

Figure 1: Schematic of conventional continuous perfusion process followed by 3 physically and logistically segregated isolation process steps (batch harvest collection, batch particle removal and batch concentration/purification)

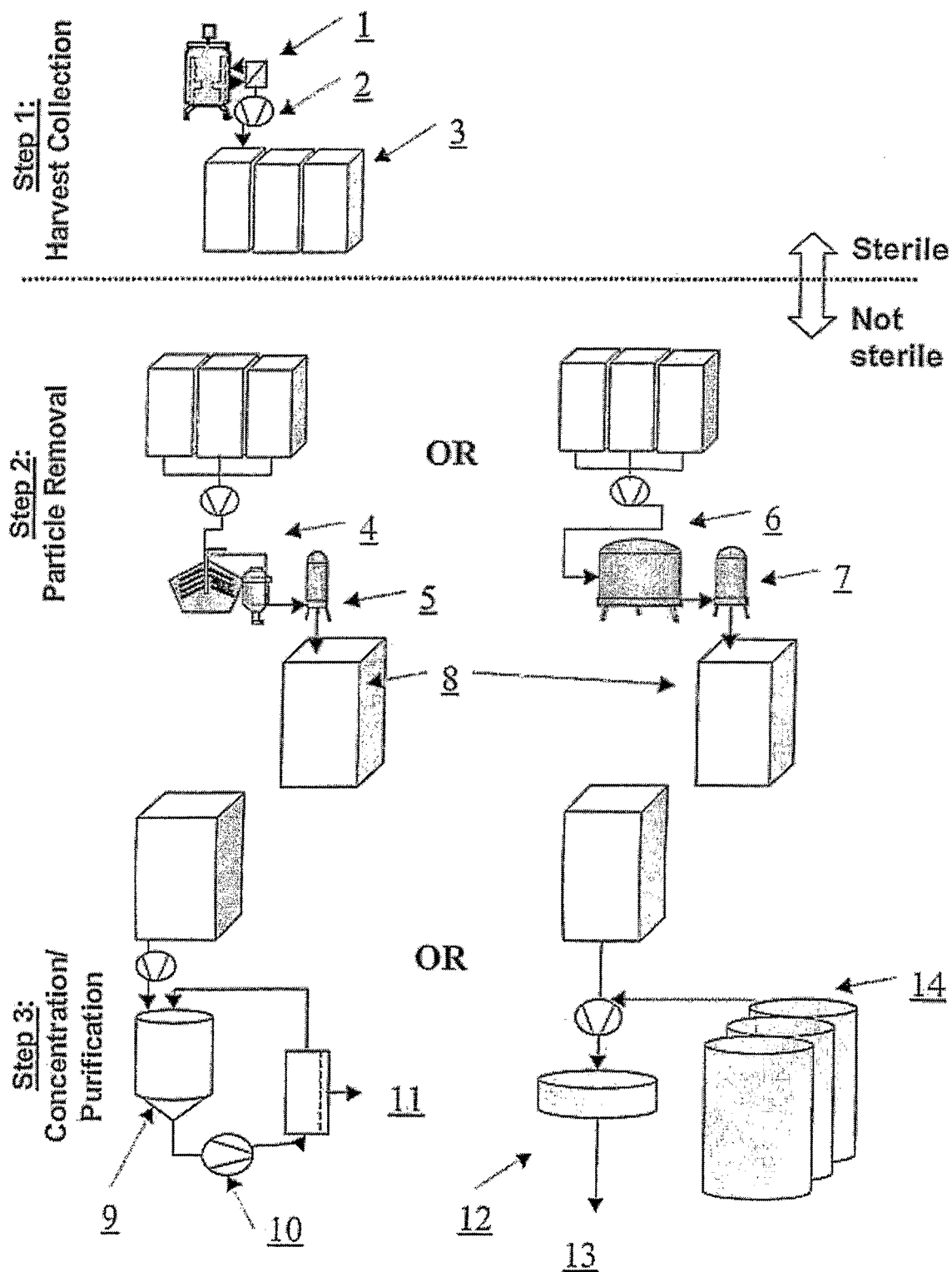


Figure 2: Schematic representation of 2 embodiments of inventive device A for continuous, integrated and sterile manufacturing. Schematic of embodiment A1 shown on left hand side and schematic of embodiment A2 shown on right hand side.

