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(71) Applicant: **3M INNOVATIVE PROPERTIES COMPANY** [US/US]; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US).

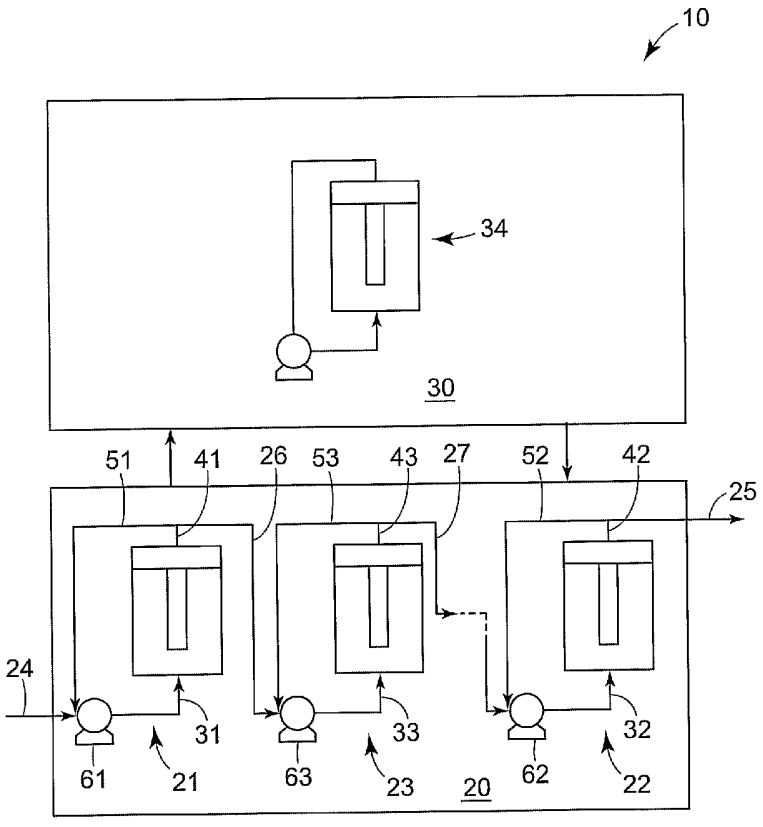
(72) Inventors: **RABINS, Andrew W.,**; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US). **GIBBENS, Kelly J.,**; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US). **NAKAMURA, Masayuki**; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US). **SESHADRI, Kannan**; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US). **FITZSIMONS, Robert T., Jr.**; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US). **CARSON, Larry J.,**; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US). **LARSEN, Stephen M.,**; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US).

(74) Agents: **LOWN, Jean A.,** et al.; 3M Center, Office of Intellectual Property Counsel, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US).

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(54) Title: METHOD AND APPARATUS FOR SEPARATING A TARGET MOLECULE FROM A LIQUID MIXTURE



(57) Abstract: A simulated moving bed apparatus and methods are described for continuously separating a target molecule from a liquid mixture, using a simulated moving bed system. The simulated moving bed system includes a plurality of filter cartridge modules in serial fluid communication. Each filter cartridge module includes a volume of stationary phase particulates adjacent a porous substrate layer. Each filter cartridge module also includes recirculation piping in fluid connection with a filter cartridge outlet and a filter cartridge inlet.

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METHOD AND APPARATUS FOR SEPARATING A TARGET MOLECULE FROM A LIQUID MIXTURE

Cross-Reference

This application claims the benefit under U.S.C. § 119(e) of U.S. Provisional No. 60/615,500 filed 01 October 2004.

Background

The present invention relates to methods and apparatus for separating a target molecule from a liquid mixture using a simulated moving bed including a plurality of cartridge filter modules.

Target molecules such as, for example, biomacromolecules are constituents or products of living cells and include, for example, proteins, carbohydrates, lipids, and nucleic acids. Detection and quantification as well as isolation and purification of these materials have long been objectives of investigators. Detection and quantification are important diagnostically, for example, as indicators of various physiological conditions such as diseases. Isolation and purification of biomacromolecules are important for therapeutic purposes such as when administered to patients having.a deficiency in the particular biomacromolecule or when utilized as a biocompatible carrier of some medicament, and in biomedical research. Biomacromolecules such as enzymes, which are a special class of proteins capable of catalyzing chemical reactions are also useful industrially; enzymes have been isolated, purified, and then utilized for the production of sweeteners, antibiotics, and a variety of organic compounds such as ethanol, acetic acid, lysine, aspartic acid, and biologically useful products such as antibodies and steroids.

In their native state in vivo, structures and corresponding biological activities of these biomacromolecules are maintained generally within fairly narrow ranges of pH and ionic strength. Consequently, any separation and purification operation must take such factors into account in order for the resultant, processed biomacromolecule to have potency.

Chromatography is a separation and purification operation that can be performed on biological product mixtures. It is a technique based on the interchange of a solute between a moving phase, which can be a gas or liquid, and a stationary phase. Separation of various solutes of the solution mixture is accomplished because of varying binding interactions of each solute with the stationary phase; stronger binding interactions generally result in longer retention times when subjected to the dissociation or displacement effects of a mobile phase compared to solutes which interact less strongly and, in this fashion, separation and purification can be effected.

Most current capture or purification chromatography is done via conventional column techniques. These techniques have severe bottlenecking issues in downstream purification, as the throughput using this technology is low. Attempts to alleviate these issues include increasing the diameter of the chromatography column, but this in turn creates challenges due to difficulties of packing the columns effectively and reproducibly. Larger column diameters also increase the occurrence of problematic channeling. Also, in a conventional chromatographic column, the absorption operation is shut down when a breakthrough of the desired product above a specific level is detected. This causes the dynamic or effective capacity of the adsorption media to be significantly less than the overall or static capacity. This reduction in effectiveness has severe economic consequences, given the high cost of some chromatographic resins.

Summary

Generally, the present invention relates to a simulated moving bed apparatus and methods for continuously separating a target molecule from a solution mixture, using a simulated moving bed system. The simulated moving bed apparatus includes a plurality of filter cartridge modules in serial fluid communication. Each filter cartridge module includes a volume of stationary phase particulates adjacent a porous substrate layer. Each filter cartridge module also includes recirculation piping in fluid connection with a filter cartridge outlet and a filter cartridge inlet.

In one embodiment, a method of continuously separating a target molecule from a solution mixture includes providing a simulated moving bed apparatus including a plurality of filter cartridge modules, flowing a solution mixture including a target molecule

continuously through the plurality of filter cartridge modules, binding the target molecule to the volume of stationary phase particulates to form a target molecule:stationary phase particulate product, removing a loaded filter cartridge module containing the target molecule:stationary phase particulate product from the solution mixture flow, separating the target molecule from the target molecule:stationary phase particulate product in the loaded filter cartridge to form an isolated target molecule product and a regenerated filter cartridge, and adding the regenerated filter cartridge module to the solution mixture flow. Each filter cartridge module includes a volume of stationary phase particulates adjacent a porous substrate layer, a filter cartridge module inlet, a filter cartridge module outlet and filter cartridge module recirculation piping connecting the filter cartridge module outlet and the filter cartridge module inlet. The plurality of filter cartridge modules are in serial fluid connection. A recirculation portion of the solution mixture flow is recirculated from the filter cartridge module outlet to the filter cartridge module inlet and a remaining portion of the solution mixture flows to a subsequent filter cartridge module inlet.

In another embodiment, a simulated moving bed apparatus for continuously separating a target molecule from a solution mixture includes a capture zone and a recovery zone. The capture zone includes a plurality of filter cartridge modules connected in series, including a first loaded filter cartridge module and a final regenerated filter cartridge module. The first loaded filter cartridge module is connected to a solution mixture feed source and the final regenerated filter cartridge module is connected to a target molecule depleted solution mixture vessel. Each filter cartridge module includes a volume of stationary phase particulates capable of binding with a target molecule adjacent a porous substrate layer, a filter cartridge module inlet, a filter cartridge module outlet, and filter cartridge module recirculation piping connecting the filter cartridge module outlet to the filter cartridge module inlet. The filter cartridge module outlet is also connected to a filter cartridge module inlet of a subsequent downstream filter cartridge module. The recovery zone is where the target molecule in a loaded filter cartridge module is separated from the volume of stationary phase particulates to form a regenerated filter cartridge module. The recovery zone includes at least one filter cartridge module.

The above summary of the present invention is not intended to describe each disclosed embodiment or every implementation of the present invention. The Figures,

Detailed Description, and Examples that follow more particularly exemplify these embodiments.

Brief Description of the Drawings

The invention may be more completely understood in consideration of the following detailed description of various embodiments of the invention in connection with the accompanying drawings, in which:

FIG. 1 is a schematic illustrative diagram of a simulated moving bed;

FIG. 2 is a schematic illustration of a cross-section of a composite filtration medium;

FIG. 3 is a perspective view of an illustrative composite filtration medium;

FIG. 4 is a perspective view of an illustrative cylindrically pleated filter element;

FIG. 5 is a perspective view of a cylindrical filter cartridge;

FIG. 6 is a schematic illustrative diagram of a simulated moving bed described in Example 1; and

FIG. 7 is a schematic illustrative diagram of a simulated moving bed described in Examples 2, 3, 4 and 5.

