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(54) **COUNTERCURRENT TANGENTIAL CHROMATOGRAPHY METHODS, SYSTEMS, AND APPARATUS**

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B01D 15/08 (2006.01)

(52) **U.S. Cl.** **210/635; 210/650; 210/656; 210/659; 210/198.2**

(58) **Field of Classification Search** 210/635, 210/650, 651, 653, 654, 655, 656, 659, 198.2, 210/321.72, 321.75, 321.1, 321.84; 530/413, 530/414, 417

See application file for complete search history.

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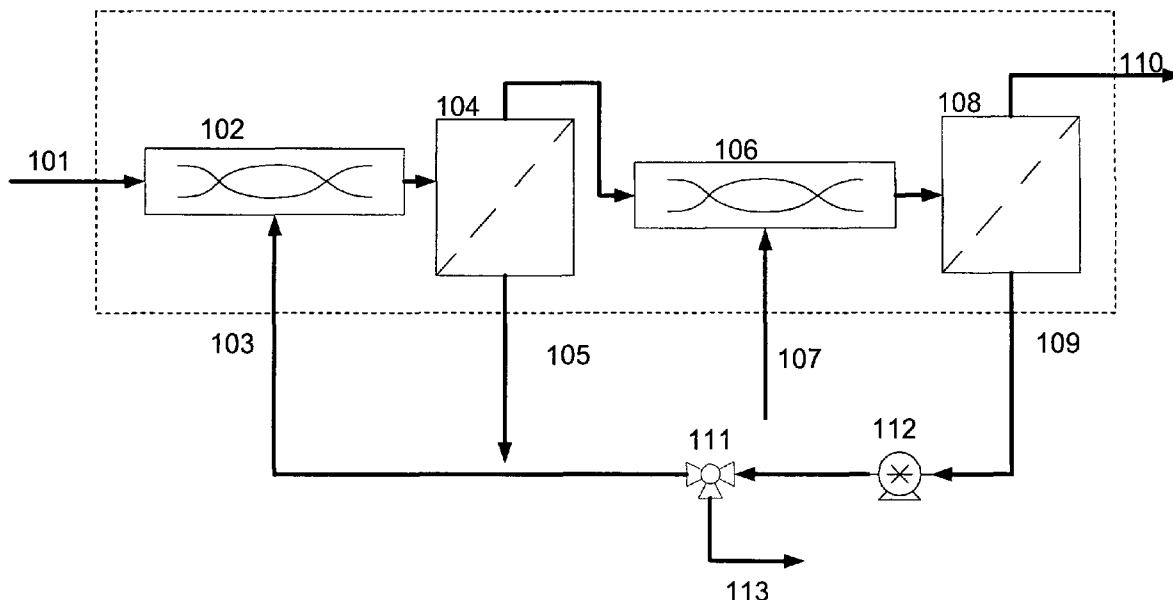
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(57) **ABSTRACT**

This invention relates to a breakthrough in the art of chromatography allowing 1) larger scale of operation; 2) faster processing time; 3) disposability; 4) reduction of media/resin expenses; and 5) a reduction of capital equipment investment. In this invention, the chromatography column is replaced by a module that consists of two or more interconnected tangential flow filters and static mixers. The chromatography resin flows through this module in a single pass, while similar operations to a regular chromatographic process are performed on the resin (binding, washing, elution, regeneration, and equilibration). The buffers for these operations are pumped into the module in a countercurrent direction to the flow of resin, and permeate solutions from later stages are recycled back into previous stages. This creates concentration gradients in the permeate solutions of the tangential flow filters in the countercurrent direction to resin flow, thus saving buffer volume and increasing process efficiency.

10 Claims, 12 Drawing Sheets

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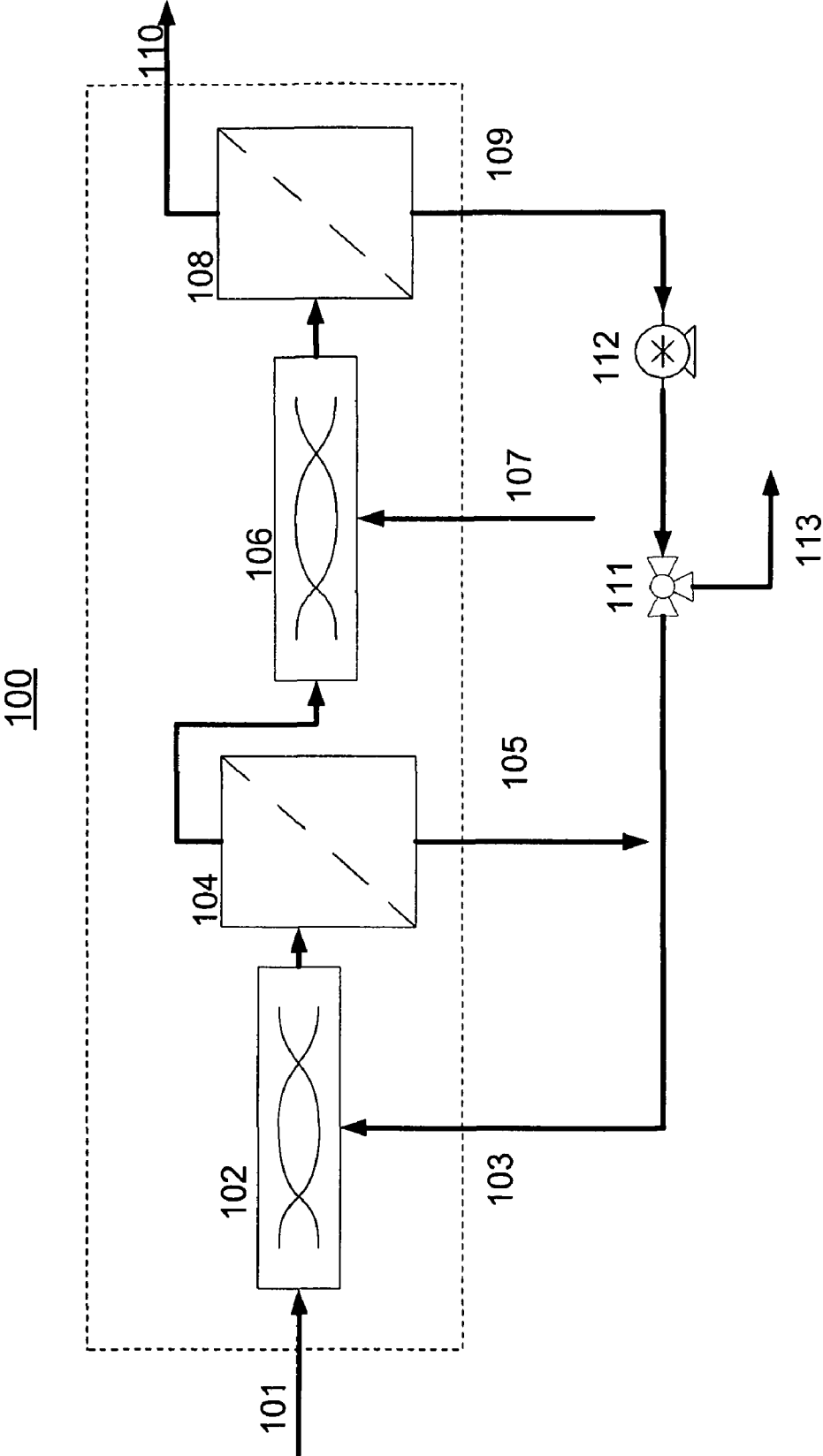