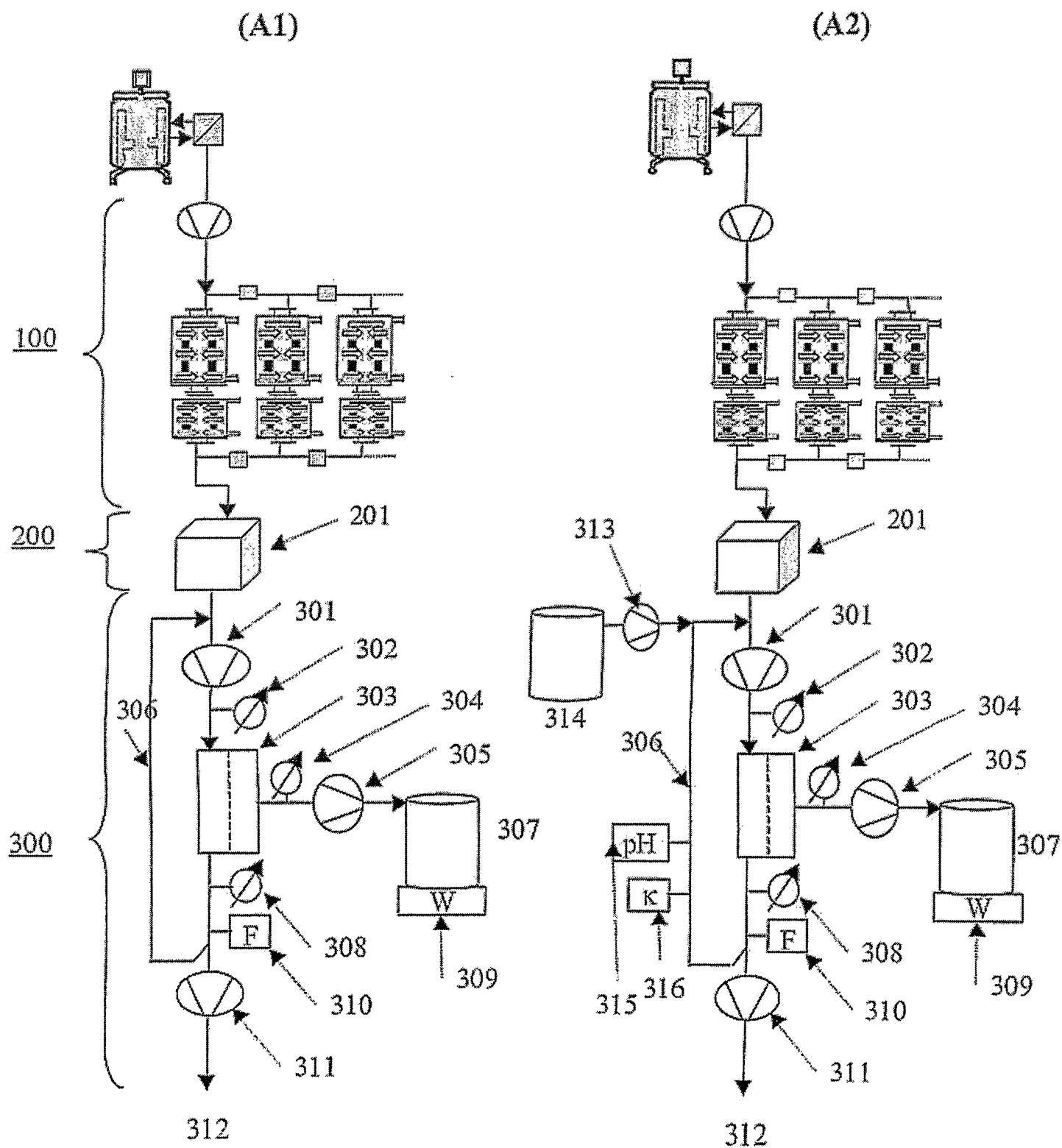


Figure 3: Schematic representation of 2 embodiments of inventive device B for continuous, integrated and sterile manufacturing. Schematic of embodiment B1 shown on left hand side and schematic of embodiment B2 shown on right hand side.