While the invention is amenable to various modifications and alternative forms, specifics thereof have been shown by way of example in the drawings and will be described in detail. It should be understood, however, that the intention is not to limit the invention to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention.

Detailed Description

For the following defined terms, these definitions shall be applied, unless a different definition is given in the claims or elsewhere in this specification.

The term "filtration layer" or "porous substrate layer" refers to a sheet-like woven or nonwoven porous material which can comprise one or more individual layers which can be combined to provide a single sheet; the average pore size can be greater than 1 micrometer and up to 50 micrometers.

The term "composite filtration medium" refers to a filtration layer comprising a layer of stationary phase particulates located on the upstream surface thereof; the medium can sustain a flux rate of at least 0.01 cm/min at a filter cartridge pressure of at most 0.25 MegaPascals (MPa).

The term "filter element" or "filtering element" or "filtration element" refers to a composite filtration medium configured for fluid passage.

The term "filter cartridge" refers to a dead end filtering device which is preferably cylindrical in shape.

The term "filter cartridge housing" refers to a support structure for a filter cartridge.

The term "separation filter assembly" refers to a housing containing a dead end filter cartridge comprising a composite filter medium on the upstream surface of which are located stationary phase particulates.

The term "dead end filter" or "dead end filtering device" refers to a filtering element in which 100% of the fluid goes through the filter.

The term "flux rate" refers to the velocity of a liquid stream passing through a filtering element and is equal to flow rate divided by the surface area of the filtration layer. Described in this way, flow of a liquid stream can be characterized and is independent of the size of the filtration layer. Flux rate also contributes to pressure drop across a filter, i.e., increased flux rates generally mean increased system pressures. In commercial filter cartridge applications, it may be desirable to provide a filter of minimum size which will process a maximum amount of liquid stream. Therefore, it is desirable that flux rate be increased by increasing the flow rate.

The term "stationary phase particulates" refers to insoluble particulates that can form a binding association with a target molecule of interest in a solution mixture. Specific binding associations can include: adsorption, ion exchange, hydrophobic, and affinity interactions.

The term "target molecule" refers to one or more chemical species for which the simulated moving bed apparatus described herein is designed to separate from a liquid feed stream or solution mixture feed stream. Target molecules can include, for example, pharmaceutical species; biomacromolecules such as, proteins and antibodies (monoclonal

or polyclonal), DNA, and RNA, expressed by bacterial, yeast, mammalian, plant, or insect cells; minerals; and manmade chemical species such as, for example, synthetic small organic molecules, peptides and polypeptides, oligosaccharides, and sugar modified proteins. In some embodiments, the target molecule can be one or more impurities or waste products, including proteins; inorganic species such as metals, metal ions, or ions such as carbonates, sulfates, oxides, phosphates, bicarbonates, and other ions commonly found in industrial, residential and biological feed streams; small organic molecules such as those that comprise, but are not limited to, dyes, pesticides, fertilizers, additives, and stabilizers; process byproducts and pollutants; DNA, RNA, phospholipids, viruses, or other cell debris from a bioprocess. In still a further embodiment, leached ligands such as, for example, protein A from an upstream affinity separation process could also be a target molecule. In other embodiments, the simulated moving bed apparatus described herein could be used to remove various chemical or biological species from a waste or drinking water stream, either via adsorption or enzymatic reaction, for example.

The term "insoluble" refers to not more than 1 part solid dissolved in 100 parts of solvent at 23 degree Celsius.

The term "filter cartridge differential pressure" refers to the difference between inlet, or upstream, and outlet, or downstream, pressures across the filter cartridge unit in a separation system.

The term "bed height" is defined as the volume of stationary phase particulate divided by the porous substrate surface area.

The recitation of numerical ranges by endpoints includes all numbers subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, and 5).

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition containing "a compound" includes a mixture of two or more compounds. As used in this specification and the appended claims, the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

Unless otherwise indicated, all numbers expressing quantities of ingredients, measurement of properties and so forth used in the specification and claims are to be

understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the foregoing specification and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by those skilled in the art utilizing the teachings of the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviations found in their respective testing measurements.

Simulated moving bed chromatography is a relatively recent technology where a series of packed columns are indexed or rotated (physically or with valving) through a system feeding a solution with a desired product or target molecule. As the packed columns are saturated, the system is indexed moving the saturated column to a recovery zone where it is washed, eluted, equilibrated and optionally regenerated. An equilibrated column is then placed on-line at the end of the column series. With conventional column chromatography, users are faced with two constraints, low throughput, and poor usage of chromatography resin due to dynamic breakthrough concerns.

In some embodiments, the simulated moving bed (SMB) system described herein is suitable for separating antibodies, immunoreactive compounds, or other proteins from a clarified fermentation broth stream, to name a few. It is intended for use as an isolation (e.g. volume reduction) and purification step. The fermentation broth can be passed through the SMB system after it has been clarified. This means that the cells, support, and cell debris has been at least mostly removed by a separation process such as centrifugation, dead end microfiltration, or tangential flow microfiltration, for example.

FIG. 1 is a schematic illustrative diagram of a simulated moving bed **10**. The simulated moving bed **10** includes a capture zone **20** and a recovery zone **30**. The capture zone **20** includes a plurality of filter cartridge modules, including, for example, a first filter cartridge module **21**, a final filter cartridge module **22** and an optional intermediate filter

cartridge module **23**. In some embodiments, the plurality of filter cartridge modules are each "dead end" filtering devices.

These filter cartridges are connected in series and in fluid connection (serial fluid connection) via feed forward lines **26** and **27**, for example. Each filter cartridge module contains a volume of stationary phase particulates capable of binding with a target molecule. The filter cartridge modules are described in greater detail below. In operation, the first filter cartridge module **21** can be termed the "loaded" filter cartridge module and refers to the filter cartridge module that contains a highest level of target molecule captured on the solid phase. The first filter cartridge module **21** is connected to a feed source **24**.

The recovery zone **30** includes at least one filter cartridge module **34** that is initially termed a saturated or "loaded" filter cartridge module that has been moved out of the capture zone **20** either physically or by valving. The recovery zone **30** regenerates the filter cartridge module **34** to form a regenerated filter cartridge module. The term "regenerated filter cartridge module" refers to a filter cartridge module that has been washed, eluted, optionally cleaned, and re-equilibrated. The wash step removes the solution mixture from the loaded filter cartridge module. The elution step removes the target molecule from the stationary phase for further processing, if desired. The cleaning step removes impurities or trace contaminants from the filter cartridge module. Re-equilibration can involve a buffer exchange to allow the regenerated filter cartridge module to be reintroduced into the capture zone. In some embodiments, at least a portion of liquids from the recovery zone **30** can be introduced into the feed source stream **24**, as desired.

The regenerated filter cartridge module can be moved back into the capture zone **20**, either physically or with manifolds and valving. The regenerated filter cartridge module can be placed as the final filter cartridge module **22**. The filter cartridge modules (solid phase) can thus be indexed countercurrent to the solution mixture flow.

The capture zone **20** can include 0, 1, 2, 3, 4, 5, 6, 7, or 8 or more intermediate filter cartridge modules **23** in serial fluid connection. The recovery zone **30** can include 0, 1, 2, 3, 4 or more filter cartridge modules. The recovery zone includes 0 modules if, for example, the cartridge is used for contamination removal and the cartridge is disposable.

The first or "loaded" filter cartridge module **21** includes a fluid inlet **31**, a fluid outlet **41**, recirculation piping **51** in fluid connection with the fluid inlet **31** and the fluid outlet **41**, and a pump **61** in fluid connection with the fluid inlet **31**, the fluid outlet **41** and the feed source **24**. The pump **61** can provide adequate fluid flow through the filter cartridge module **21**. A feed forward line **26** allows at least a portion of the fluid flow to flow forward to the next filter cartridge module (**23** in **FIG. 1**) in series.

The final or "regenerated" filter cartridge module **22** includes a fluid inlet **32**, a fluid outlet **42**, recirculation piping **52** in fluid connection with the fluid inlet **32** and the fluid outlet **42**, and a pump **62** in fluid connection with the fluid inlet **32**, the fluid outlet **42** and a feed forward line **27** from a previous filter cartridge module in series. The pump **62** can provide adequate fluid flow through the filter cartridge module **22**. The fluid outlet **42** is shown in fluid connection with a target molecule depleted solution mixture stream line **25**. This target molecule depleted solution mixture stream line **25** can be in fluid connection with a target molecule depleted solution mixture vessel (not shown.).

The optional intermediate cartridge module **23** includes a fluid inlet **33**, a fluid outlet **43**, recirculation piping **53** in fluid connection with the fluid inlet **33** and the fluid outlet **43**, and a pump **63** in fluid connection with the fluid inlet **33**, the fluid outlet **43** and a feed forward line **26** from a previous filter cartridge module in series. The pump **63** can provide adequate fluid flow through the filter cartridge module **23**.