Figure 1

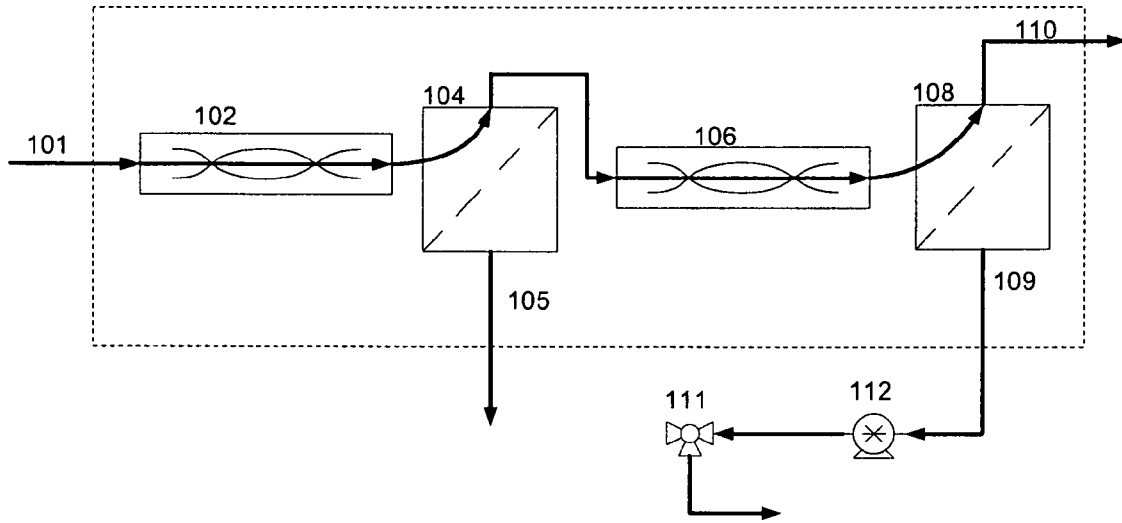


Figure 2A

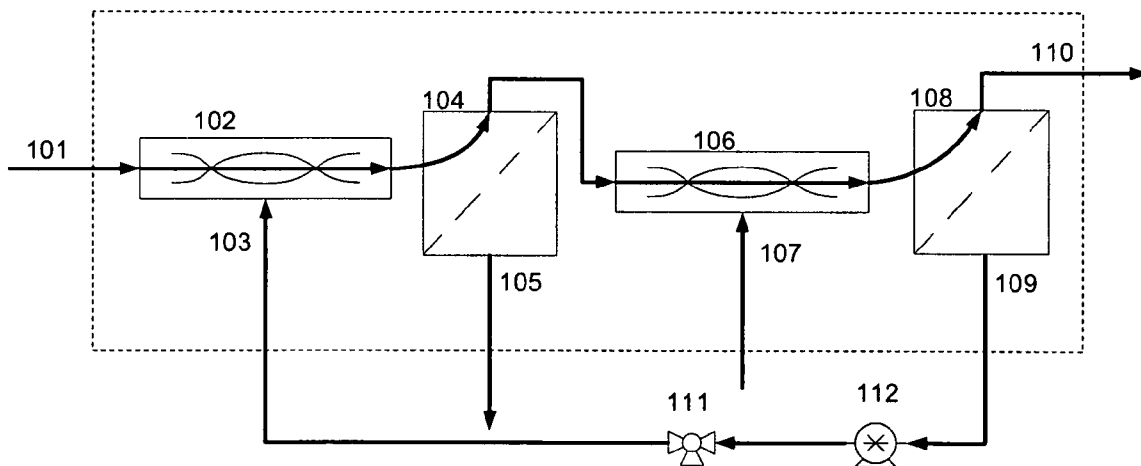


Figure 2B

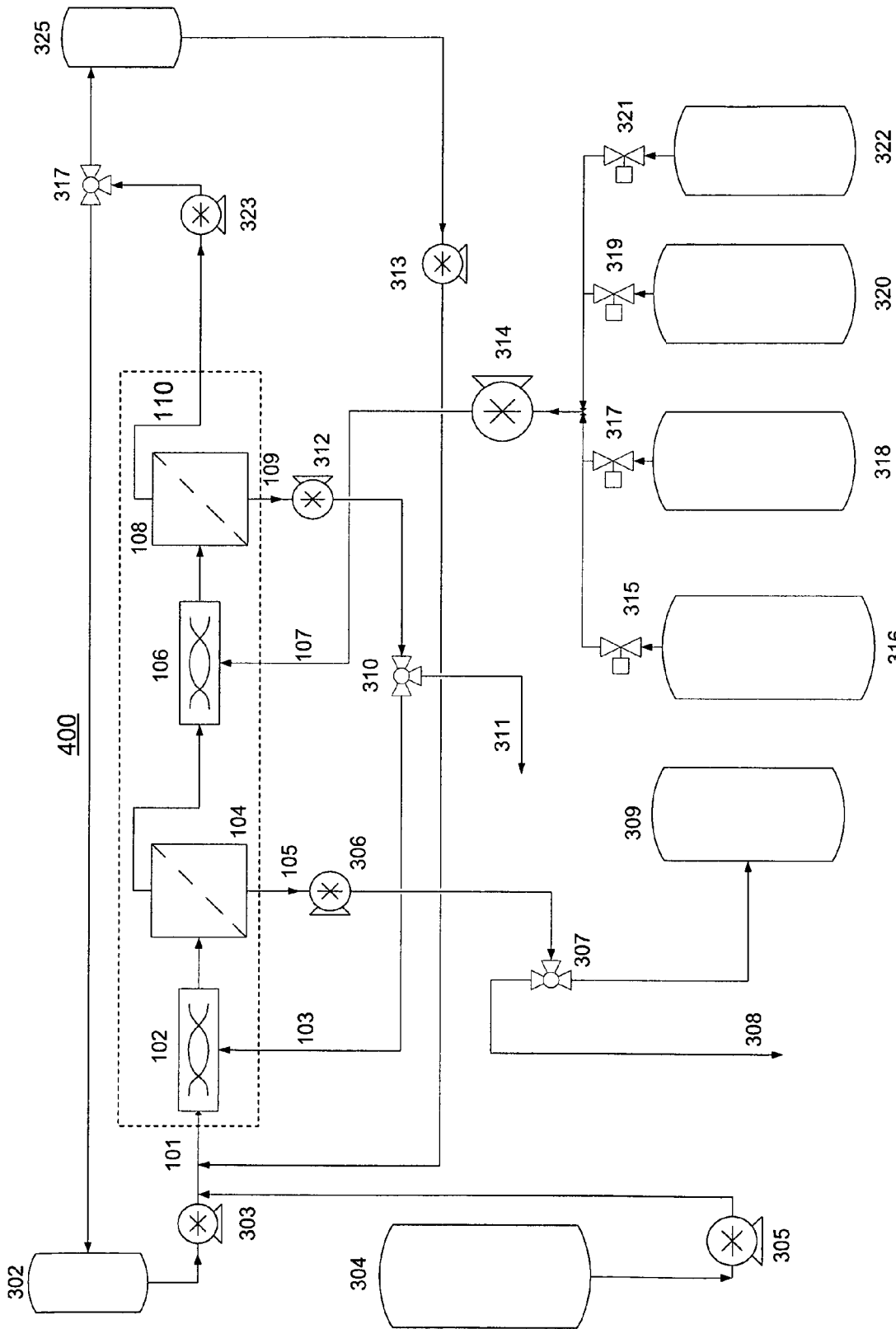


Figure 4

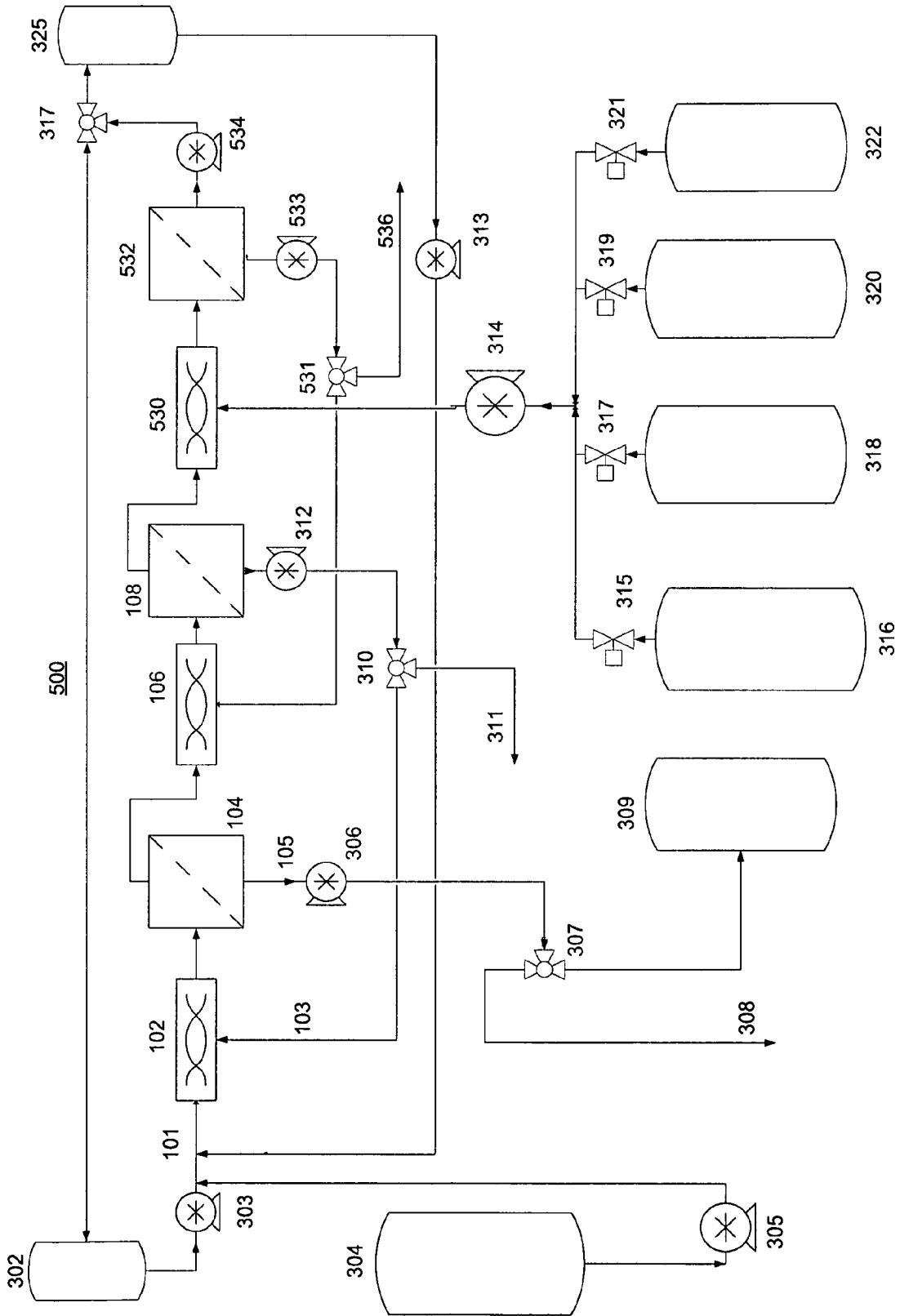


Figure 5

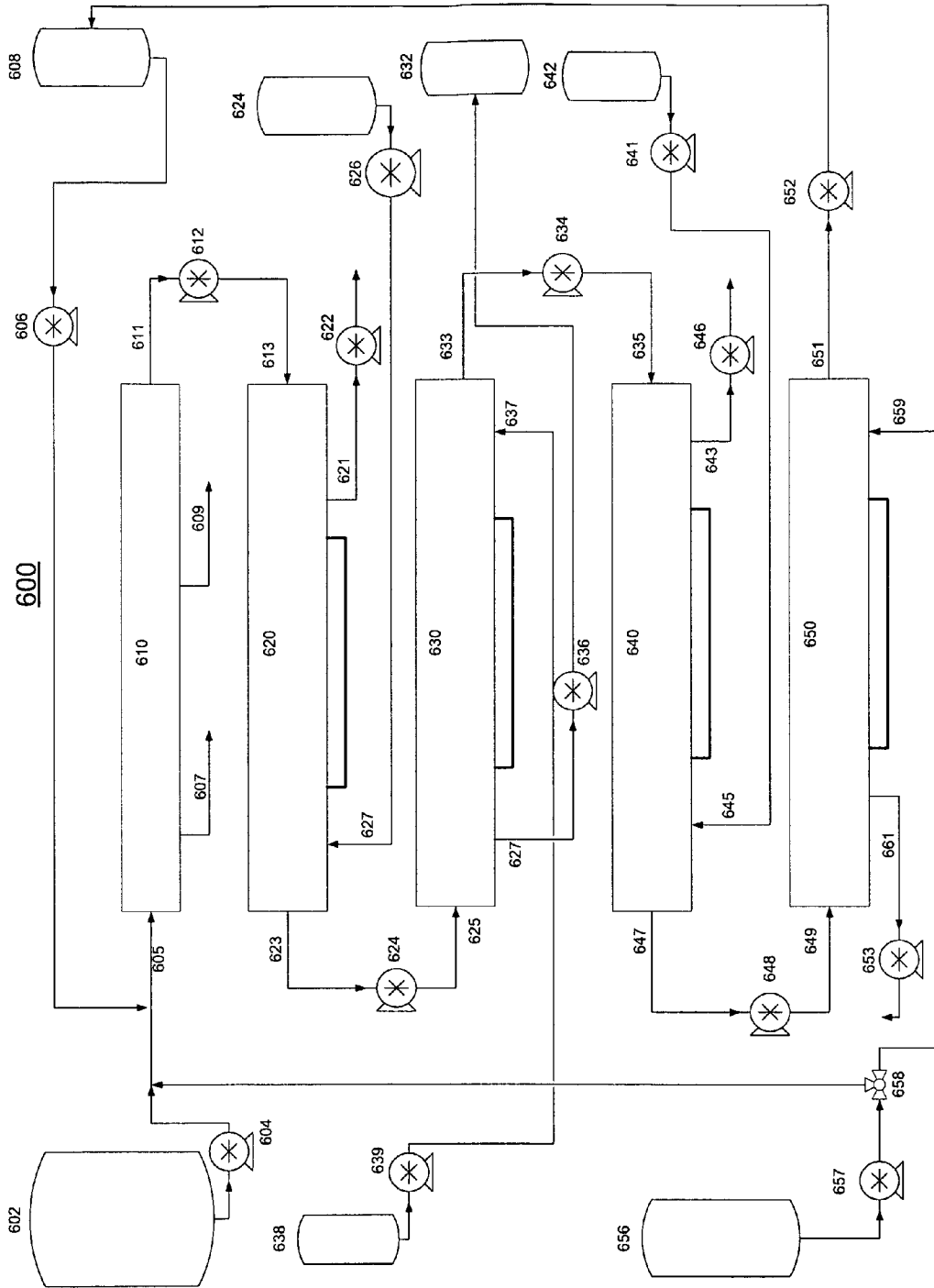


Figure 6

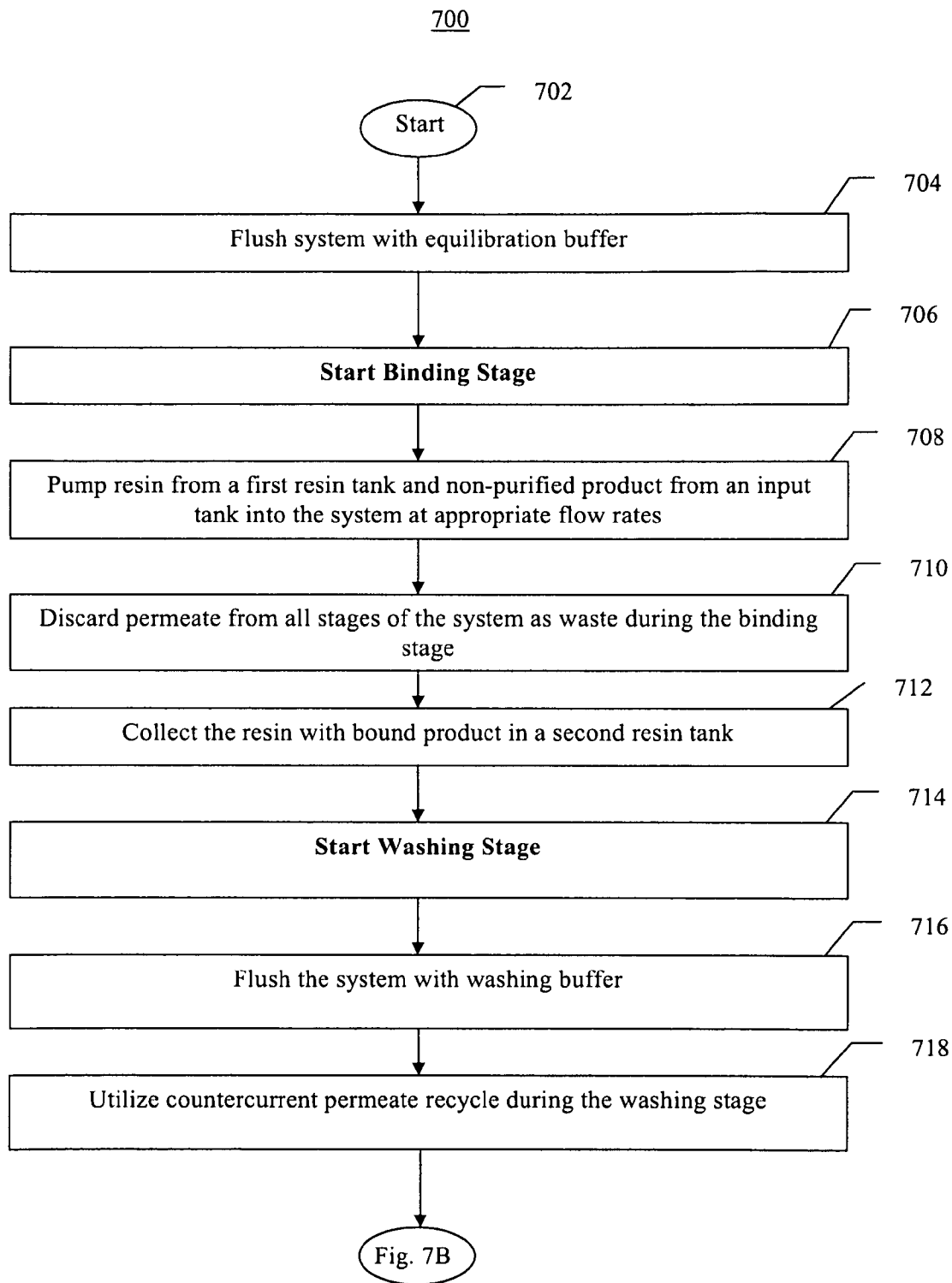


Figure 7A

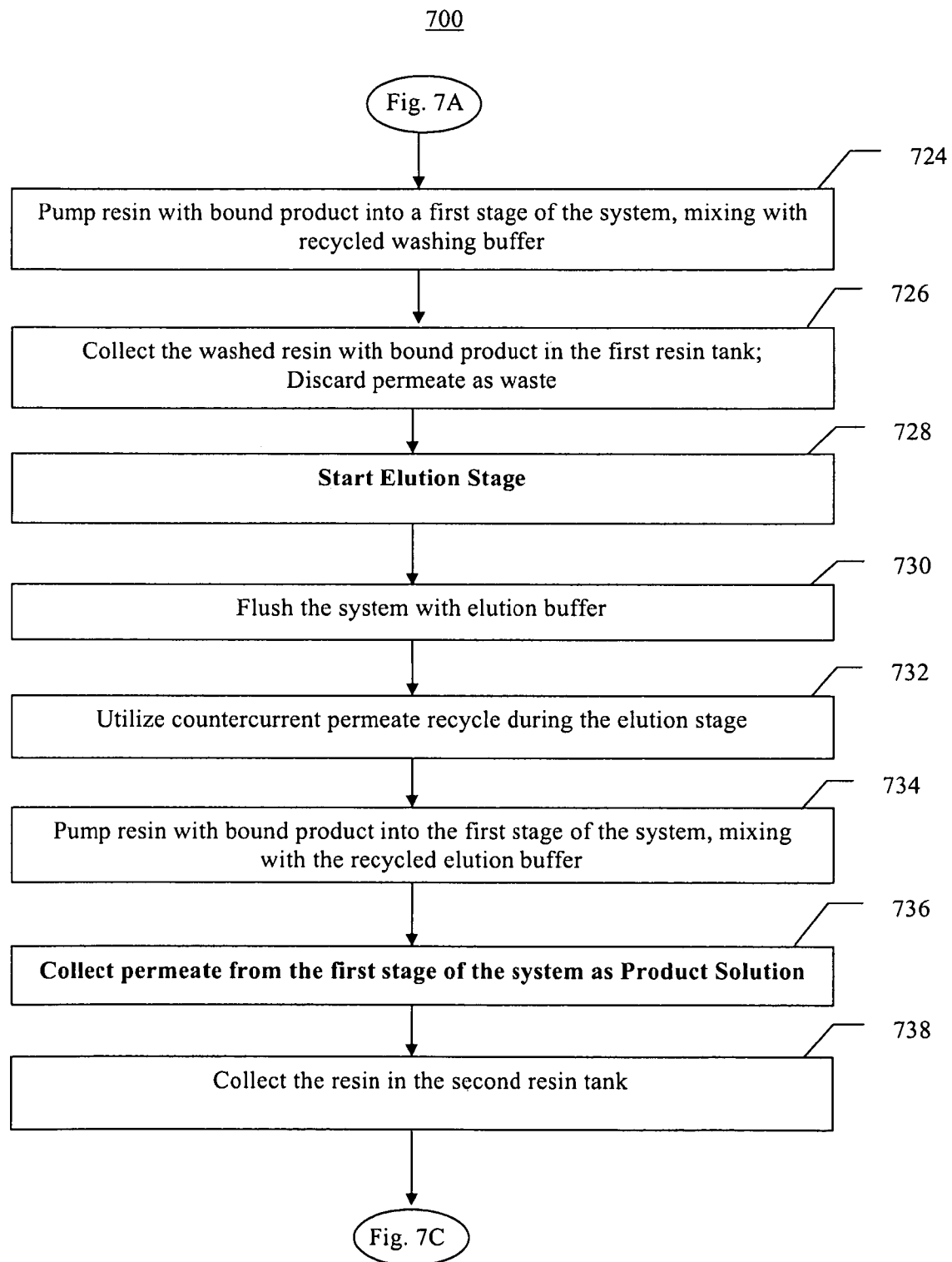


Figure 7B

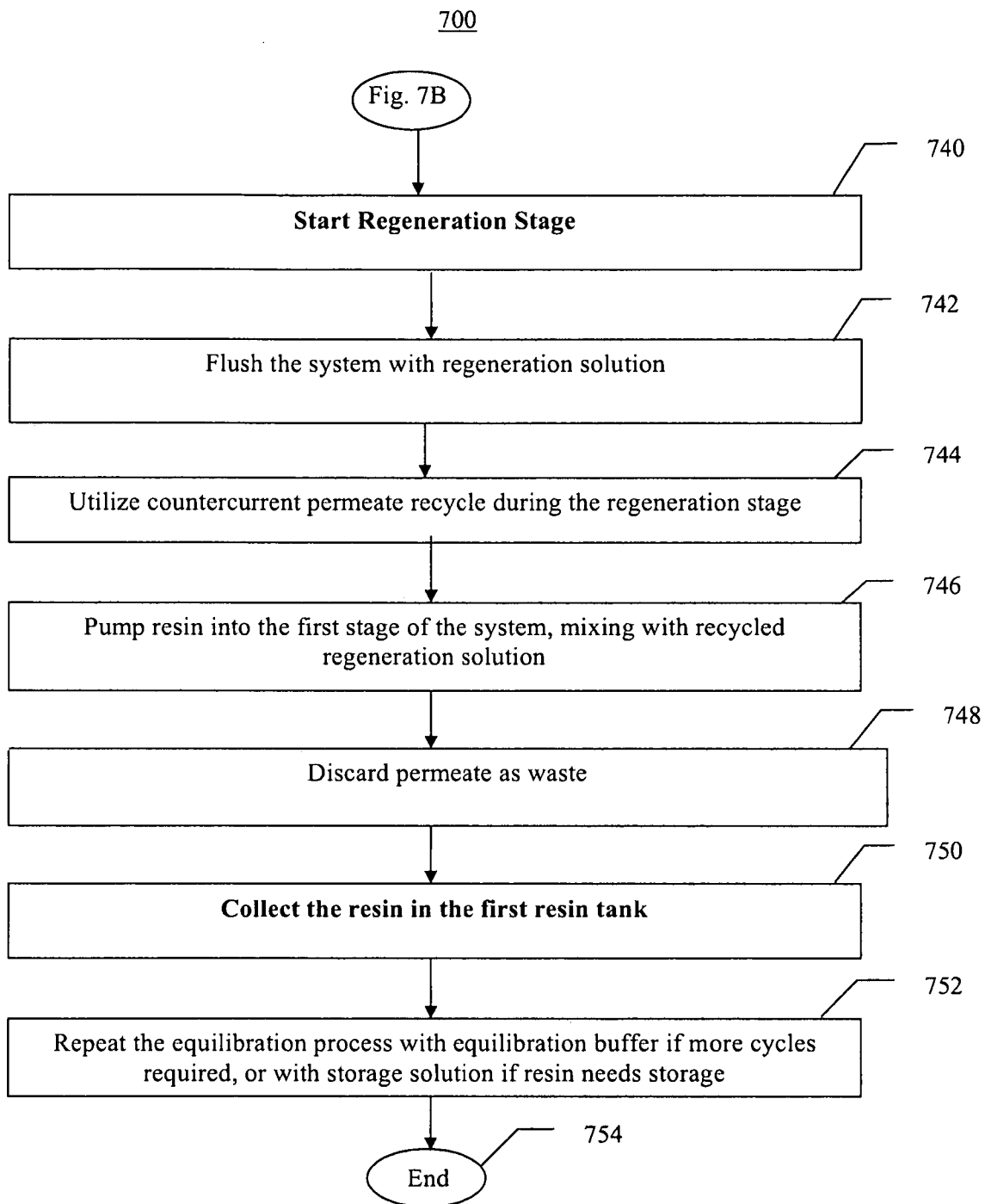


Figure 7C

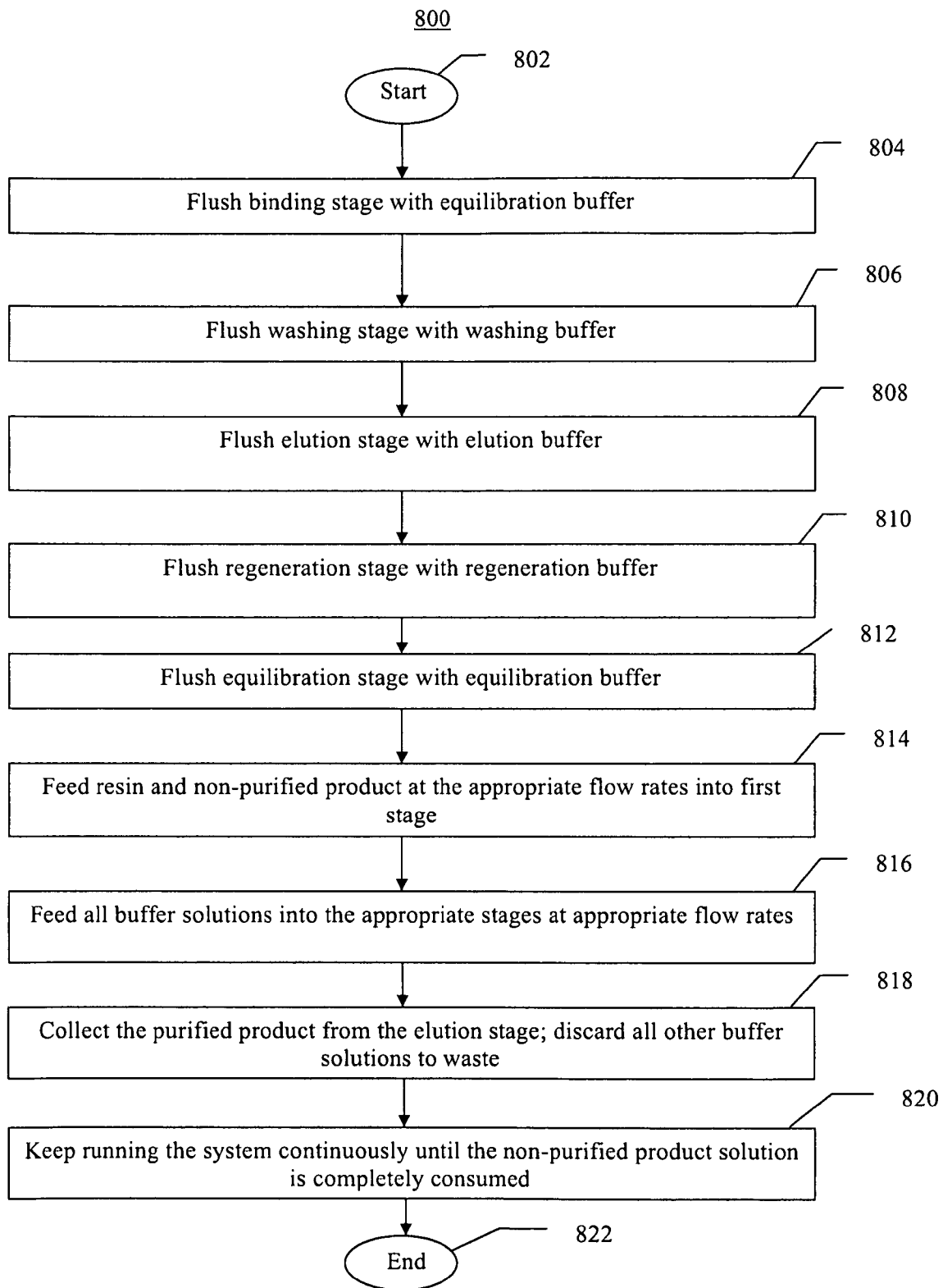


Figure 8

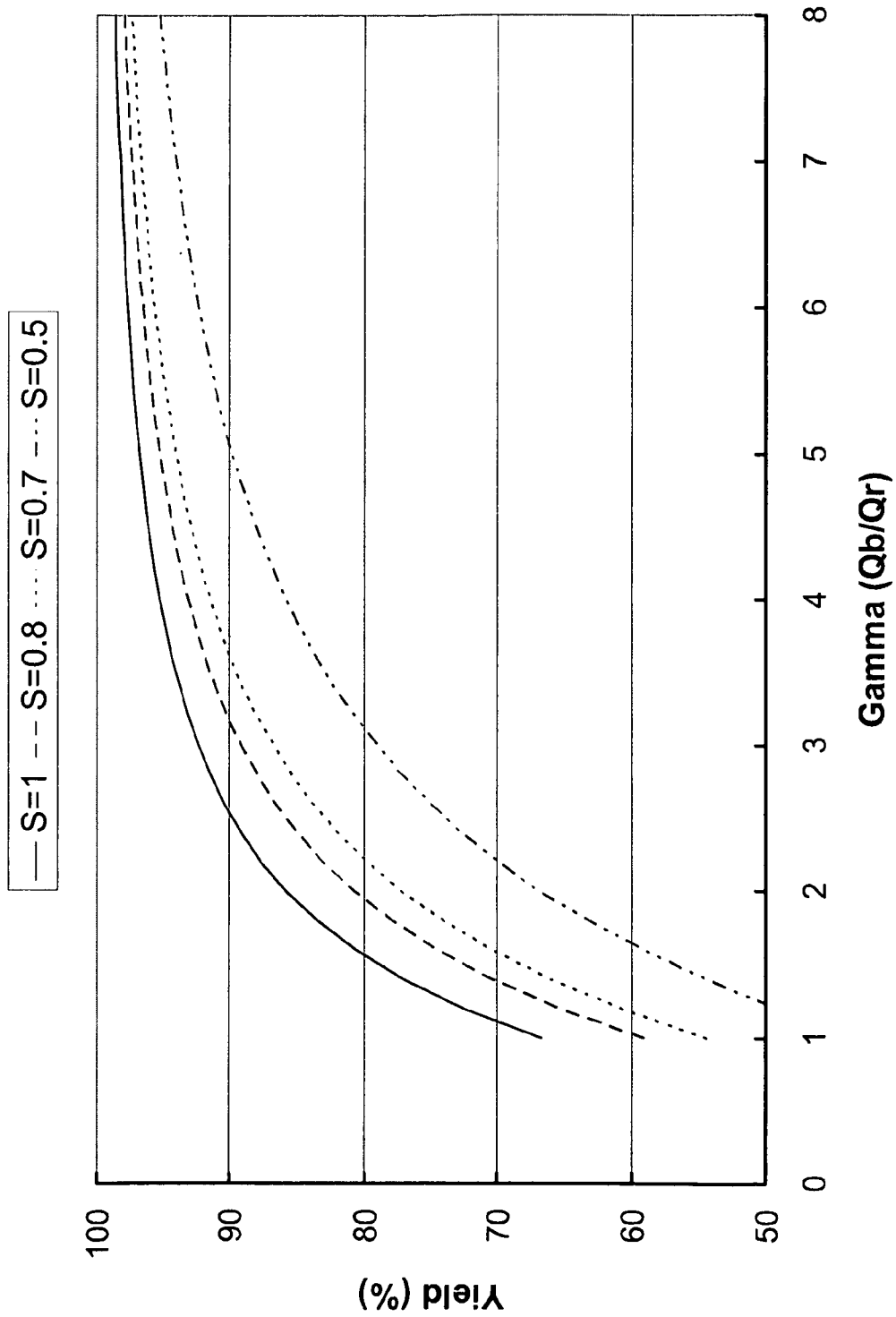


Figure 9

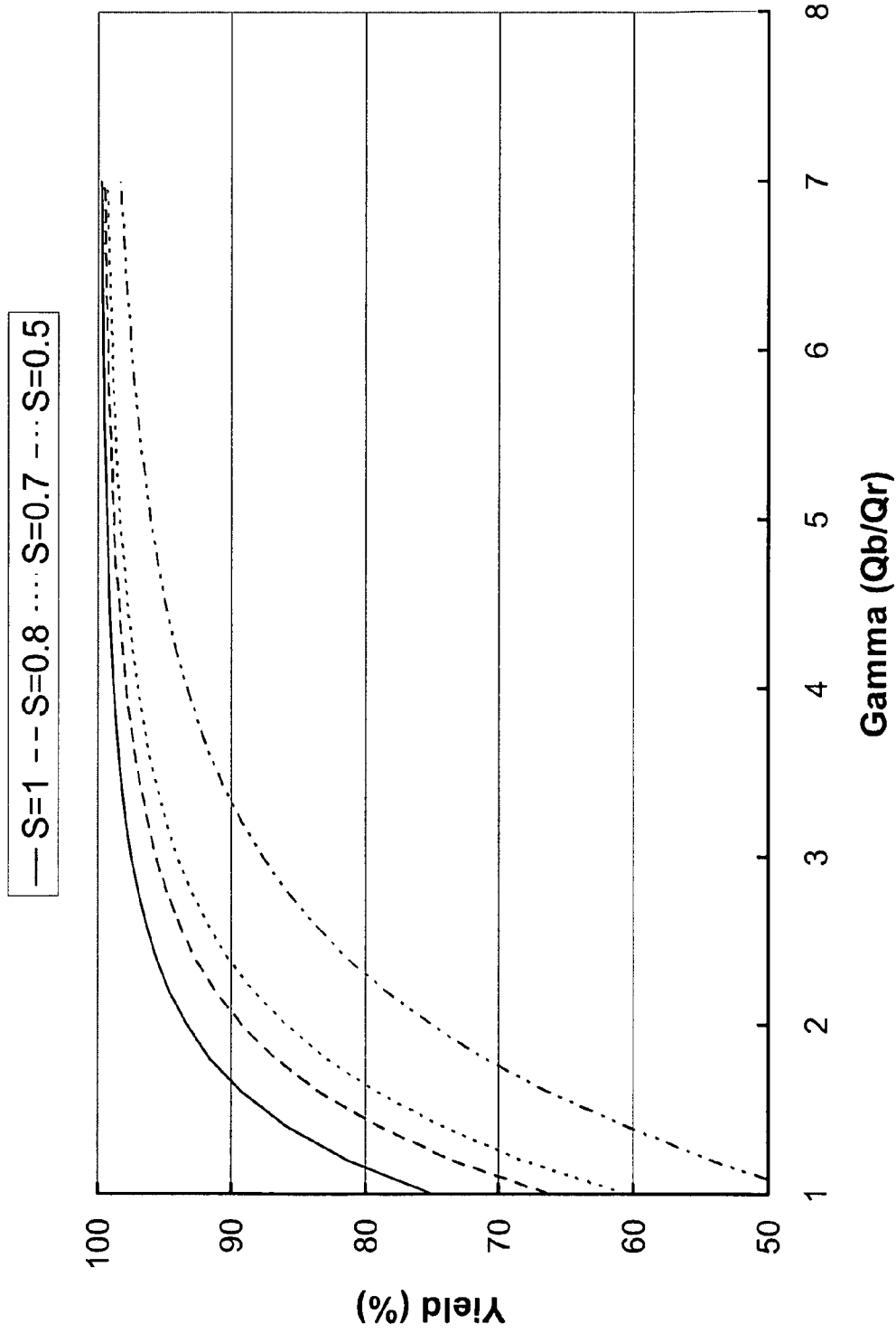


Figure 10

1