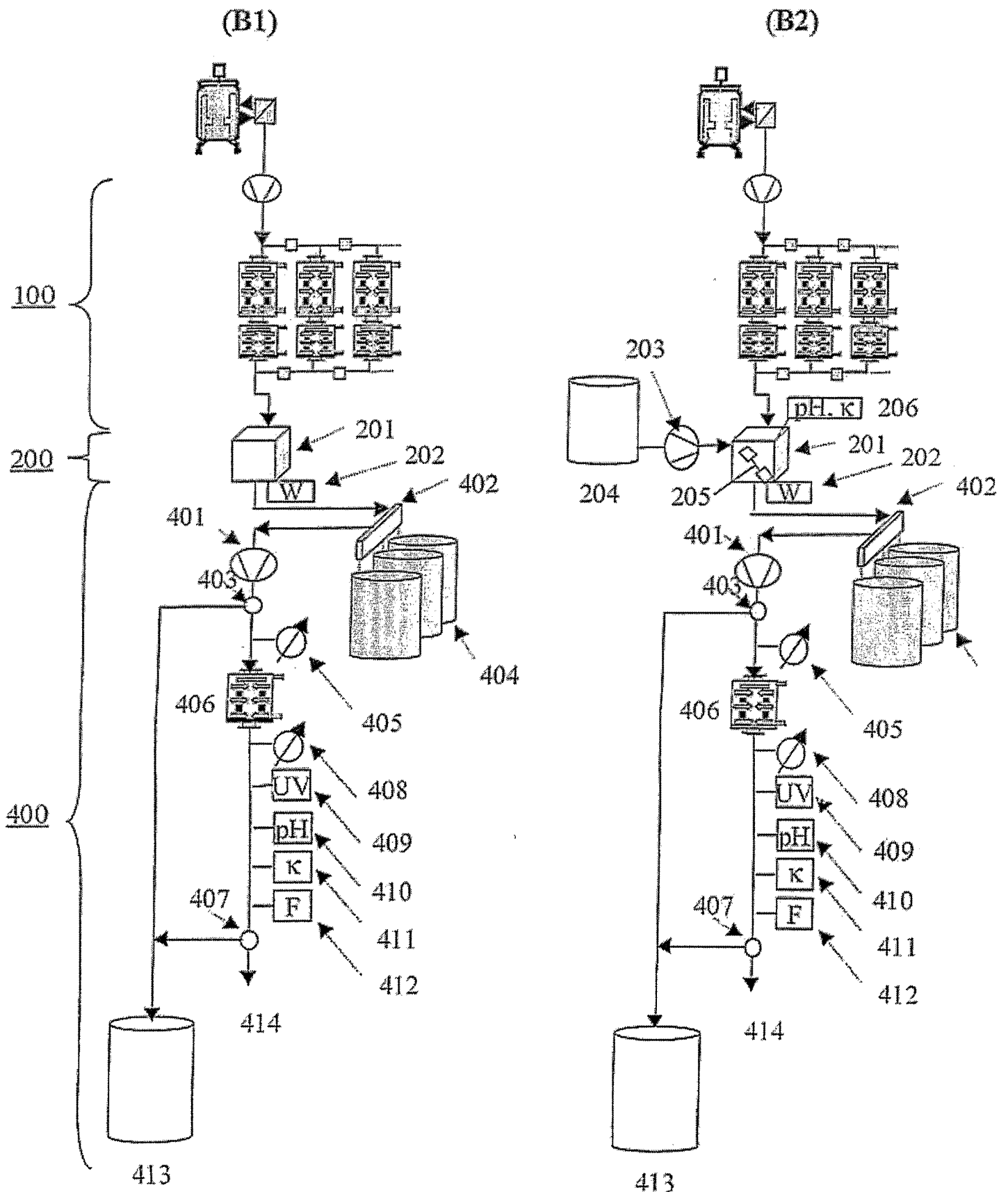


Figure 4: Schematic of an embodiment of inventive integrated, continuous particle removal system (100), an element of both inventive device A and inventive device B.

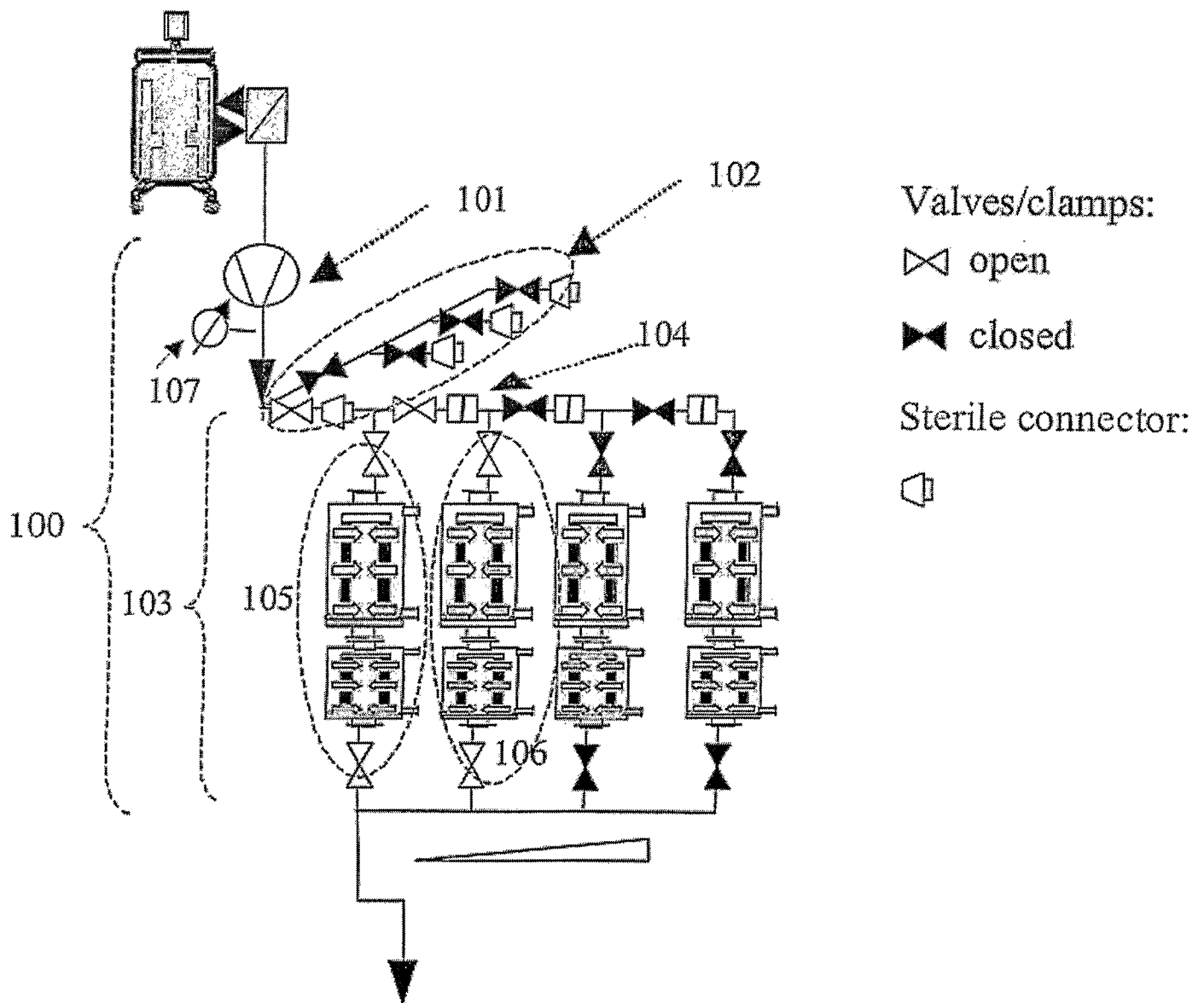
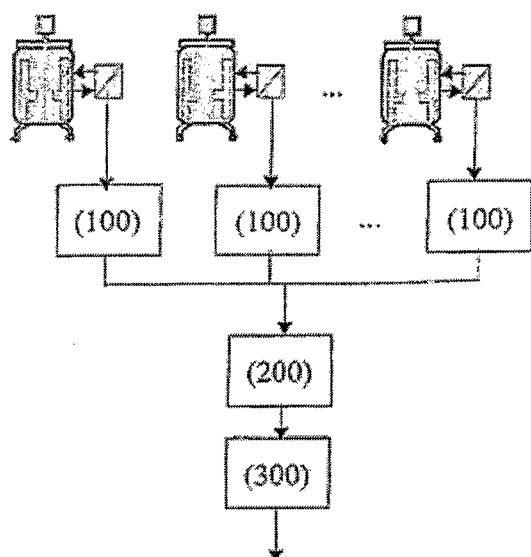
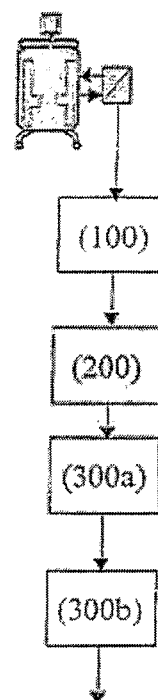