In one embodiment, a method of continuously separating a target molecule from a solution mixture includes providing a simulated moving bed apparatus including a plurality of filter cartridge modules, flowing a solution mixture including a target molecule continuously through the plurality of filter cartridge modules, binding the target molecule to the volume of stationary phase particulates to form a target molecule:stationary phase particulate product, removing a loaded filter cartridge module containing the target molecule:stationary phase particulate product from the solution mixture flow, separating the target molecule from the target molecule:stationary phase particulate product from the loaded filter cartridge to form an isolated target molecule product and a regenerated filter cartridge, and adding the regenerated filter cartridge module to the solution mixture flow. Each filter cartridge module includes a volume of stationary phase particulates adjacent a porous substrate (i.e., filtration) layer, a filter cartridge module inlet, a filter cartridge

module outlet and filter cartridge module recirculation piping connecting the filter cartridge module outlet and the filter cartridge module inlet. The plurality of filter cartridge modules are in serial fluid connection. A recirculation portion of the solution mixture flow is recirculated from the filter cartridge module outlet to the filter cartridge module inlet and a remaining portion of the solution mixture flows to a subsequent filter cartridge module inlet, such as the filter cartridge module inlet next in series.

In one illustrative embodiment, the removing step and the adding step occur simultaneously. In another illustrative embodiment, the removing step, the flowing step, and the adding step occur simultaneously. In another illustrative embodiment, the separating step and the binding step occur simultaneously. In still another illustrative embodiment, the removing step, the separating step, the flowing step, the binding step, and the adding step occur simultaneously.

While one pump is shown associated with one filter cartridge module, it is understood that more than one pump can be associated with each filter cartridge module. In addition, it is also understood that more than one filter cartridge module can be associated with each pump. In some embodiments no pump may be used.

Each filter cartridge module in the capture zone has a solution mixture flowing through it at a specified rate defined as liters/minute of solution mixture per liter of stationary phase particulate in the filter cartridge module. This flow rate can be any useful value. In some embodiments, this flow rate is at least 3 liter/minute per liter of stationary phase particulate, or at least 5 liter/minute per liter of stationary phase particulate, or at least 7 liter/minute per liter of stationary phase particulate, or from 3 to 20 liter/minute per liter, or from 5 to 20 liter/minute per liter, or from 7 to 20 liter/minute per liter.

Each filter cartridge module in the capture zone can recirculate a portion of the solution mixture exiting the filter cartridge module back to the inlet of the same filter cartridge module and allow the remaining solution mixture flow portion to proceed to a subsequent filter cartridge module for further separation. This recirculation portion can be expressed as a recirculation percent of total flow through the filter cartridge module. This recirculation percent can be any useful amount such as, for example, 25% or greater, 50% or greater, 60% or greater, 70% or greater, 75% or greater, 80% or greater, 85% or greater, 90% or greater, or 95% or greater; or 50 to 95%, or 75 to 95%, or 80 to 95%.

Each filter cartridge module in the capture zone has a solution mixture pressure drop or differential pressure across the filter cartridge inlet and the filter cartridge outlet. In some embodiments, this pressure drop is 172 kPa (25 psi) or less, 138 kPa (20 psi) or less, 103 kPa (15 psi) or less, 69 kPa (10 psi) or less, or 34 kPa (5 psi) or less. In an illustrative embodiment, the filter cartridge module has a flow rate of at least 7 liter/minute per liter of stationary phase particulate (mean diameter from 5 to 30 micrometers) with a pressure drop of 103 kPa (15 psi) or less. In another illustrative embodiment, the filter cartridge module has a flow rate of at least 5 liter/minute per liter of stationary phase particulate (mean diameter from 5 to 30 micrometers) with a pressure drop of 69 kPa (10 psi) or less. In a further illustrative embodiment, the filter cartridge module has a flow rate of at least 3 liter/minute per liter of stationary phase particulate (mean diameter from 5 to 30 micrometers) with a pressure drop of 34 kPa (5 psi) or less.

The filter cartridge modules described herein differ from conventional chromatography packed columns in at least several respects. Rather than a tube filled with media operated at a low flow rate, the filter cartridge modules depend on a relatively thin layer of media supported on a porous membrane (bed height) operated at a high flow rate. This allows for a lower pressure drop, better external mass transfer, as well as the use of smaller media particles than can be employed with traditional chromatography packed columns.

In many embodiments, the filter cartridge module has a stationary phase particulate bed height (volume of stationary phase particulate divided by the porous substrate surface area) of less than 1 centimeter or in a range from 0.02 to 0.20 centimeters, or from 0.02 to 0.14 centimeters or from 0.04 to 0.10 centimeters, or from 0.05 to 0.08 centimeters, as desired. In illustrative embodiments, the porous substrate surface area is in a range from 100,000 to 200,000 square centimeters.

In one illustrative embodiment, the filter cartridge has a height of one meter with 15 liters of protein A beads, having a mean diameter from 5 to 30 micrometers and a bed height from 0.04 to 0.10 centimeters, supported on the porous substrate.

FIG. 2 is a schematic illustration of a cross-section of a composite filtration medium **110** including an illustrative nonwoven web as a surface filtration layer **111** which can be one or more individual layers, upon the upstream surface of which are located

insoluble stationary phase particulates **112**. The filtration layer **110** can include an upstream pre-filter layer **113**, filtration layers **114** that can include a plurality of filtration layers having increasingly finer downstream porosity, and a downstream cover layer **115**.

FIG. 3 is a perspective view of a nonpleated portion of a pattern of embossed shapes **122** on composite filtration medium **120** utilized to produce an illustrative embodiment of the filter cartridge module. Embossing can be conducted to increase surface area and more completely define the surface filtering element. The insoluble stationary phase particulates are omitted from the illustration for clarity.

FIG. 4 is a perspective view of an illustrative embodiment of one longitudinally extended cylindrically pleated filter element **130**. Radial pleats **132** of composite filtration (porous substrate) element **130** are shown, and stationary phase particulates **112** are omitted for clarity. In some embodiments, the composite filtration element **130** includes 10 or more, 20 or more, 30 or more, 40 or more, or 50 or more pleats **132**.

FIG. 5 is a perspective view which illustrates one embodiment of inner and outer supplemental support members for the composite filtration element **130** (not shown) within a cylindrical filter cartridge **140**. External support structure **141**, such as a scrim or screen with a plurality of holes, can provide additional support in an inward-out fluid flow mode to reduce the likelihood of rupturing the filter element. Similarly, inner support structure **142** consisting of a scrim or screen, or a porous casing or similar construction can provide support to prevent the filter element **130** (not shown) from collapsing under high pressure applications in an outward-in fluid flow situation. In both cases, the supplemental support structures can be attached to endpieces **143** of the filter cartridge to provide an integral unit. One example of a useful filter cartridge module is described in U.S. Patent 5,468,847.

Referring to **FIG. 2**, the stationary phase particulates **112** bind or strongly associate with the target molecule of interest in solution mixtures by one or a combination of the following interactions: adsorption, ion exchange, hydrophobic association, size exclusion, and/or affinity binding.

A partial listing of adsorption stationary phase particulates include: hydroxyapatite (available from BioRad Laboratories, Richmond, CA), alumina (available from EM separations, Gibbstown, NJ), silicagel (available from EM Separations), silica (available

from Celite), zirconia (disclosed in U.S. 5,015,373), zeolites, montmorrilonite clays, titania, zinc hydroxide, and polymer coated zirconia.

A partial listing of ion exchange stationary phase particulates are commercially available, for example, from Amersham, Toso Haas, EM Separations, J T Baker, and Ciphergen. Suitable ion exchange resins include: agarose, dextran, polyacrylic, polyimine, vinyl polymers, acrylamide polymers, polysaccharides, cellulosic polymers, and other polymers such as polystyrene modified with divinylbenzene, acrylate/ethylene glycol co-polymers, respectively modified to contain diaminoethyl, quaternary ammonium groups in case of anion exchange and carboxymethyl, sulfo and sulfopropyl groups for cation exchange. Other supports for ion exchange resins include silica, both neat and stabilized with inorganic species such as zirconia. Mixed function adsorbents include, for example dipolar ion exchange adsorbents (sulfanilic acid coupled to epoxy activated agarose). Polyethyleneimine can be used to purify nucleic acids, and precipitates nucleoproteins. Hydrophobic interaction particulates can contain short aliphatic chain ($C_4 - C_{10}$) and phenyl groups, while reverse phase adsorbents containing longer aliphatic chains (C_8-C_{18}), can be used for smaller proteins. Reverse phase chromatography supports include those based on silica coupled with organosilanes, titania, poly(styrene-divinylbenzene), aluminum oxide, etc.

A partial listing of size exclusion particulates include: porous glass, silica based supports modified by diols, diol-bonded silica subsequently modified by metal oxides such as zirconia, polymer based particles containing crosslinked agarose and dextran, or both; allyl dextran crosslinked with methylene bis-acrylamide, polymers synthesized as tentacle polymers (Fractogel from Merck), cellulose, polyhydroxymethacrylate, crosslinked polysaccharides, and copolymerized acrylates.