COUNTERCURRENT TANGENTIAL CHROMATOGRAPHY METHODS, SYSTEMS, AND APPARATUS

REFERENCE TO RELATED APPLICATIONS

This applications claims priority from provisional applica-
tion U.S. Ser. No. 61/150,240, filed on Feb. 5, 2009, and
entitled "Counter Current Tangential Chromatography," the
entirety of which is hereby incorporated by reference herein.

FIELD OF THE INVENTION

The present invention is generally related to chromatogra-
phy. More specifically, this invention relates to a method,
system, and apparatus of tangential chromatography using
countercurrent flow to facilitate separation of the desired
product and enhance efficiency of the entire chromatography
process.

BACKGROUND OF THE INVENTION

There has been a significant and sustained growth in new
drug production featuring monoclonal antibodies and other
proteins, approximately 15-20% annually. This growth is due
to expanding drug pipelines, as well as more efficient cell
lines and bioreactor growth optimizations. The annual bio-
production costs are currently estimated at \$2.6 billion. One
of the most significant investments a drug manufacturer has to
make is process chromatography (approximately 30% or
\$850 million annually).

Chromatography is an integral part of drug production; its
purpose in the biotechnology industry is to purify the product
proteins from contaminating species. The industry has started
to recognize that the efficiency of the chromatography steps
which are used to purify the product proteins are no longer
keeping up with production demands. There are multiple
reasons for this:

First, no significant improvements have been made to the
column chromatography process in the past 30 years—most
of the work in the industry has been focused on new resin
development. A notable exception is membrane chromatog-
raphy which was recently adopted by the industry.