Figure 5: Schematic representations of additional embodiments of inventive device A which combine multiple elements to either increase overall plant capacity (A3) or concentration and separation performance (A4).

(A3)



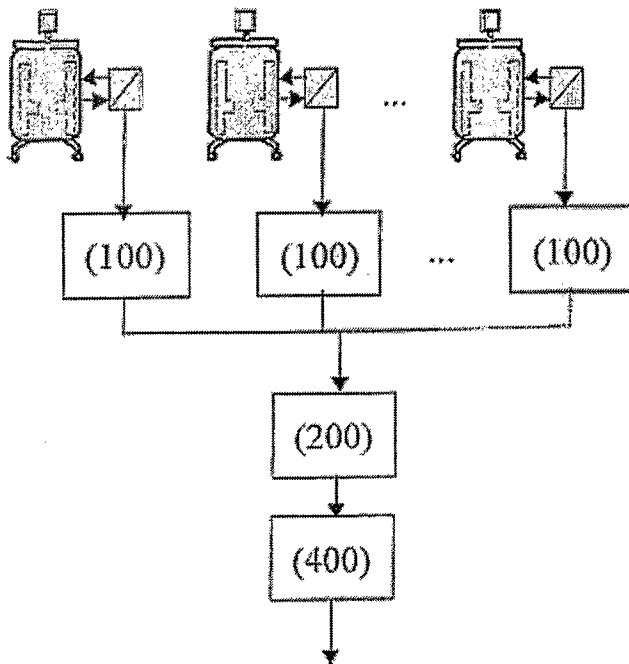
(A4)



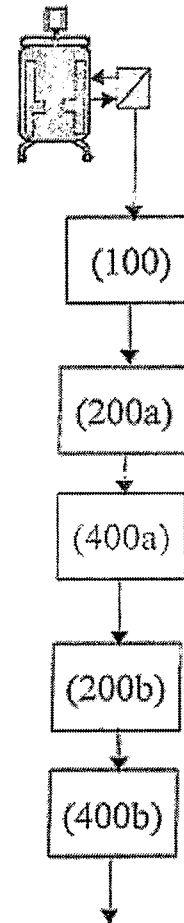
6/17

Figure 6: Schematic representations of additional embodiments of inventive device B.

(B3)

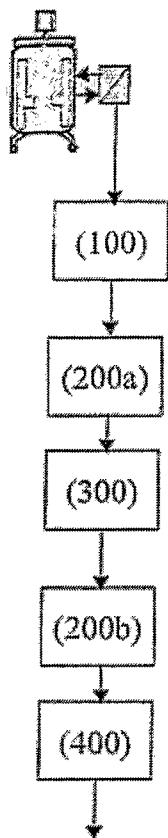


(B4)