Affinity chromatography particulates are commercially available from Amersham, Millipore, Tosoh, EM Separations, Ciphergen, and Applied Biosystems, among others. The base supports can include agarose, cellulose and vinyl polymers. Some particulates may also contain inorganic supports in the core such as silica (ProSep from Millipore) and stainless steel (Streamline from Amersham), or composites (such as ceramic and acrylic from BioSepra). The base supports can be modified or provided with groups that can be used to immobilize ligands. These groups include azlactone, CNBr, epoxy, hydrazides,

NHS, tresyl, thiophilic groups, and hydroxyapatite. The ligands (with specific biomacromolecule affinities) can be chosen from a wide-ranging list including: arginine and benzamidine (serine proteases); calmodulin (ATPases, protein kinases, phosphodiesterases, and neurotransmitters); gelatin (fibronectins); glutathione(transferases, glutathione dependent proteins, and fusion proteins); heparin (growth factors, coagulation proteins, steroid receptors, restriction endonucleases, lipoproteins, and lipases); Proteins A and G (IgG and subclasses); Protein A mimetic synthetic ligands (IgG, IgM, and other immunoglobulins); L-lysine (plasminogen, plasminogen activator, and ribosomal RNA); concanavalin A and lectins (glycoproteins, membrane proteins, glycolipids, and polysaccharides); DNA (DNA polymerase, RNA polymerase, T-4 polynucleotide kinase, and exonucleases); and AMP, ADP, NADH and NADPH based chromatography (enzymes such as NADH- and NADPH-dependent dehydrogenases, reductases, kinases, phosphatases, and transferases).

Other affinity methods include those without a biospecific interaction, such as immobilized metals such as Fe, Co, Ni, Cu, Al and Zn that form the basis of IMAC (immobilized metal affinity chromatography) wherein the purification is by the formation of coordinate bonds. Other affinity matrices use thiophilic ligands such as vinyl sulfone linked mercaptoethanol, pyridyl disulfide linkages, etc., that have affinity for γ-globulins and monoclonal antibodies.

Affinity matrices can also be utilized for separation of cells (cell affinity chromatography or CAC). Matrices for CAC include agarose, cellulose, dextran, polyacrylamide, glass, polystyrene, trisacryl (modified so as to have reactive groups such as –OH, -OCN, -CH-CH_2O, -NH_2, to couple specific proteins such as avidin, IgG, anti-IgG, anti-IgE, etc. to capture specific cell types such as lymphocytes, stem cells, neuronal cells, mast cells, basophils, erythrocytes, etc.). Other variations include the use of a tentacle approach to couple ligands to grafted polymer chains (such as Fractogel from Merck, which is a polyglycidylmethacrylate grafted gel); the ligands could be from any of those described above, and could be coupled either directly (for thiophilic adsorption/IMAC) or indirectly (for dye-ligand affinity chromatography through amino intermediates, or immobilized proteins via azlactone intermediates)

Particulates for ultrafiltration and concentration such as Sephadex G-25 (commercially available from Pharmacia), polyethylene glycol, or carboxymethyl cellulose can also be used. These materials may be loaded into the cartridge filter module in a slurry form, which may be made from the particulates as received, or may require preswelling in some solvents or buffers, or may have to be pretreated.

The sizes of the stationary phase particulates useful in the invention can have a mean diameter that ranges from 1 to 200 micrometers, or 3 to 50 micrometers, or 5 to 40 micrometers, or 5 to 30 micrometers, or 5 to 15 micrometers. Illustrative stationary phase particulates include, for example, ProSep-A™ Ultra resin, commercially available from Millipore Inc., (Billerica, MA), and MabSelect™ resin, commercially available from GE Healthcare (Chalfont St. Giles, UK). In some embodiments, each filter cartridge module includes 5 to 25 liters of stationary phase particulates, or 10 to 20 liters of stationary phase particulates.

The present invention should not be considered limited to the particular examples described herein, but rather should be understood to cover all aspects of the invention as fairly set out in the attached claims. Various modifications, equivalent processes, as well as numerous structures to which the present invention can be applicable will be readily apparent to those of skill in the art to which the present invention is directed upon review of the instant specification.

EXAMPLES

Examples 1-3 are based on results from mathematical modeling. They are meant to illustrate how a system would perform. Although some of our assumptions and the parameters from the model are based on experimental data, the Examples as a whole are based on a prediction from a model.

The following Examples disclose a system to purify a monoclonal antibody by Protein A affinity separation. The filter cartridge modules described in each example was composed of a filter cartridge, such as the horizontally pleated filter 743B made commercially available from 3M (St. Paul, MN), a cartridge filter housing, and a volume of solid material (stationary phase particulate product) used for the separation. The solid material used for the separation in Examples 1-3 was 15 liters of ProSep-A Ultra™ resin. As shown in **FIGs. 6** and **7**, corresponding with each filter cartridge module, there is a pump, an inlet stream, and an outlet stream. The outlet stream is split into two streams, one stream is recirculated back just upstream of the pump for that filter cartridge module and the other is the feed stream for the next filter cartridge module (except for the final filter cartridge module.) The liquid volume of each filter cartridge module and associated piping was estimated to be 50 L. Four filter cartridge modules are connected in series for the capture step for Example 1 (**FIG. 6**). Six filter cartridge modules are connected in series for the capture step for Examples 2 and 3 (**FIG. 7**). A feed stream enters at the inlet of the first filter cartridge module. The outlet from the final filter cartridge module, after the recirculation split, is the depleted target molecule stream or waste stream.

Filter cartridge modules can be indexed when the final filter cartridge module waste stream reaches a specified level such as, for example, 1% or 2% of the feed stream target molecule concentration. The index time determination can be completed by mathematical modeling, in-line sensors or in-line testing, as desired.

At the index time, the filter cartridge modules index and rotate in the system (either physically or by valving). The filter cartridge module that is most upstream in the capture step is removed from the capture series, and is then sent to the recovery zone. At the same time, the regenerated filter cartridge module that has finished the recovery cycle is added at the most downstream position in the series, and the remaining modules in the capture series are indexed upstream by one position. It is assumed that right after the index time,

the added regenerated filter cartridge module contains no target molecule and the waste stream returns to a zero concentration of target molecule.

A mathematical model was used for the kinetics and modeling of Examples 1 – 3 below. The governing equation (change in concentration over change in time) used was:

$$\partial q/\partial t = K(C-C^*)$$

where q is the antibody concentration in the resin (mg/mL), t is time (min), K is the kinetic rate constant (min^{-1}), C is the antibody concentration in the solution mixture (mg/mL), and C* is the antibody concentration in the solution mixture that would be in equilibrium with q. The Langmuir isotherm describes the relationship between C and q at equilibrium, and has the form:

$$q = (Q_{max} \bullet C)/(K_d + C)$$

where Q_{max} is the maximum antibody concentration in the resin (mg/mL), and K_d is the reciprocal of the Langmuir equilibrium constant (mg/mL).

Model parameters were estimated for ProSep-A Ultra™ resin. The kinetic rate constant K was estimated from batch uptake kinetics in which a monoclonal antibody solution was recirculated through one filter cartridge module and aliquots were taken with time. Batch adsorption kinetics were measured with 161 mL of ProSep A Ultra, using 3.4 L of a monoclonal antibody solution at a concentration of 0.7 mg/mL. The resultant plot of q (IgG adsorption) versus time was used to fit the rate constant K. The adsorption equilibrium isotherm was assumed to be of the Langmuir type. The Langmuir constants K_d and Q_{max} were taken from literature for 700 Angstrom porous glass (assumed to be equivalent to ProSep-A Ultra™ resin). (McCue, J. Chrom. A, 989(2003) 139-153.)

Example 1

This simulated moving bed apparatus **200** example includes four filter cartridge modules **201, 202, 203, 204** in series in the capture zone, and one filter cartridge module **250** in the recovery zone (see **FIG. 6**.)

When the system **200** is first started the stationary phase particulate does not contain any IgG antibody. The antibody concentration in the liquid phase in each module is also zero. Filter cartridge modules start out filled with buffer. The feed **210** is introduced at a flow rate of 12 L/min having an antibody concentration of 1 mg/mL. The filter cartridge modules index when the waste stream **220** reaches an antibody

concentration at 1% of the feed concentration, or 0.01 mg/mL. The waste stream **220** is removed at a flow rate of 12 L/min. Thus, each feed stream **211, 212, 213** flows at a rate of 12 L/min.

The flow rate entering each filter cartridge module **201, 202, 203, 204** at each inlet **221, 222, 223, 224** is 150 L/min. The flow rate being recirculated through recirculation piping **216, 217, 218, 219** is 138 L/min. Thus, 92% of the solution mixture is recirculated through each filter cartridge module **201, 202, 203, 204**. The pressure drop across each filter cartridge modules **201, 202, 203, 204** inlet and outlet would be less than 69 kPa (10 psi.)

The simulated moving bed is indexed when the concentration of the target molecule reaches 0.01 mg/mL (1% of the feed target molecule concentration.) The index times were determined by the mathematical modeling. The first index time is longer than subsequent index times. The index time reaches a steady value even though the antibody concentrations in the liquid phase, C, and in the stationary phase particulate, q, in each filter cartridge module are changing with time. This is referred to as pseudo steady state. The first index time was 122 min. Subsequent index times were 84 minutes.

It was assumed that C (mg of IgG per mL of solution mixture) in each of the filter cartridge modules at a given time was the same as the concentration in the outlet from that filter cartridge module. Table 1 shows the concentrations C and q (mg of IgG per mL of stationary phase particulate) in each filter cartridge module just before indexing at pseudo steady state.

