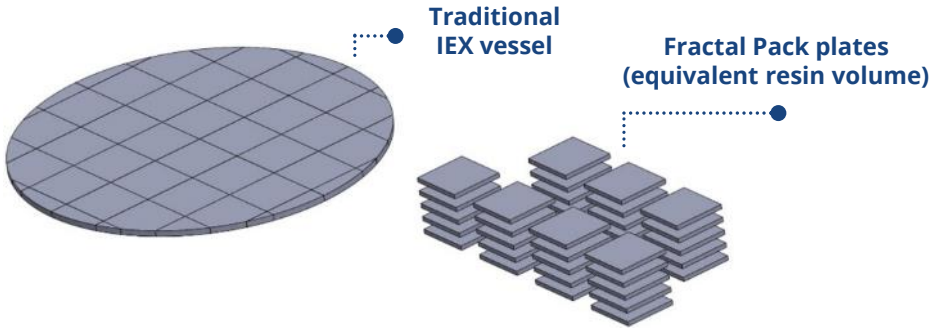
ARi has pioneered and perfected the use of fractal-based fluid distribution technology in both conventional IEX and Fractal Pack systems. A Fractal Distributor provides an effective alternative to technologies based on the use of pressure drop to achieve fluid distribution. The symmetry of fractals provides consistent scaling to all equipment sizes (unlike distribution methods employing pressure drop) and guarantees uniform distribution of fluid across the surface of a resin bed with very low pressure drop.



A plug of blue dye exiting the outlets of an ARi Fractal Distributor. The symmetry of fractals provides plug flow characteristics, ensuring that the dye uniformly exits each outlet at precisely the same time.



Sanitary Fractal Pack pilot system containing multiple resin beds



The uniform flow distribution provided by ARi’s Fractal Distributors maximizes utilization of the resin or adsorbent, allowing for the use of beds as shallow as 3 – 6 inches.

ARi’s Fractal Pack ion exchange system converts the large surface area of a traditional column to a compact set of plates with equivalent resin volume, significantly reducing the required footprint

The low pressure drop of shallow beds enables the use of smaller resin beads with improved mass transfer kinetics. This allows for higher resin loading and increased regeneration efficiency.

LACTOFERRIN PURIFICATION

ARi's Fractal Pack system is ideally suited for protein separation applications and has been commercially implemented for the purification of lactoferrin, a valuable protein present in dairy process streams such as milk and whey.

Because the intrinsic charge characteristics of lactoferrin are considerably different compared to most other whey proteins, it is possible to separate lactoferrin into a pure fraction using a simple IEX process. The Fractal Pack IEX system has been tested in a demonstration-scale trial at a U.S. dairy plant and successfully separated lactoferrin from both whey and skim milk using cation exchange chromatography.

Although ARi's lactoferrin separation technology has been proven to be effective in the dairy industry, testing with a customer's specific feed material is recommended to optimize the process and offer the lowest-cost solution.

Fractal Pack Lactoferrin System Performance	
Feedstock	Sweet Whey, Skim Milk
Lactoferrin Product Purity	85-90% purity

Testing in a U.S. dairy with a previous prototype Fractal Pack system

PROTEIN SEPARATIONS

ARi has experience developing and optimizing IEX processes to separate various proteins from impurities, or to purify specific proteins from dairy and fermentation sources.

Small differences between individual protein molecules and between non-protein impurities have been exploited to produce purified protein products via IEX that are:

- **Free of unwanted impurities**
- **Greater than 95% pure**
- **Tailored for specific ratios of proteins within a purified mixture**

ARi is able to develop customized protein separation solutions that are specific to each customer's particular needs. The operational flexibility of ARi's IEX systems, such as the Fractal Pack, allows for process modifications to tune the system's productivity and product composition.

Proteins Separated by Industrial Ion-exchange	
β-lactoglobulin	**Osteopontin**
α-lactalbumin	**Caseins**
Bovine Serum Albumin	**Tannins**
Immunoglobulins	**Procyanidins**
Lactoferrin	**Enzymes**
Lactoperoxidase	**Antibodies**

WORKING WITH US

ARi develops customized ion exchange solutions for new applications. ARi provides a wide spectrum of support for your unique process, from lab-scale testing to industrial installation.

Provide **proof of concept** information

LABORATORY-SCALE

Generate data to **model the separation**

Generate engineering data for scale-up

PILOT-SCALE

Demonstrate the separation process at a **small scale**

ARi's IEX systems can be **reliably scaled up** to industrial capacities

INDUSTRIAL-SCALE

Consistent performance is maintained through uniform fluid distribution

On-site support for troubleshooting and optimization

TECHNICAL SUPPORT

Onsite **technical audits and training**

CHROMATOGRAPHY SYSTEMS INSTALLED IN MORE THAN 20 COUNTRIES WORLDWIDE

AMALGAMATED RESEARCH LLC
2531 Orchard Dr. E.
Twin Falls, Idaho, 83301
USA

P: +1 (208) 735-5400
F: +1 (208) 733-8604
info@arifractal.com
www.arifractal.com

AMALGAMATED RESEARCH LLC