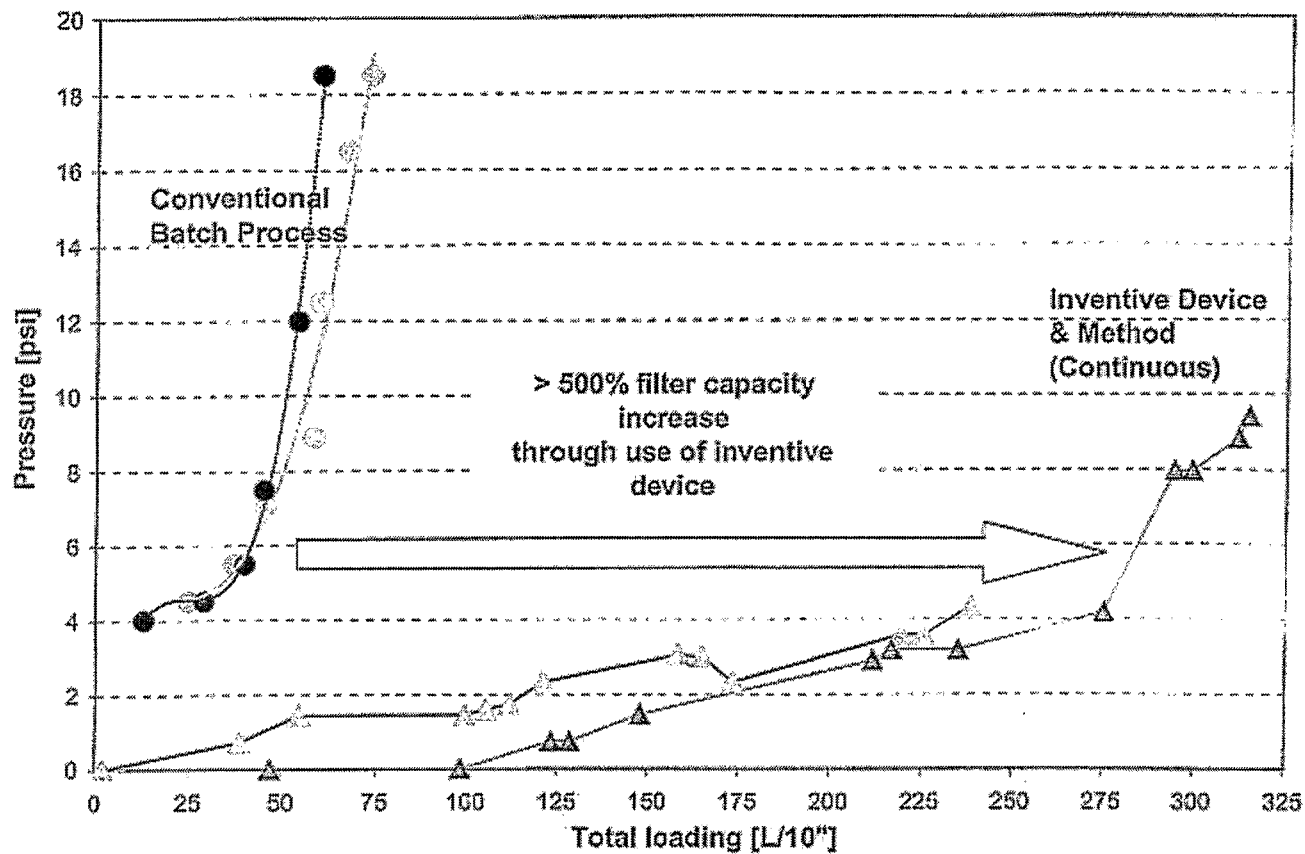
7/17

Figure 7: Additional embodiment of inventive devices that combines the elements of device A and device B in series to increase overall concentration and separation performance.



8/17

Figure 8: Example comparison of total load capacities per 10" of filter capsule for conventional batch process and embodiment of inventive device and method for continuous particle removal (integrated continuous filtration process) using identical commercial filter capsules. Example method producing recombinant blood coagulation Factor VIII shown.



9/17

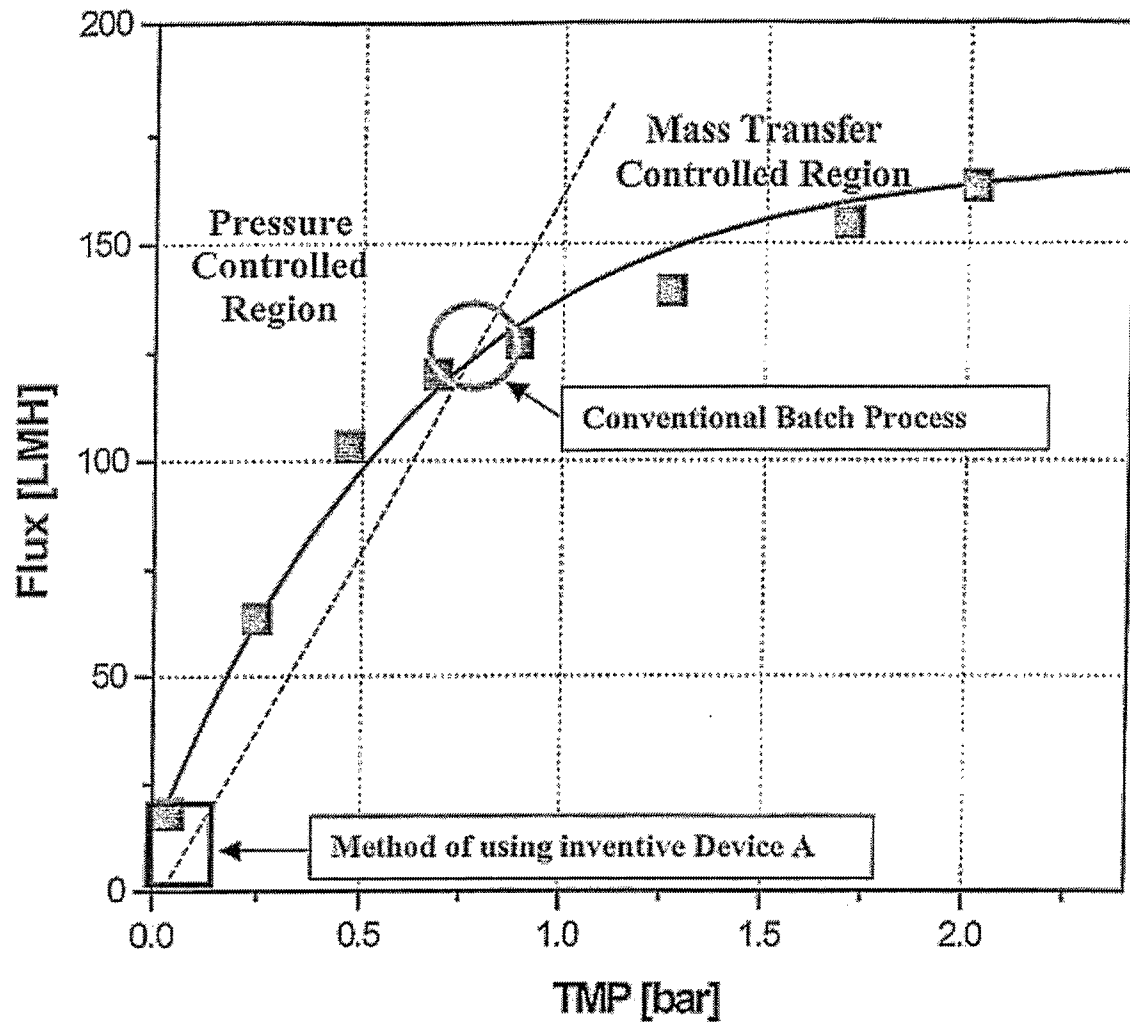


Figure 9: Example of pressure-flux curve (specific permeate flux in LMH = liters/hour/m² over transmembrane pressure) and determination of operation point. The circle shows the typical operation point that will be adjusted via the TMP for conventional batch processes. The rectangle shows the operational region that will be adjusted via the permeate pump as per the method of using the inventive device A.