Table 1

Filter Cartridge Module	C (mg IgG/mL)	q (mg IgG/mL stationary phase particulate)
201′	0.66	63
202	0.17	22
203	0.04	5.4
204	0.01	1.1

Example 2

This simulated moving bed apparatus **300** example includes six filter cartridge modules **301, 302, 303, 304, 305, 306** in series in the capture zone, and one filter cartridge module **350** in the recovery zone (see **FIG. 7**).

When the system **300** is first started the stationary phase particulate does not contain any IgG antibody. The antibody concentration in the liquid phase in each module is also zero. Filter cartridge modules start out filled with buffer. The feed **310** is introduced at a flow rate of 34 L/min having an antibody concentration of 1 mg/mL. The filter cartridge modules index when the waste stream (**320**) reaches an antibody concentration at 2% of the feed concentration, or 0.02 mg/mL. The waste stream **320** is removed at a flow rate of 34 L/min. Thus, each feed stream **311, 312, 313, 314, 315** flows at a rate of 34 L/min.

The flow rate entering each filter cartridge module **301, 302, 303, 304, 305, 306** at each inlet **321, 322, 323, 324, 325, 326** is 150 L/min. The flow rate being recirculated through recirculation piping **331, 332, 333, 334, 335, 336** is 116 L/min. Thus, 77% of the solution mixture is recirculated through each filter cartridge module **301, 302, 303, 304, 305, 306**. The pressure drop across each filter cartridge modules **301, 302, 303, 304, 305, 306** inlet and outlet would be less than 69 kPa (10 psi.)

The simulated moving bed is indexed when the concentration of the target molecule reaches 0.02 mg/mL (2% of the feed target molecule concentration.) The index times were determined by the mathematical modeling. The first index time is longer than subsequent index times. The index time reaches a steady value even though the antibody concentrations in the liquid phase, C, and in the stationary phase particulate, q, in each filter cartridge module are changing with time. This is referred to as pseudo steady state. The first index time was 61 min. Subsequent index times were 29 minutes.

It was assumed that C (mg of IgG per mL of solution mixture) in each of the filter cartridge module at a given time was the same as the concentration in the outlet from that filter cartridge module. Table 2 shows the concentrations C and q (mg of IgG per mL of stationary phase particulate) in each filter cartridge module just before indexing at pseudo steady state.

Table 2

Filter Cartridge Module	C (mg IgG/mL)	q (mg IgG/mL stationary phase particulate)
301	0.78	63
302	0.39	37
303	0.19	18
304	0.09	7.9
305	0.04	3.3
306	0.02	1.1

Example 3

This simulated moving bed apparatus **300** example includes six filter cartridge modules **301, 302, 303, 304, 305, 306** in series in the capture zone, and one filter cartridge module **350** in the recovery zone (see **FIG. 7**).

When the system **300** is first started the stationary phase particulate does not contain any IgG antibody. The antibody concentration in the liquid phase in each module is also zero. Filter cartridge modules start out filled with buffer. The feed **310** is introduced at a flow rate of 30 L/min having an antibody concentration of 2 mg/mL. The filter cartridge modules index when the waste stream **320** reaches an antibody concentration at 1% of the feed concentration, or 0.02 mg/mL. The waste stream **320** is removed at a flow rate of 30 L/min. Thus, each feed stream **311, 312, 313, 314, 315** flows at a rate of 30 L/min.

The flow rate entering each filter cartridge module **301, 302, 303, 304, 305, 306** at each inlet **321, 322, 323, 324, 325, 326** is 150 L/min. The flow rate being recirculated through recirculation piping **331, 332, 333, 334, 335, 336** is 120 L/min. Thus, 80% of the solution mixture is recirculated through each filter cartridge module **301, 302, 303, 304, 305, 306**. The pressure drop across each filter cartridge modules **301, 302, 303, 304, 305, 306** inlet and outlet would be less than 69 kPa (10 psi.)

The simulated moving bed is indexed when the concentration of the target molecule reaches 0.02 mg/mL (1% of the feed target molecule concentration.) The index times were determined by the mathematical modeling. The first index time is longer than subsequent index times. The index time reaches a steady value even though the antibody concentrations in the liquid phase, C, and in the stationary phase particulate, q, in each filter cartridge module are changing with time. This is referred to as pseudo steady state. The first index time was 30 min. Subsequent index times were 16 minutes.

It was assumed that C (mg of IgG per mL of solution mixture) in each of the filter cartridge module at a given time was the same as the concentration in the outlet from that filter cartridge module. Table 3 shows the concentrations C and q (mg of IgG per mL of stationary phase particulate) in each filter cartridge module just before indexing at pseudo steady state.

Table 3

Filter Cartridge Module	C (mg IgG/mL)	q (mg IgG/mL stationary phase particulate)
301	1.25	63
302	0.55	31
303	0.24	14
304	0.11	5.5
305	0.05	2.1
306	0.02	0.6

Example 4

This example includes six modules in series in the capture zone, and one module in the recovery zone (see **FIG. 7**). This system was used to remove IgG from a feed solution by Protein A affinity separation. A module was composed of a filter capsule manufactured by Pall (Part Number 12121), and a solid material used for the separation. Each module contains 13 mL of a Protein A Biosupport Medium (prepared according to US 5,907,016 Example 4) having a mean particle diameter of 28 micrometers and a bed height of 0.04 cm. Corresponding to each module, there was a pump, an inlet stream, and an outlet

stream. The outlet stream was split into two streams, one is recycled back just upstream of the pump for that module and the other was the feed for the next module. The liquid volume of the module and piping was estimated to be 112 mL. Six modules are connected in series for the capture step. The feed stream was the inlet to the first module. The outlet from the sixth module, after the recycle split, was the waste, or effluent, stream. The overall process includes capture, wash, elution, optional cleaning, and re-equilibration. The modules were cleaned after the final elution for the batch, otherwise the module was re-equilibrated and placed back into the capture zone. At the index time, the modules index and rotate in the system by changing valves that direct the flow of the process streams. The module that was most upstream in the capture step was taken out of the series, and then washed with 10mM PBS (sodium phosphate in 140 mM NaCl, pH 7.2), then eluted with elution buffer (0.5 M acetic acid, pH 2.45), then re-equilibrated with PBS. These three steps of wash, elution, and re-equilibration occur in the recovery zone. At the same time, a regenerated filter cartridge module was inserted at the most downstream position in the series, and the remaining modules in the capture series were shifted upstream one position.

When the system was started the stationary phase particulate does not contain any IgG antibody. The antibody concentration in the liquid phase in each module was also zero. The modules were initially filled with 10mM PBS buffer. The feed solution (containing polyclonal human IgG from EQUITECH-BIO, Inc.; Kerrville, TX in PBS buffer) was introduced at a flow rate of about 20 mL/min and an antibody concentration of 1.0 mg/mL, with a total flow of 980 mL/min through each module. The cartridges were indexed two times. The time between indexing was 30 minutes for each cycle. At the end of the last cycle all of the cartridges in the series were washed with PBS, eluted with 0.5 M acetic acid (pH 2.45), washed again with PBS, then regenerated with a guanidine buffer (2M guanidine HCl, 10mM TRIS (tris(hydroxymethyl)aminomethane) at pH 7.5). Concentrations were calculated from absorption measured by UV-Vis spectrophotometer at 280nm.

Cartridge Position for Cycle 1:

Inlet -A-B-C-D-E-F-Outlet

Offline (G)

Cycle 1

	Volume (mL)	Concentration (mg/mL)	IgG (mg)
Effluent	577	0	0
Module A Wash 1	443	0.209	93
Module A Wash 2	499	0.037	18
Module A Elution	457	0.688	314
Module A Post Elution Wash	507	0.061	31

Feed Volume	783	mL
Cycle Duration	30	min
Average Flow Rate	26	mL/min
Feed Concentration	1.05	mg/mL
Total Challenge	822	mg
Total Eluted	314	mg
Samples	210	mL

Module A was washed, eluted, and re-equilibrated during the second cycle.

Cartridge Position for Cycle 2:

Inlet -B-C-D-E-F-G- Outlet

Offline (A) In the recovery zone

Cycle 2

	Volume (mL)	Concentration (mg/mL)	IgG (mg)
Effluent	475	0..011	5
Module B Wash 1	485	0.215	104
Module B Elution	479	0.637	305
Module B Post Elution Wash	490	0.209	102

Feed Volume	510	mL
Cycle Duration	30	min
Average Flow Rate	17	mL/min
Feed Concentration	1.05	mg/mL
Total Challenge	535	mg
Total Eluted	305	mg
Samples	25	mL

Module B was washed, eluted, and re-equilibrated after the second cycle has ended, during the third cycle.