Second, upstream technology has improved tremendously
in the same time period—the bioreactors are larger (up to
20,000 liters), and the titers are much higher (up to 15 g/L
compared with 1-2 g/L five years ago). As a result of longer
fermentation times, there are generally more impurities in the
bioreactor effluent solution. All of the above reasons result in
a much heavier load for the downstream purification.

Third, column chromatography has inherent physical limi-
tations. Columns larger than 2 meter in diameter do not scale
up. The largest columns in the market are 2 meter diameter
and 40 cm bed height. They fit 1,250 L of resin. Assuming a
binding capacity of 30 g/L of resin (common Protein A resin
capacity for monoclonal antibodies), a single cycle can bind
38 kg. A 20,000 L bioreactor with an output of 10 g/L would
produce a load of 200 kg. This means that the biggest column
in the market would have to run at least 6 full cycles to process
a single batch. The operation can take up to 24 hrs and can
result in a significant bottleneck for the manufacturing pro-
cess.

Finally, in the present marketplace, disposability in the
manufacturing process is gaining popularity. Disposable pro-
cess steps save labor, do not require cleaning validation and
are easier to run for the manufacturing personnel. Strides have
been made in most downstream processes to have disposable

2

systems. These are—bioreactors (up to 2,000 L volume Xcel-
lerex Corp.), microfiltration (KleenPak TFF technology from
Pall Corp.), depth filtration (POD, Millipore Corp.), sterile
filtration (all major manufacturers), tangential flow filtration
(all major manufacturers) and membrane chromatography
(Mustang, Pall Corp., Sartobind, and Sartorius Corp.). The
column chromatography technology because of its inherent
limitation cannot be a part of the disposable trend. Therefore,
it is currently impossible to have a completely disposable
downstream process—a purification train must include a
chromatography step which cannot be disposable.

Therefore, it was recognized by the present inventor that a
breakthrough in the state of the art would include solutions to
the above problems. It was recognized that the industry needs
1) larger scale of operation; 2) faster processing time; 3)
disposability; 4) reduction of media/resin expenses; and 5) a
reduction of capital equipment investment.

It is against this background that various embodiments of
the present invention were developed.

BRIEF SUMMARY OF THE INVENTION

Therefore, one embodiment of the present invention is a
scalable, reliable and disposable technology that utilizes a
principle of recycling to significantly increase process effi-
ciency, increase the scale of operation, and decrease resin
costs.

In the present invention, the chromatography column is
replaced by a module that consists of two or more intercon-
nected tangential flow filters and static mixers. The chroma-
tography resin flows through this module in a single pass,
while similar operations to a regular chromatographic pro-
cess are performed on the resin (binding, washing, elution,
regeneration, and equilibration). The buffers for these opera-
tions are pumped into the module in a countercurrent direc-
tion to the flow of resin, and permeate solutions from later
stages are recycled back into previous stages. This creates
concentration gradients in the permeate solutions of the tan-
gential flow filters in the countercurrent direction to resin
flow, thus saving buffer volume and increasing process effi-
ciency. The permeate solutions from binding, washing,
equilibration and regeneration operations are put to waste.
The permeate solution from the elution operation is the puri-
fied product stream which is collected in a separate product
tank.

Accordingly, one embodiment of the present invention is a
module **100** for countercurrent tangential chromatography
(see FIG. 1) comprising a first input port (**101**) for receiving
an input solution; a first mixer (**102**) for mixing the input
solution with a recycled solution from a second input port
(**103**) to produce a first mixer output; a stage I filter (**104**)
for concentrating the first mixer output to produce stage I reten-
tate, wherein stage I permeate solution exits the module from
the stage I filter via a first output port (**105**); a second mixer
(**106**) for mixing the stage I retentate from the stage I filter
(**104**) and an optional buffer solution from a second input port
(**107**); and a stage II filter (**108**) for concentrating an output
from the second mixer (**106**) to produce stage II retentate
which exits the module from the stage II filter via a second
output port (**110**), wherein stage II permeate solution exits the
module from the stage II filter via a third output port (**109**).

Another embodiment of the present invention is the mod-
ule described above wherein the input solution comprises
resin and unpurified product solution.

Another embodiment of the present invention is the mod-
ule described above wherein the stage I permeate solution is
waste.

Another embodiment of the present invention is the module described above wherein the stage I permeate solution is product.

Another embodiment of the present invention is the module described above wherein the second mixer receives clean buffer solution.

Another embodiment of the present invention is the module described above wherein the second mixer does not receive clean buffer solution.

Another embodiment of the present invention is the module described above wherein the third output port (109) is connected via a pump (112) and a three-way valve (111) to the second input port (103). (See FIG. 1.)

Another embodiment of the present invention is the module described above wherein the three-way valve (111) sends an output from the third output port (109) either to waste or product (113), or to the second input port (103). (See FIG. 1.)

Another embodiment of the present invention is the module described above wherein the stage I filter and the stage II filter are tangential flow filters.

Yet another embodiment of the present invention is a system (300) for countercurrent tangential chromatography (see FIG. 3) comprising a module (100) for countercurrent tangential chromatography; a first resin tank (302) for storing clean resin connected to a first input port (101) of the module; an input tank (304) for storing input solution connected to the first input port (101); a pump (112) and a three-way valve (111) interconnecting a second input port (103) with a third output port (109) of the module; a second resin tank (325) for storing resin connected to a second output port (110) of the module via a pump (323) and the three-way valve (317), and connected to the first input port (101) of the module via a pump (313); a product tank (309) for capturing product solution connected via a three-way valve (307) and a pump (308) to the first output port (105) of the module; and one or more buffer tanks (316, 318, 320, 322) for storing buffer solution connected to the third input port (107) of the module via a pump (314), wherein the first resin tank (302) is connected via 3-way valve (317) to second resin tank (325) and pump (323).

Another embodiment of the present invention is the system described above further comprising an additional countercurrent tangential chromatography stage comprising a third mixer (530) and a third filter (532), for increasing efficiency of the system. (See FIG. 5.)

Other embodiments of the present invention include the methods corresponding to the systems above, the systems constructed from the modules described above, and the methods of operation of the systems and modules described above. Other features and advantages of the various embodiments of the present invention will be apparent from the following more particular description of embodiments of the invention as illustrated in the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a module for countercurrent tangential chromatography according to one embodiment of the present invention.

FIG. 2A shows direction of flow in the module during binding mode, while FIG. 2B shows direction of flow in the module during elution, washing, equilibration, and regeneration modes.

FIG. 3 shows a block diagram of a countercurrent tangential chromatography system operating in batch mode according to one embodiment of the present invention.

FIG. 4 shows another block diagram of the countercurrent tangential chromatography system of FIG. 3.

FIG. 5 shows a block diagram of another countercurrent tangential chromatography system according to another embodiment of the present invention.

FIG. 6 shows a block diagram of another countercurrent tangential chromatography system operating in continuous mode, according to yet another embodiment of the present invention.

FIGS. 7A, 7B, and 7C show a flowchart of a process of countercurrent tangential chromatography operating in batch mode, according to yet another embodiment of the present invention.

FIG. 8 shows a flowchart of a process of countercurrent tangential chromatography operating in continuous mode, according to yet another embodiment of the present invention.

FIG. 9 shows results of a mathematical model of a two-stage countercurrent tangential chromatography system according to the principles of the present invention, showing a ratio of buffer to resin flow-rates (γ) vs. percent yield for various sieving coefficients.

FIG. 10 shows results of a mathematical model of a three-stage countercurrent tangential chromatography system according to the principles of the present invention, showing a ratio of buffer to resin flow-rates (γ) vs. percent yield for various sieving coefficients.

DETAILED DESCRIPTION OF THE INVENTION

Definitions: The following terms of art shall have the below ascribed meanings throughout this Specification.

Binding mode is a stage of operation during which resin and unpurified product form a reversible complex.

Washing mode is a stage of operation during which resin with bound product is washed with a washing buffer to rid the resin of impurities.

Elution mode is a stage of operation during which the complex of resin and the product is reversed and the purified product is collected.

Regeneration mode is a stage of operation during which the resin is cleaned for the purpose of reuse or for later cycles.

Equilibration mode is a stage of operation during which the system is equilibrated in a neutral buffer.

As stated in the Summary of the Invention section, in the present invention, the chromatography column is replaced by a module that consists of two or more interconnected tangential flow filters and static mixers. The chromatography resin flows through this module in a single pass, while similar operations to a regular chromatographic process are performed on the resin (binding, washing, elution, regeneration, and equilibration). The buffers for these operations are pumped into the module in a countercurrent direction to the flow of resin, and permeate solutions from later stages are recycled back into previous stages. This creates concentration gradients in the permeate solutions of the tangential flow filters in the countercurrent direction to resin flow, thus saving buffer volume and increasing process efficiency. The permeate solutions from binding, washing, equilibration and regeneration operations are put to waste. The permeate solution from the elution operation is the purified product stream which is collected in a separate product tank.

Accordingly, FIG. 1 shows a block diagram of a module 100 for countercurrent tangential chromatography (inside the dotted line). Input solution enters at port 101, and the input solution and any input from port 103 (none during binding mode) are mixed inside static mixer 102. The output from the static mixer 102 enters a tangential flow filter 104, from which the permeate exits the module at port 105. The retentate from

5

tangential flow filter **104** is fed into static mixer **106**, which may receive pure buffer at port **107**. The output from static mixer **106** is fed into a tangential flow filter **108**, from which permeate is pumped via pump **112** out of the module at port **109**. Three-way valve **111** is utilized to direct flow either to waste **113** or to port **103**. The retentate from tangential flow filter **108** exits the module at port **110**. During washing, elution, equilibration and regeneration, three-way valve **111** directs the flow to port **103**, where it mixes with input **101** in static mixer **102**. During binding, three-way valve **111** directs the flow to waste **113**.