10/17

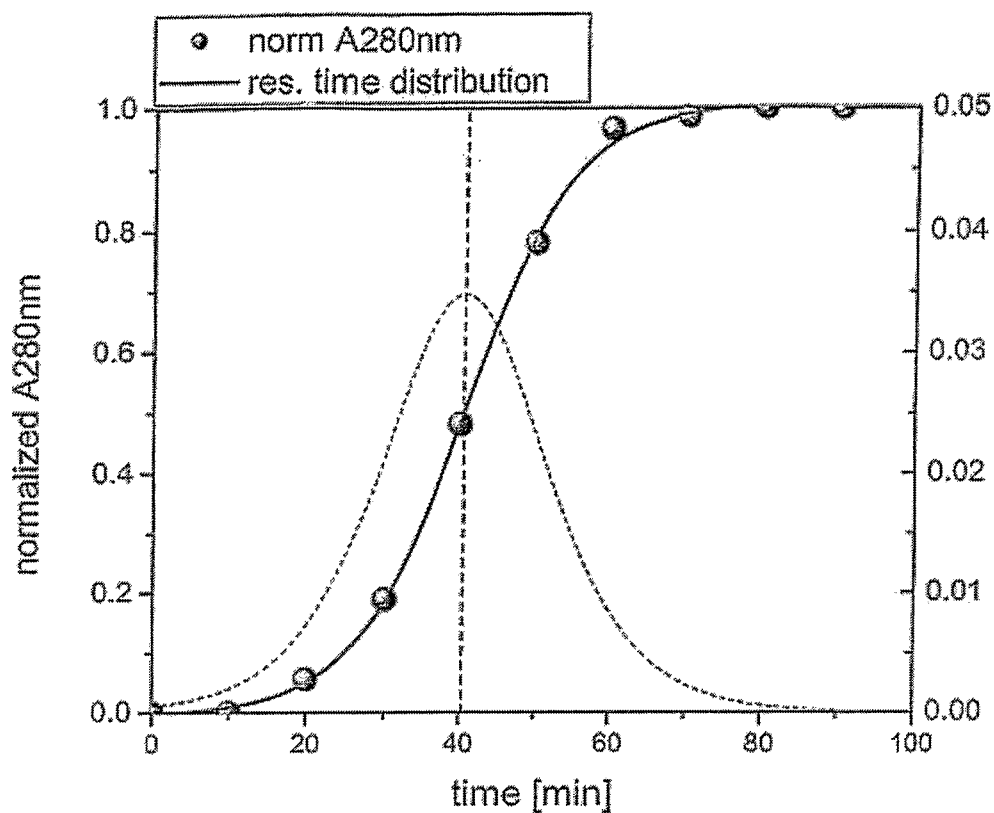


Figure 10: Example of residence time distribution and mean residence time of integrated continuous UF system (300) as per method of using inventive device A. Measured for disposable continuous system with 290 cm² module (62.5 cm length), 120 LMH crossflow, 0.2 LMH retentate flow, 2 LMH permeate flow.