Cartridge Position for Cycle 3:

Inlet -C-D-E-F-G-A-Outlet

Offline (B) In the recovery zone

Cycle 3

	Volume (mL)	Concentration (mg/mL)	IgG (mg)
Effluent	401	0.010	4
All Modules Wash 1	3260	0.014	47
Elutions			
Module C Elution	475	0.537	255
Module D Elution	465	0.320	149
Module E Elution	485	0.153	74
Module F Elution	486	0.095	46
Module G Elution	492	0.054	27
Module A Elution	485	0.037	18
All Modules Post Elution Wash	1851		

Feed Volume	445	mL
Cycle Duration	30	min

Average Flow Rate	14.8	mL/min
Feed Concentration	1.05	mg/mL
Total Challenge	467	mg
Total Eluted	569	mg
Samples	45	mL

Module C, D, E, F, G, and A were washed together, eluted separately, and all cartridges were regenerated together with 2M guanidine TRIS buffer after cycle 3.

Samples were taken every three minutes from each cartridge for Cycle 1, and every three minutes from the last cartridge in the series for Cycle 2 and Cycle 3.

Module	Time (min)	IgG Concentration (mg/mL)
A	3	0.071
A	6	0.121
A	9	0.193
A	12	0.260
A	15	0.341
A	18	0.411
A	21	0.467
A	24	0.527
A	27	0.605
A	30	0.646
B	3	0.015
B	6	0.015
B	9	0.023
B	12	0.040
B	12	0.037
B	15	0.063
B	18	0.086
B	21	0.104
B	24	0.146
B	27	0.198
B	30	0.239
C	3	0.008
C	6	0.008
C	9	0.009

C	12	0.010
C	15	0.013
C	18	0.016
C	21	0.015
C	24	0.026
C	27	0.038
C	30	0.036
D	3	0.006
D	6	0.007
D	9	0.008
D	12	0.008
D	15	0.009
D	18	0.009
D	21	0.002
D	24	0.008
D	27	0.011
D	30	0.012
E	3	0.006
E	6	0.006
E	9	0.007
E	12	0.009
E	15	0.009
E	18	0.008
E	21	0.001
E	24	0.006
E	27	0.007
E	30	0.008
F	3	0.004
F	6	0.005
F	9	0.004
F	12	0.006
F	15	0.007
F	18	0.007
F	21	0.002
F	24	0.006
F	27	0.006
F	30	0.007
G	33	0.007
G	36	0.010
G	39	0.010
G	42	0.010
G	45	0.010
G	48	0.010

G	51	0.010
G	54	0.010
G	57	0.014
G	60	0.009
A	63	0.018
A	66	0.009
A	69	0.009
A	72	0.011
A	75	0.020
A	78	0.014
A	81	0.015
A	84	0.014
A	84	0.014
A	87	0.015
A	90	0.020

Example 5

This example was identical to Example 4 except the target feed flow rate was 40 mL/min and the total flow through each module was 890 mL/min.

Cycle 1

	Volume (mL)	Concentration (mg/mL)	IgG (mg)
Effluent	1248	0.011	13
Module A Wash 1	460	0.247	114
Module A Elution	530	0.661	351
Module A Post Elution Wash	503	0.172	87

Feed Volume	1458	mL
Cycle Duration	30	min
Average Flow Rate	48.6	mL/min
Feed Concentration	1.0	mg/mL
Total Challenge	1458	mg
Total Eluted	351	mg
Samples	210	mL

Cycle 2

	Volume (mL)	Concentration (mg/mL)	IgG (mg)
Effluent	1011	0.030	30
Module B Wash 1	522	0.246	128
Module B Elution	541	0.671	363
Module B Post Elution Wash	528	0.211	111

Feed Volume	1047	mL
Cycle Duration	30	min
Average Flow Rate	35	mL/min
Feed Concentration	1.0	mg/mL
Total Challenge	1047	mg
Total Eluted	363	mg
Samples	35	mL

Cycle 3

	Volume (mL)	Concentration (mg/mL)	IgG (mg)
Effluent	957	0.041	39
All Modules Wash 1 Part 1	1907	0.170	324
All Modules Wash 1 Part 2	616	0.053	33
Module C Elution	461	0.761	351
Module D Elution	490	0.616	302
Module E Elution	507	0.494	250
Module F Elution	499	0.447	223
Module G Elution	504	0.327	165
Module A Elution	512	0.261	134
All Modules Post Elution Wash	1981	0.177	350

Feed Volume	992	mL
Cycle Duration	30	min
Average Flow Rate	33	mL/min
Feed Concentration	1.0	mg/mL
Total Challenge	992	mg
Total Eluted	1425	mg
Samples	35	mL

As described above, samples were taken every three minutes from each cartridge for Cycle 1, and every three minutes from the last cartridge in the series for Cycle 2 and Cycle 3.

Module	Time (min)	Concentration (mg/mL)
A	3	0.1567
A	6	0.2976
A	9	0.4145
A	12	0.5246
A	15	0.6033
A	18	0.6797
A	21	0.7647
A	24	0.7951
A	27	0.8291
A	30	0.8642
B	3	0.0322
B	6	0.0729
B	9	0.1354
B	12	0.1931
B	15	0.2578
B	18	0.3640
B	21	0.4484
B	24	0.4957
B	27	0.5575
B	30	0.6224
C	3	0.0080
C	6	0.0147
C	9	0.0311
C	12	0.0495

C	15	0.0693
C	18	0.1093
C	21	0.1459
C	24	0.1798
C	27	0.2358
C	30	0.3068
D	3	0.0088
D	6	0.0093
D	9	0.0161
D	12	0.0210
D	15	0.0298
D	18	0.0491
D	21	0.0619
D	24	0.0577
D	27	0.0819
D	30	0.1140
E	3	0.0087
E	6	0.0090
E	9	0.0120
E	12	0.0163
E	15	0.0164
E	18	0.0349
E	21	0.0431
E	24	0.0300
E	27	0.0399
E	30	0.0515
F	3	0.0069
F	6	0.0081
F	9	0.0105
F	12	0.0131
F	15	0.0134
F	18	0.0293
F	21	0.0211
F	24	0.0235
F	27	0.0324
F	30	0.0393
G	33	0.0089
G	36	0.0173
G	39	0.0267
G	43	0.0258
G	45	0.0274
G	48	0.0351
G	51	0.0323

G	54	0.0331
G	57	0.0345
G	60	0.0369
A	63	0.0167
A	66	0.0257
A	69	0.0287
A	72	0.0334
A	75	0.0431
A	78	0.0408
A	81	0.0407
A	84	0.0465
A	87	0.0500
A	90	0.0572

WE CLAIM:

1. A method of continuously separating a target molecule from a solution mixture comprising steps of:

providing a simulated moving bed apparatus comprising a plurality of filter cartridge modules, each filter cartridge module comprising a volume of stationary phase particulates adjacent a porous substrate layer, each filter cartridge module having a filter cartridge module inlet, a filter cartridge module outlet and filter cartridge module recirculation piping connecting the filter cartridge module outlet and the filter cartridge module inlet, the plurality of filter cartridge modules being in serial fluid connection;

flowing a solution mixture comprising a target molecule continuously through the plurality of filter cartridge modules, wherein a recirculation portion of the solution mixture flow is recirculated from the filter cartridge module outlet to the filter cartridge module inlet and a remaining portion of the solution mixture flows to a subsequent filter cartridge module inlet;

binding the target molecule to the volume of stationary phase particulates to form a target molecule:stationary phase particulate product;

removing a loaded filter cartridge module containing the target molecule:stationary phase particulate product from the solution mixture flow;

separating the target molecule from the target molecule:stationary phase particulate product from the loaded filter cartridge to form an isolated target molecule product and a regenerated filter cartridge; and

adding the regenerated filter cartridge module to the solution mixture flow.

2. A method according to claim 1, wherein the providing step comprises providing a simulated moving bed apparatus comprising a plurality of filter cartridge modules, each filter cartridge module comprising a volume of stationary phase particulates adjacent a porous substrate layer and the volume of stationary phase particulates having a bed height of less than 1 centimeter.

3. A method according to claim 1, wherein the providing step comprises providing a simulated moving bed apparatus comprising a plurality of filter cartridge modules, each filter cartridge module comprising a volume of stationary phase particulates adjacent a porous substrate layer and the volume of stationary phase particulates having a mean diameter in a range from 5 to 30 micrometers.

4. A method according to claim 1, wherein the flowing step comprises flowing a solution mixture comprising a target molecule continuously through the plurality of filter cartridge modules, wherein a recirculation portion of the solution mixture flow is recirculated from the filter cartridge module outlet to the filter cartridge module inlet and the recirculation portion is 75% or greater of the solution mixture flow.

5. A method according to claim 1, wherein the flowing step comprises flowing a solution mixture comprising a target molecule wherein the target molecule comprises a biomacromolecule.

6. A method according to claim 1, wherein the flowing step comprises flowing a solution mixture comprising a target molecule wherein the target molecule comprises a protein.

7. A method according to claim 1, wherein the removing step and the adding step occur at the same time.

8. A method according to claim 1, wherein the removing step, the flowing step and the adding step occur at the same time.

9. A method according to claim 1, wherein the separating step, and the binding step occur at the same time.

10. A method according to claim 1, wherein the removing step, the separating step, the flowing step, the binding step, and the adding step occur at the same time.

11. A method according to claim 1, wherein the removing step comprises removing a loaded filter cartridge module containing a first concentration of target molecule per milliliter of stationary phase particulate and wherein the adding step comprises adding the regenerated filter cartridge module containing a second concentration of target molecule per milliliter of stationary phase particulate, and the first concentration of target molecule per milliliter of stationary phase particulate being greater than the second concentration of target molecule per milliliter of stationary phase particulate.