FIG. 2A shows direction of flow in module **100** of FIG. 1 during binding mode. Note the single-pass nature of the flow, and the fact that no flow enters at port **103** and port **107**. The mixture of resin and non-purified product solution enter at the left through port **101** (through mixer **102**), flows through filter **104** (with permeate exiting as waste at port **105**), flows through mixer **106**, flows through filter **108** (with permeate exiting as waste at port **109** via pump **112** and three-way valve **111**), and exits the module at the right through port **110**.

FIG. 2B shows direction of flow in module **100** during elution, washing, regeneration, and equilibration modes. Note the single-pass nature of the flow, and the fact that flow is recycled in a countercurrent direction from port **109** to port **103** via pump **112** and three-way valve **111**. Note how in this configuration, clean buffer solution enters at port **107**, and recycled buffer solution enters at port **103**. Note also the countercurrent nature of the flow, where the solution being processed flows left-to-right as in FIG. 2A, while recycled buffer solution flows from right-to-left via pump **112** and three-way-valve **111**, in a direction that is "counter-current" to the left-to-right flow of the solution being processed.

FIG. 3 shows a block diagram of a countercurrent tangential chromatography system **300** operating in batch mode according to another embodiment of the present invention. Module **100** operates in the same way as shown and described in relation to FIG. 1. Input port **101** of module **100** is connected to pumps **303**, **305** and **313**. Pump **303** pumps resin from first resin tank **302**. Pump **313** pumps resin from second resin tank **325**. Pump **305** pumps unpurified product solution from input tank **304**. Port **103** of module **100** is connected via three-way valve **111** and pump **112** to port **109** of module **100**, as shown in FIG. 1. Waste exits the system at **113**. Output from port **105** is connected to pump **306**, which is connected to a three-way valve **307**. Three-way valve **307** is connected to product tank **309** and waste **308**. Port **107** receives input into module **100** via pump **314**, which is connected to equilibration tank **316**, washing tank **318**, elution tank **320** and regeneration tank **322** via valves **315**, **317**, **319** and **321**. Output from port **110** is pumped via pump **323** and three-way valve **317** to the first resin tank **302** and a second resin tank **325**.

The system in FIG. 3 is designed to treat the resin using a batch-mode operation. The resin is sequentially treated by different chromatographic processes (binding, washing, elution, regeneration, and equilibration) as it cycles from the first resin tank **302** to the second resin tank **325** and vice versa. For example, during the first stage (binding), resin passes from tank **302** to tank **325** from left to right through module **100** via pump **303**. During the next stage (washing) resin passes from tank **325** to tank **302** from left to right through module **100** via pump **313**. The other stages (elution, regeneration, and equilibration) alternate tanks in a similar manner. The countercurrent operation during washing, elution, regeneration, and equilibration allows greater efficiency and buffer conservation.

6

FIG. 4 shows a block diagram **400** of the countercurrent tangential chromatography system shown in FIG. 3, with the internal structure of module **100** shown. FIG. 4 is identical to FIG. 3 with the exception that the internals of module **100** are shown as in FIG. 1.

FIG. 5 shows a block diagram **500** of another countercurrent tangential chromatography system **500** according another embodiment of the present invention. This embodiment is similar to FIGS. 3 and 4, except for addition of an additional (third) stage of countercurrent filtration, made up of mixer **530**, filter **532**, pump **533**, and three-way valve **531**, interconnected as shown in FIG. 5. Waste exits at **536**. The embodiment shown in FIG. 5 operates in a manner analogous to the system shown in FIGS. 3 and 4, with the exception that flow passes through a third stage, which increases process efficiency and decreases buffer utilization, but introduces some complexity into the design. Mathematical modeling described below shows that using more than three stages produces no appreciable improvement in process efficiency while introducing substantial complexity. Hence, more than three stages, while possible according to the principles of the present invention and within the scope of the present invention, are not described further.

FIG. 6 shows a block diagram **600** of another countercurrent tangential chromatography system operating in continuous mode, according to yet another embodiment of the present invention. Modules **610** ("binding stage"), **620** ("washing stage"), **630** ("elution stage"), **640** ("regeneration stage") and **650** ("equilibration stage") operate in an analogous manner to the operation of module **100** shown in FIGS. 1, 2A and 2B. The thick black line on modules **620**, **630**, **640** and **650** represent a connection of a third output port (**109**) and a second input port (**103**) via pump (**112**) and three-way valve (**111**) on each module shown. These pumps and three-way valves are not shown in FIG. 6 for clarity, but they are present in each module **620**, **630**, **640** and **650** as shown in FIG. 1. Note that module **610** ("binding stage") does not have its third output port (**609**) nor its second input port (**607**) interconnected, since in this module both port **609** and port **607** go to waste.

Binding stage module **610** is connected at port **605** via pump **604** to non-purified product tank **602**, via pump **606** to resin tank **608**, and via three-way valve **658** and pump **657** to equilibration buffer tank **656**. Ports **607** and **609** on module **610** go to waste.

Washing stage module **620** is connected at port **613** via pump **612** to an output port **611** of the binding stage module **610**. Port **621** goes to waste via pump **622**. Washing buffer enters at port **627** via pump **626** from washing buffer tank **624**.

Elution stage module **630** is connected at port **625** via pump **624** to output port **623** of washing stage module **620**. Elution buffer enters at port **637** via pump **639** from elution buffer tank **638**. Purified product exits module **630** at port **627** via pump **636** into product storage tank **632**.

Regeneration module **640** is connected at port **635** via pump **634** to output port **633** of module **630**. Waste exits at port **643** via pump **646**. Regeneration buffer enters at port **645** via pump **641** from regeneration buffer tank **642**.

Equilibration module **650** is connected at port **649** via pump **648** to output port **647** of regeneration module **640**. Resin is pumped out of port **651** via pump **652** into the resin storage tank **608**. Waste is pumped from module **650** at port **661** via pump **653**. Equilibration buffer enters at port **659** via the three-way valve **658** and the pump **657** from the equilibration buffer tank **656**.

Accordingly, unlike the system of FIG. 3, which is designed to treat the resin/product in alternating batch-mode, with resin alternating between the first and the second resin tanks, the system of FIG. 6 is designed to treat the resin/product in a single continuous pass, with resin flowing continuously from the resin tank 608, through modules 610, 620, 630, 640, and 650, and returning to resin tank 608. The continuous nature of the system shown in FIG. 6 allows a fixed amount of resin to be used for processing an essentially unlimited amount of unpurified product, subject only to the lifetime of the resin.

FIGS. 7A, 7B, and 7C show a flowchart of a process 700 of countercurrent tangential chromatography operating in batch mode, according to yet another embodiment of the present invention. Process 700 begins at step 702. The system is flushed with equilibration buffer, as shown in step 704. In step 706, the binding stage is started (emphasis in bold). Resin and non-purified product is pumped into the system at appropriate flow rates, as shown in step 708. The permeate solutions are discarded from all stages as waste during the binding stage only, as shown in step 710. The resin is collected with bound product as shown in step 712.

In step 714, the washing stage is started (emphasis in bold). The system is flushed with washing buffer, as shown in step 716. The countercurrent permeate is recycled and utilized during the washing stage to improve process efficiency and conserve buffer solution according to the principles of the present invention, as shown in step 718. Resin is pumped with bound product back into the first stage of the system, where it mixes with the recycled wash buffer, as shown in step 724. The washed resin with bound product is collected in the first resin tank, while permeate solution is discarded as waste, as shown in step 726.

In step 728, the elution stage is started (emphasis in bold). The system is flushed with elution buffer, as shown in step 730. The countercurrent permeate is recycled and reused during the elution stage in order to improve process efficiency and to conserve buffer solution, as shown in step 732. Resin bound with product is pumped back into the first stage of the system, where it mixes with the recycled elution solution, as shown in step 734.

In step 736, permeate solution from the first stage is collected as product solution (emphasis in bold). Resin is collected in the second resin tank, as shown in step 738.

In step 740, the regeneration stage is started (emphasis in bold). The system is flushed with regeneration solution, as shown in step 742. The countercurrent permeate is recycled and reused during the regeneration stage, in order to improve process efficiency and to conserve buffer solution, as shown in step 744. The resin is pumped into the first stage, where it mixes with the recycled regeneration solution, as shown in step 746. The permeate solution is discarded as waste, as shown in step 748.

In step 750, the resin is collected in the first resin tank (emphasis in bold), hence completing the cycle and allowing the reuse of resin.

Finally, the equilibration process using equilibration buffer may be repeated if more cycles are required, as shown in step 752. Alternatively, equilibration process may be performed with storage solution if the resin requires storage, as shown in step 752. The process 700 ends in step 754.

FIG. 8 shows a flowchart of a process 800 of countercurrent tangential chromatography operating in continuous mode, according to yet another embodiment of the present invention. Process 800 begins in step 802. The binding stage (Module 610 of FIG. 6) is flushed with equilibration buffer, as shown in step 804. The washing stage (Module 620 of FIG. 6) is flushed with washing buffer, as shown in step 806. The elution stage (Module 630 of FIG. 6) is flushed with elution buffer, as shown in step 808. The regeneration stage (Module

640 of FIG. 6) is flushed with regeneration buffer, as shown in step 810. The equilibration stage (Module 650 of FIG. 6) is flushed with equilibration buffer, as shown in step 812. Resin and non-purified product are fed at the appropriate flow rates into the first stage of the system (Module 610 of FIG. 6), as shown in step 814. All buffer solutions are fed into the appropriate stages at appropriate flow rates, as shown in step 816. The purified product is collected from the elution stage (Module 630 of FIG. 6), while all other buffer solutions are discarded to waste, as shown in step 818. The entire system is kept running continuously until the non-purified product solution is completely consumed, as shown in step 820. The process 800 ends in step 822.