11/17

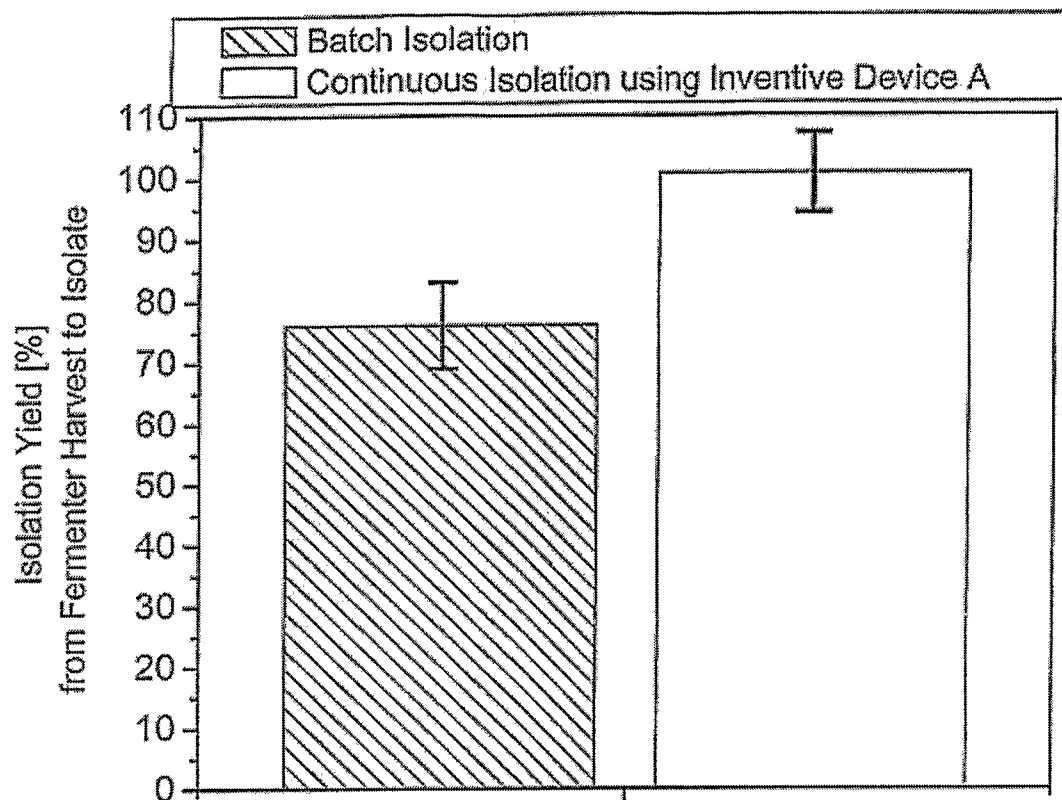


Figure 11: Example of isolation of rFVIII from plasma-protein free continuous perfusion fermentation using an embodiment of inventive device A. Comparison of average isolation yield of inventive continuous method compared to average yield of batch isolation, including one standard deviation spread. 3 consecutive batches were used for determining batch yield, while 3 consecutive points (days) were used for the continuous process.

12/17

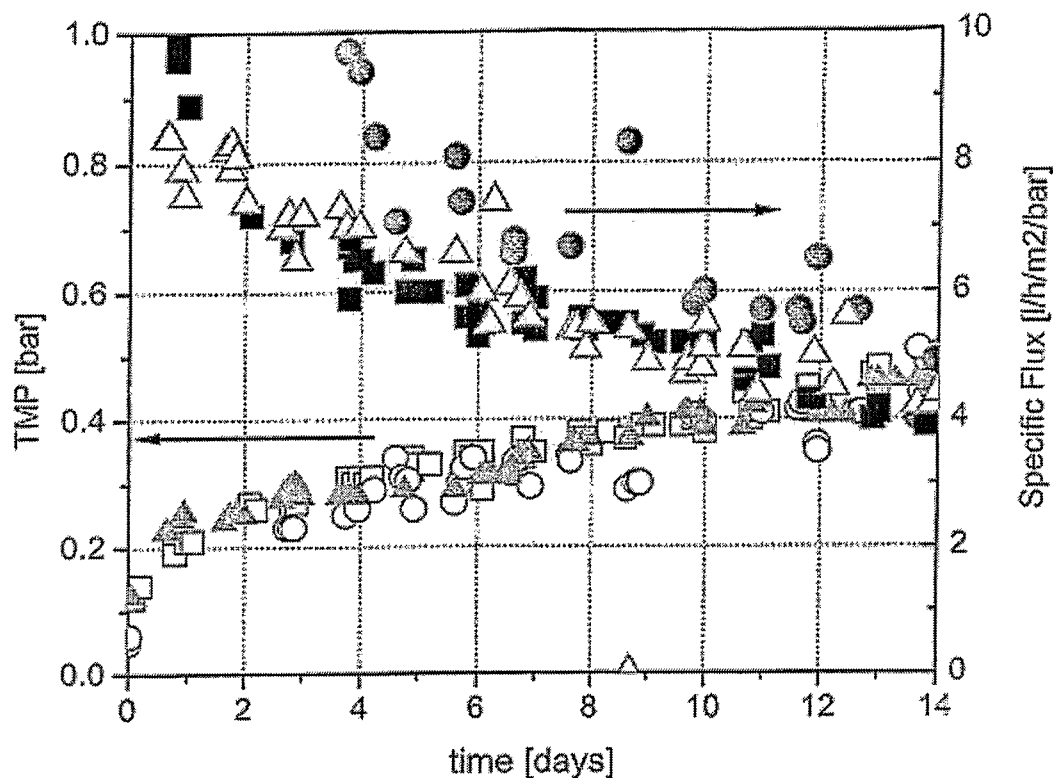


Figure 12: Examples of performance of inventive device A. Transmembrane pressure and specific flux of integrated continuous ultrafiltration system (300) as a function of continuous process time for 3 different examples shown. Triangles = 100 kD membrane, recombinant blood coagulation Factor VIII (rFVIII); Squares = 10 kD membrane, recombinant interleukin-2; Circles = 50 kD membrane, genetically engineered glycoprotein (Mr > 100 kD). All examples shown are from plasma protein-free continuous perfusion fermentation.