12. A method according to claim 3, wherein the flowing step comprises flowing a solution mixture comprising a target molecule continuously through the plurality of filter cartridge modules, wherein the solution mixture flows through each filter cartridge at a rate of at least 5 liters/minute per liter of stationary phase particulates in each filter cartridge module and each filter cartridge has a pressure drop from the filter cartridge module inlet to the filter cartridge module outlet of 69 kPa (10 psi) or less.

13. A method according to claim 3, wherein the flowing step comprises flowing a solution mixture comprising a target molecule continuously through the plurality of filter cartridge modules, wherein the solution mixture flows through each filter cartridge at a rate of at least 7 liters/minute per liter of stationary phase particulates in each filter cartridge module and each filter cartridge has a pressure drop from the filter cartridge module inlet to the filter cartridge module outlet of 103 kPa (15 psi) or less.

14. A simulated moving bed apparatus for continuously separating a target molecule from a solution mixture comprising:

a capture zone comprising a plurality of filter cartridge modules connected in series including a first loaded filter cartridge module and a final regenerated filter cartridge module, the first loaded filter cartridge module connected to a solution mixture feed source and the final regenerated filter cartridge module connected to a target molecule depleted solution mixture vessel, each filter cartridge module comprising a volume of stationary phase particulates capable of binding with a target molecule adjacent a porous substrate

layer, each filter cartridge having a filter cartridge module inlet and a filter cartridge module outlet and filter cartridge module recirculation piping connecting the filter cartridge module outlet to the filter cartridge module inlet, and the filter cartridge module outlet being connected to a filter cartridge module inlet of a subsequent downstream filter cartridge module; and

a recovery zone where the target molecule in a loaded filter cartridge module is separated from the volume of stationary phase particulates to form a regenerated filter cartridge module, the recovery zone comprising at least one filter cartridge module.

15. A simulated moving bed according to claim 14, wherein the capture zone further comprises a solution mixture pump in fluid connection with the filter cartridge module inlet and the filter cartridge module recirculation piping.

16. A simulated moving bed according to claim 14, wherein the plurality of filter cartridge modules further comprises one or more intermediate filter cartridge modules connected in series between the first loaded filter cartridge module and the final regenerated filter cartridge module.

17. A simulated moving bed according to claim 14, wherein the capture zone further comprises at least one solution mixture pump in fluid connection with each of the plurality of filter cartridge modules.

18. A simulated moving bed according to claim 14, wherein the stationary phase particulates have a mean particle size in a range of 5 to 30 micrometers.

19. A simulated moving bed according to claim 14, wherein the volume of stationary phase particulates has a bed height of less than 1 centimeter.

20. A simulated moving bed according to claim 14, wherein each of the plurality of filter cartridge modules comprise a volume of 5 to 25 liters of stationary phase particulates capable of binding with a target molecule.