Modeling

Product recovery is one of the most important cost drivers in chromatography. This is because the protein molecules are of extremely high value. A capture chromatography process should have a recovery of at least 90%. Therefore, it was decided to model the product recovery stage of the present invention (the elution stage).

The following assumptions were made in this model:

1. The tangential flow (TFF) membranes in the module are able to process the slurry of resin and elution buffer at appropriate conversion factors (upwards of 80%).

2. The kinetics of desorption of the product molecule from the resin is fast.

3. The sieving coefficient of the TFF membrane is constant through out the process.

4. The system is "dead-space" free.

The impact on the percent yield (% recovery) of the following variables are explored in this model:

1. "Gamma (γ)" is the ratio of elution buffer flow-rate to resin buffer flow rate, and governs the dilution of the product, buffer usage, and washing efficiency. This variable can be controlled by the operator.

2. "s" is the sieving coefficient of the TFF membrane for the product molecule. s equals the product concentration in the permeate divided by the product concentration in the retentate. This is an inherent property of the membrane and cannot be changed by the operator.

3. "N" is the number of stages; the present model explores a two-stage and a three-stage system in operation. As the number of stages increases, the washing efficiency and product recovery increases, with all other variables are held constant. However, more stages increase the complexity and cost of the system.

Model equations were derived by using material balances and solving for % yield. It became convenient to introduce a new variable $\alpha = \gamma \cdot s$.

Equation 1 shows the percent-yield for a two-stage system as a function of α :

$$\% \text{ Yield} = \left(1 - \frac{1}{(1 + \alpha + \alpha^2)}\right) * 100\% \quad (1)$$

Equation 2 shows the percent-yield for a three-stage system as a function of α :

$$\% \text{ Yield} = \left(1 - \frac{1}{(1 + \alpha + \alpha^2 + \alpha^3)}\right) * 100\% \quad (2)$$

FIGS. 9 and 10 show the results of this model; gamma (γ) is the independent variable, and percent (%) yield is the

dependent variable. Percent yield curves are generated for specific sieving coefficients for both models ($s=0.5, 0.7, 0.8, 1.0$).

FIG. 9 shows the results for a two-stage countercurrent tangential chromatography system showing the ratio of buffer to resin flow-rates (γ) vs. percent yield for sieving coefficients $s=0.5, 0.7, 0.8, 1.0$.

FIG. 10 shows the results for a three-stage countercurrent tangential chromatography system showing the ratio of buffer to resin flow-rates (γ) vs. percent yield for sieving coefficients $s=0.5, 0.7, 0.8, 1.0$.

The results of the model show that greater than 95% yield can be achieved by both the two-stage and the three-stage systems. Sieving coefficients for these processes are expected to be within a range of [0.8-1.0] because the membranes used in this system would be microporous and would therefore be expected to pass the product molecule relatively freely. The two-stage system would need a higher buffer to feed ratio (γ) than the three-stage system to achieve the same percent (%) yield. Therefore, the recommended operating gamma (γ) for a two-stage system is 4 to 6, and for a three-stage system the recommended operating gamma (γ) is 3 to 4.

A modeling example is described here of protein A capture of 20,000 L bioreactor harvest, 5 g/L IgG concentration, in a three-stage countercurrent tangential chromatography system operating in batch mode, as shown in FIG. 5. This example is illustrative of one of many modes of operation of the present invention.

This modeling example makes the following assumptions:

1. Residence time=0.5 min (hypothetical "small" protein A bead)
2. Resin capacity=30 g/L
3. General Electric® hollow fibers are used as the TFF membrane. The areas and hold up volumes are used from existing large scale General Electric® modules.
4. Flux=100 LMH
5. An 80% conversion factor is assumed in the TFF filters.

TABLE 1

Modeling results	
Volume	20000 L
Product conc.	5 g/L
Total product	100 kg
Total Membrane area	300 m ²
# of stages	3
Washing dilution factor	4
Resin Volume	300 L
Resin Capacity	30 g/L
Flux	100 LMH
One cycle processes	9 kg MAB
One cycle Volume	1800 L
Residence time	0.5 min
Static mixer volume	100 L
Total Flow	200 L/min
Resin Flow rate	28.6 L/min
Feed Flow rate	171.4 L/min
Feed Flux	34.3 LMH
Binding stage time	0.175 hrs
Wash Volume 4 Resin Volumes (RV)	1200 L
Washin stage Time	0.120 hrs
Elution Volume 4 Resin Volumes (RV)	1200 L
Elution stage Time	0.12 hrs
Regeneration Buffer volume (4 RV) optional	1200 L
Wash Time	0.120 hrs
Equilibration Buffer volume (4 RV)	1200
Wash Time	0.120 hrs
Total Cycle time	0.66 hrs
# of cycles	12
Total Processing Time	7.9 hrs

The results of this model show the following:

1. 20,000 L of unpurified product can be processed with 300 L of resin which represents a factor of 4 decrease from conventional column chromatography.

2. The operation can be performed in a single 8-hr shift.

3. Number of cycles can be decreased by increasing resin volume.

4. Efficiency and process time could be increased by increasing flux.

10 The inventor recognizes numerous and substantial advantages of the present invention to the downstream purification process, including:

1. Current technology could be readily adapted to this process because existing components are readily available in the market. Namely, the tangential flow filters (cassettes, hollow fibers and ceramic membranes) and chromatography resins are readily available. It might be advantageous to develop a new line of resins specifically designed for this invention by using smaller beads than in conventional column chromatography. This would nullify mass transfer limitations, increase dynamic binding capacity, and make the process more efficient.

2. Tangential chromatography systems according to the principles of this invention may be scaled as large as necessary, similarly to any tangential flow system. This is not the case with conventional column chromatography—the largest scalable columns in the market are currently limited to 2 meters in diameter.

3. Continuous-mode countercurrent tangential chromatography can be designed as shown in FIG. 6. In general, continuous processes are more efficient and require a smaller system size.

4. There is potential to run this system in a completely disposable manner. This is because much smaller amounts of resin are needed for this operation than in column chromatography (this would be true for cheaper resin kinds such as ion exchange resins). Additionally, the tangential flow filters at smaller scales could be used on a disposable basis as well.

5. The use of resin could be an order of magnitude lower than in conventional chromatography, causing significant cost savings by as much as 80%.

Therefore, the present inventor recognizes numerous applications of the present invention to the \$850+ million/year process chromatography market.

45 U.S. Pat. No. 4,780,210 to Jen-Chang Hsia entitled "Tangential flow affinity ultra-filtration" describes a process for trypsin purification. More particularly, it relates to a process of biochemical purification which combines the processing techniques of affinity chromatography and tangential ultra-filtration, and is capable of being operated on a continuous flow or semi-continuous-flow basis, for use in the purification (or separation) of molecules of biological interest. The process of the present invention is verifiably different because of the countercurrent and single-pass nature, along with various other improvements. The process described in U.S. Pat. No. 4,780,210 is not suitable for the biotech market.

Accordingly, while the methods disclosed herein have been described and shown with reference to particular operations performed in a particular order, it will be understood that these operations may be combined, sub-divided, or re-ordered to form equivalent methods without departing from the teachings of the present invention. Accordingly, unless specifically indicated herein, the order and grouping of the operations is not a limitation of the present invention.

65 Finally, while the invention has been particularly shown and described with reference to particular embodiments thereof, it will be understood by those skilled in the art that

11

various other changes in the form and details may be made without departing from the spirit and scope of the invention, as defined in the appended claims.

What is claimed is:

1. A module, comprising:

a first input port for receiving an input solution;

a first mixer for mixing the input solution with a recycled solution from a second input port to produce a first mixed output;

a stage I filter for concentrating the first mixed output to produce stage I retentate, wherein stage I permeate exits the module from the stage I filter via a first output port;

a second mixer for mixing the stage I retentate from the stage I filter and an optional buffer solution from a third input port to produce a second mixed output; and

a stage II filter in series with the stage I filter for concentrating the second mixed output to produce stage II retentate which exits the module from the stage II filter via a second output port, wherein stage II permeate exits the module from the stage II filter via a third output port, wherein the input solution from input port flows through the stage I filter and the stage II filter in a single pass, and

12

recycled solution from the third output port flows countercurrent to the input solution into the second input port.

2. The module of claim 1, wherein the input solution comprises resin slurry and unpurified product solution.

3. The module of claim 1, wherein the stage I permeate is waste.

4. The module of claim 1, wherein the stage I permeate is product.

5. The module of claim 1, wherein the second mixer receives clean buffer solution.

6. The module of claim 1, wherein the second mixer does not receive clean buffer solution.

7. The module of claim 1, wherein the third output port is connected via a pump and a three-way valve to the second input port.

8. The module of claim 7, wherein the three-way valve sends an output from the third output port either to waste, or to the second input port.

9. The module of claim 1, wherein the stage I filter and the stage II filter are tangential flow filters.

10. The module of claim 1, wherein the first mixer and the second mixer are static mixers.

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