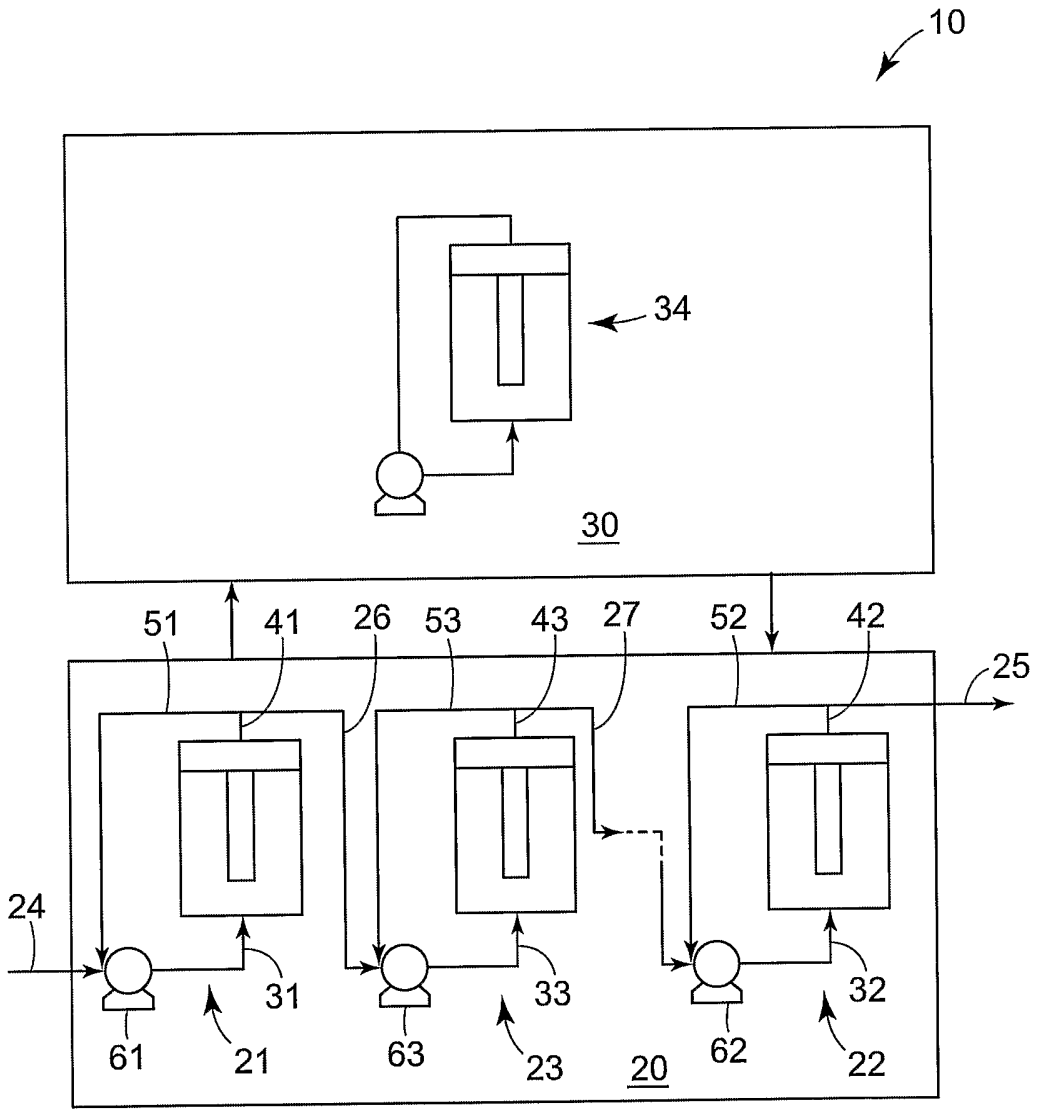


FIG. 1

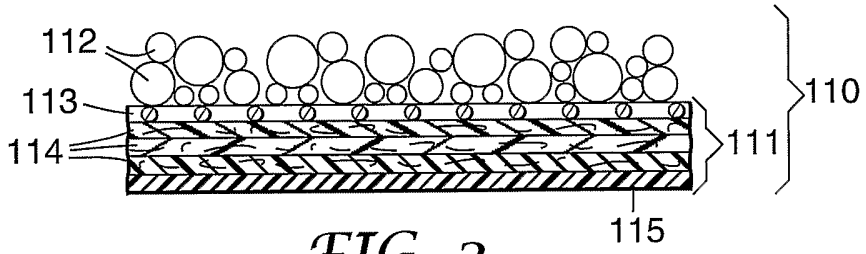


FIG. 2

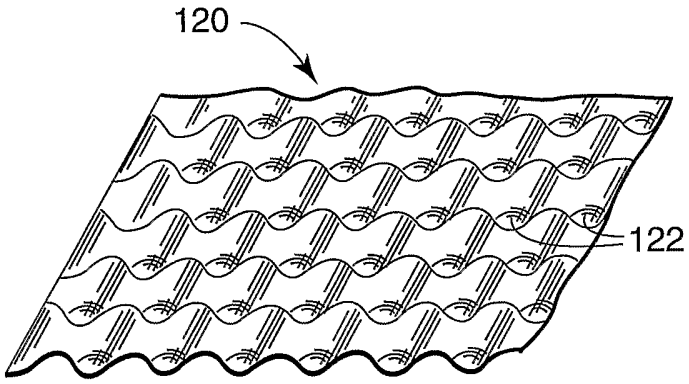


FIG. 3

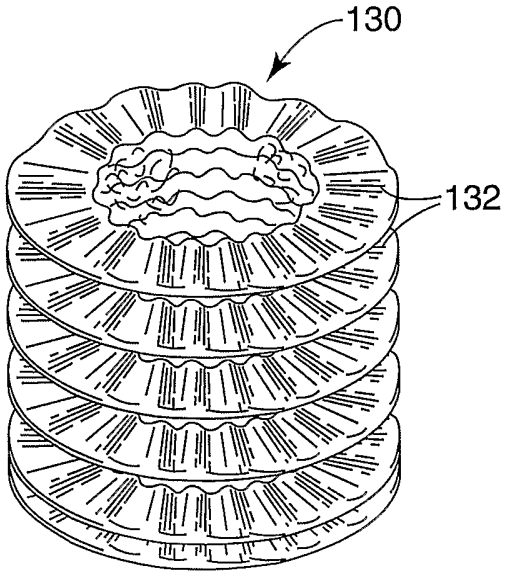


FIG. 4

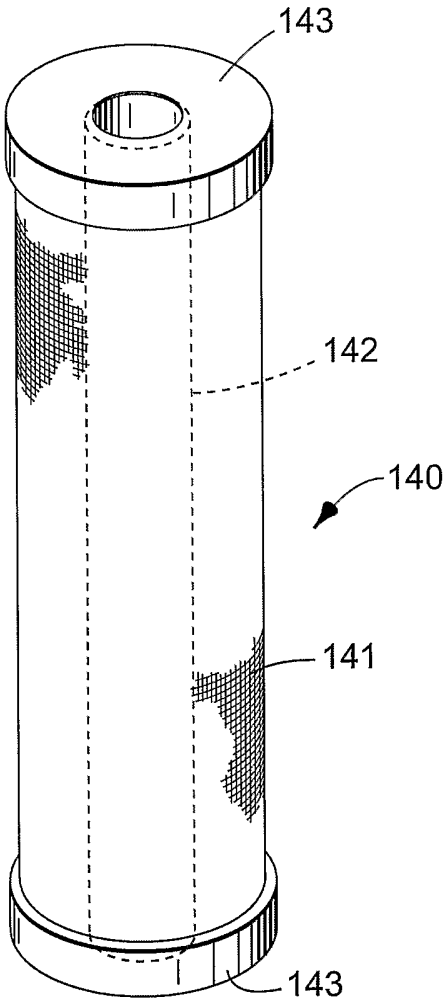


FIG. 5

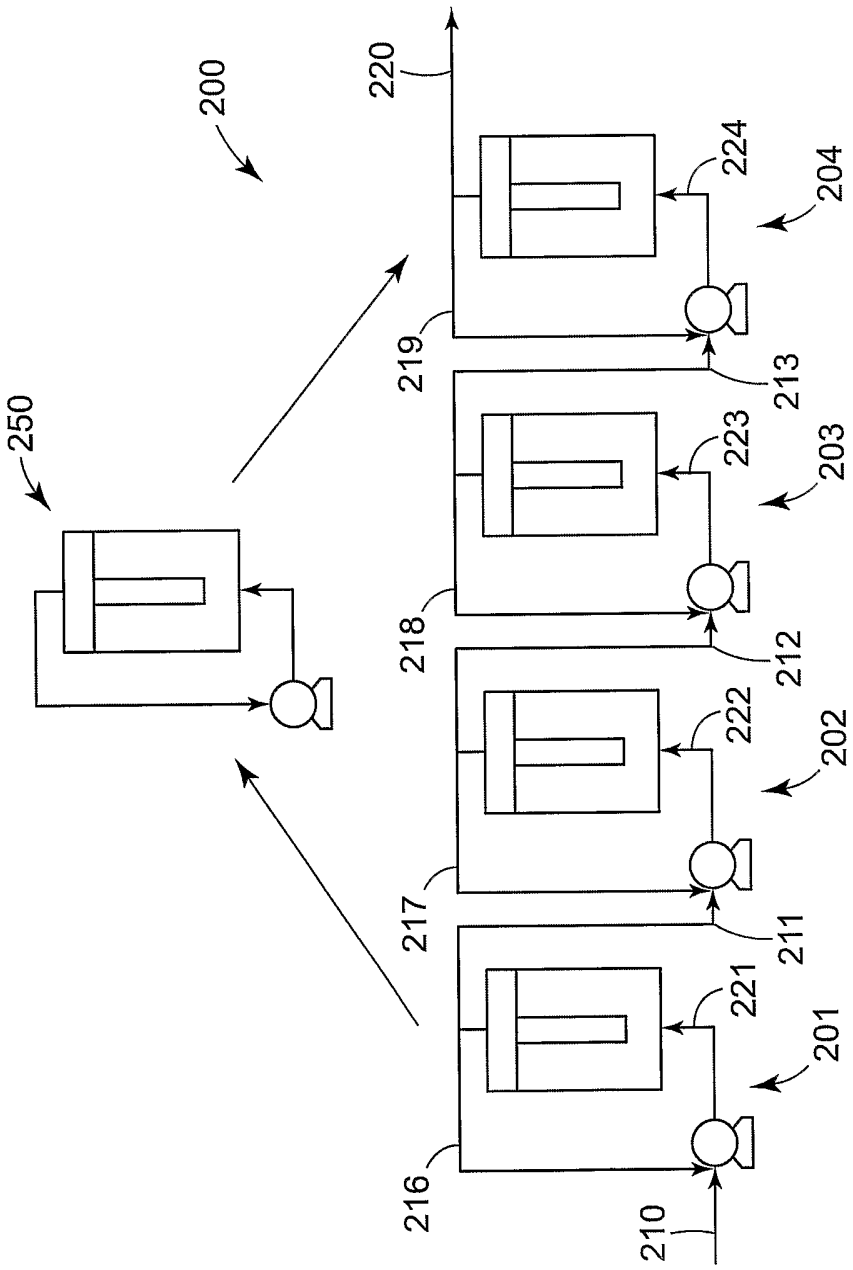

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FIG. 6

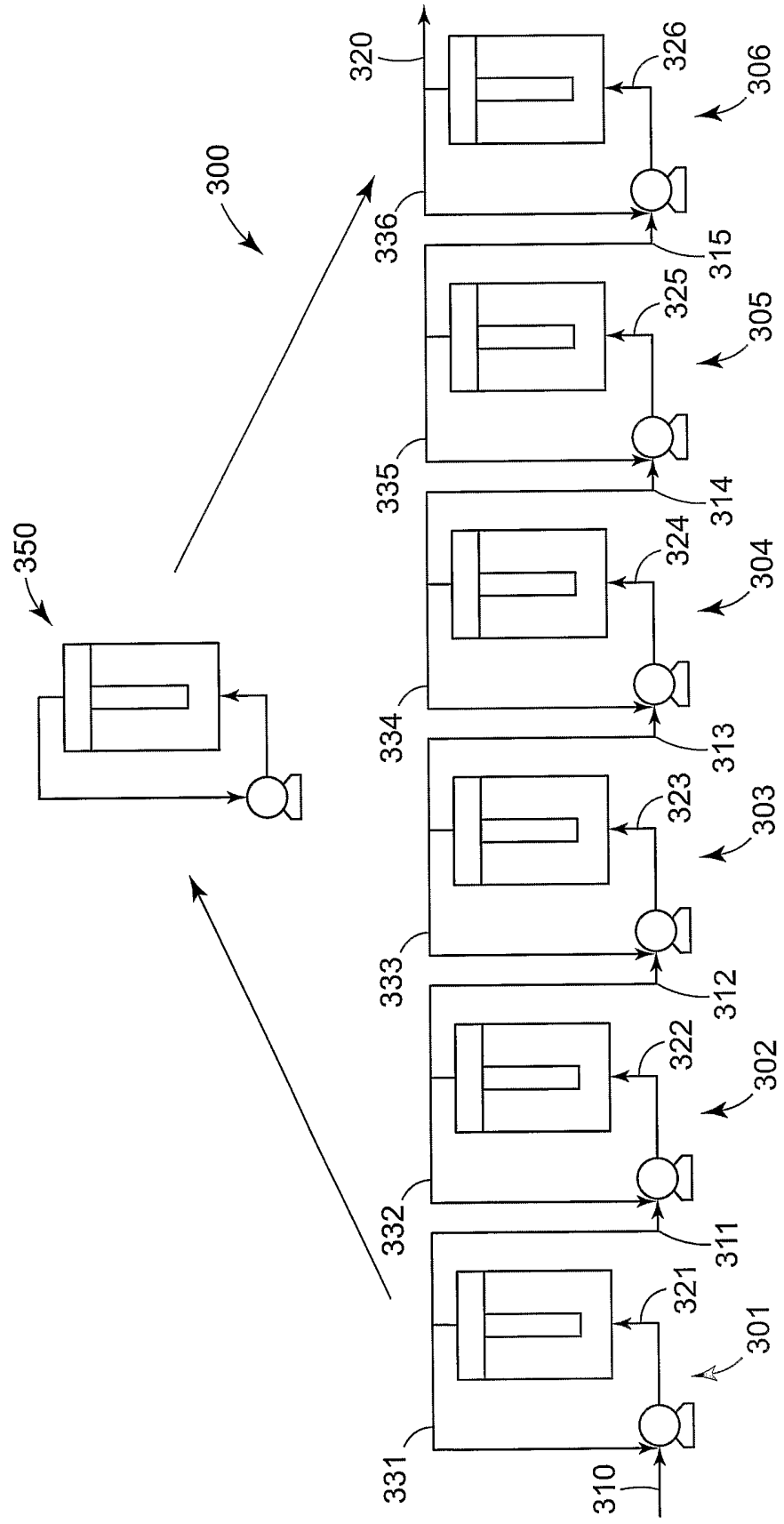

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FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/035247

A. CLASSIFICATION OF SUBJECT MATTER
B01D15/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 January 2006	30/01/2006

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Persichini, C

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