13/17

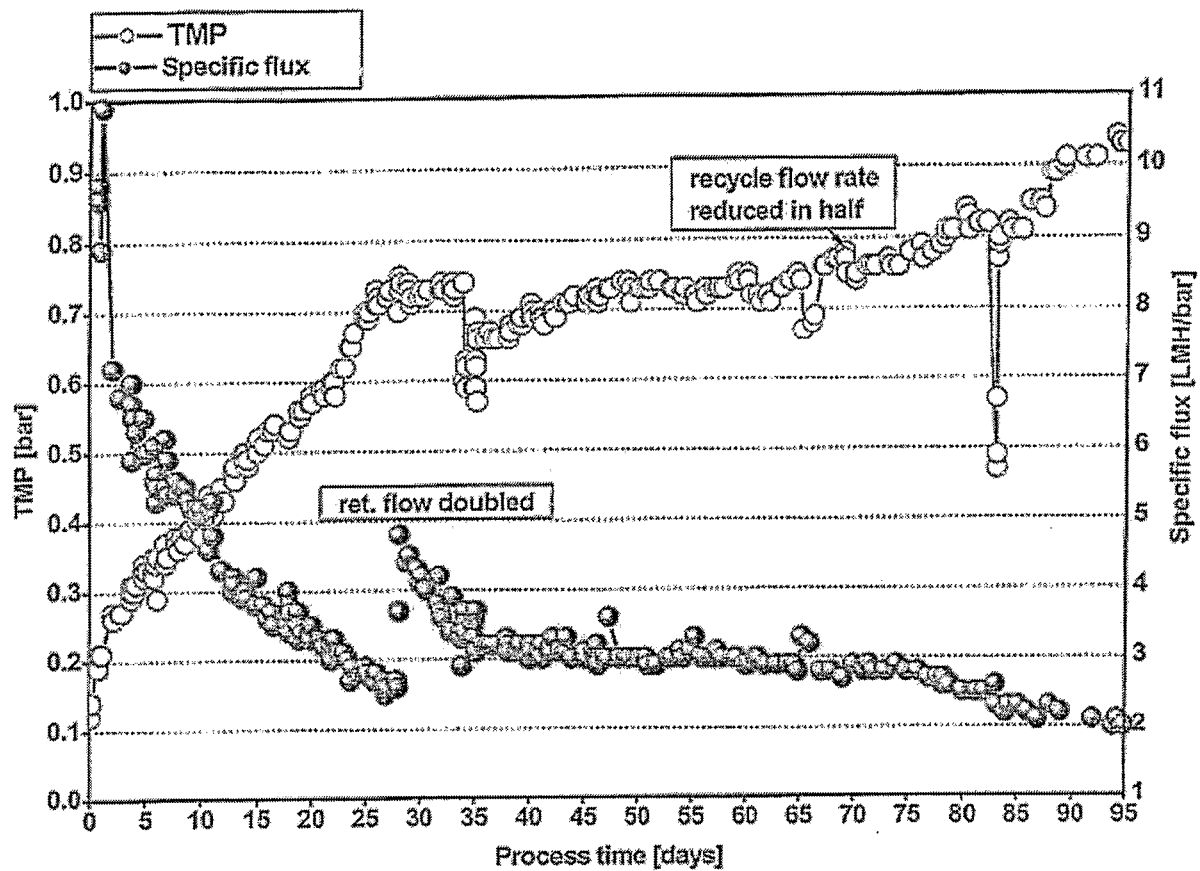


Figure 13: Example of long-term performance of inventive device A directly coupled to continuous perfusion fermentation of cell-line co-expressing 2 protein products (Green fluorescent protein GFP and IL-2SA). Transmembrane pressure and specific flux of inventive continuous ultrafiltration system (300) as a function of continuous process time shown. 10kD membrane was used.

14/17

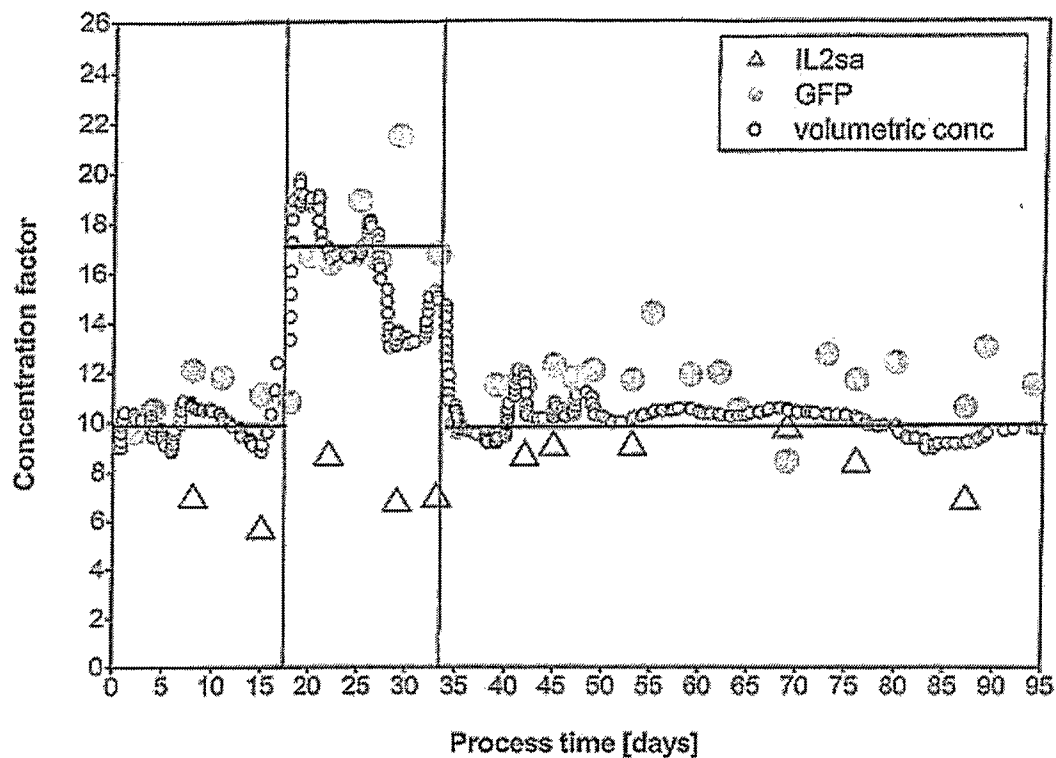


Figure 14: Example of long-term performance of inventive device A directly coupled to continuous perfusion fermentation of cell-line co-expressing 2 protein products (Green fluorescent protein GFP and IL-2SA). 10kD membrane was used. Concentration factor of both protein products, as determined by specific assays, and volumetric concentration factor shown as a function of continuous process time.

15/17

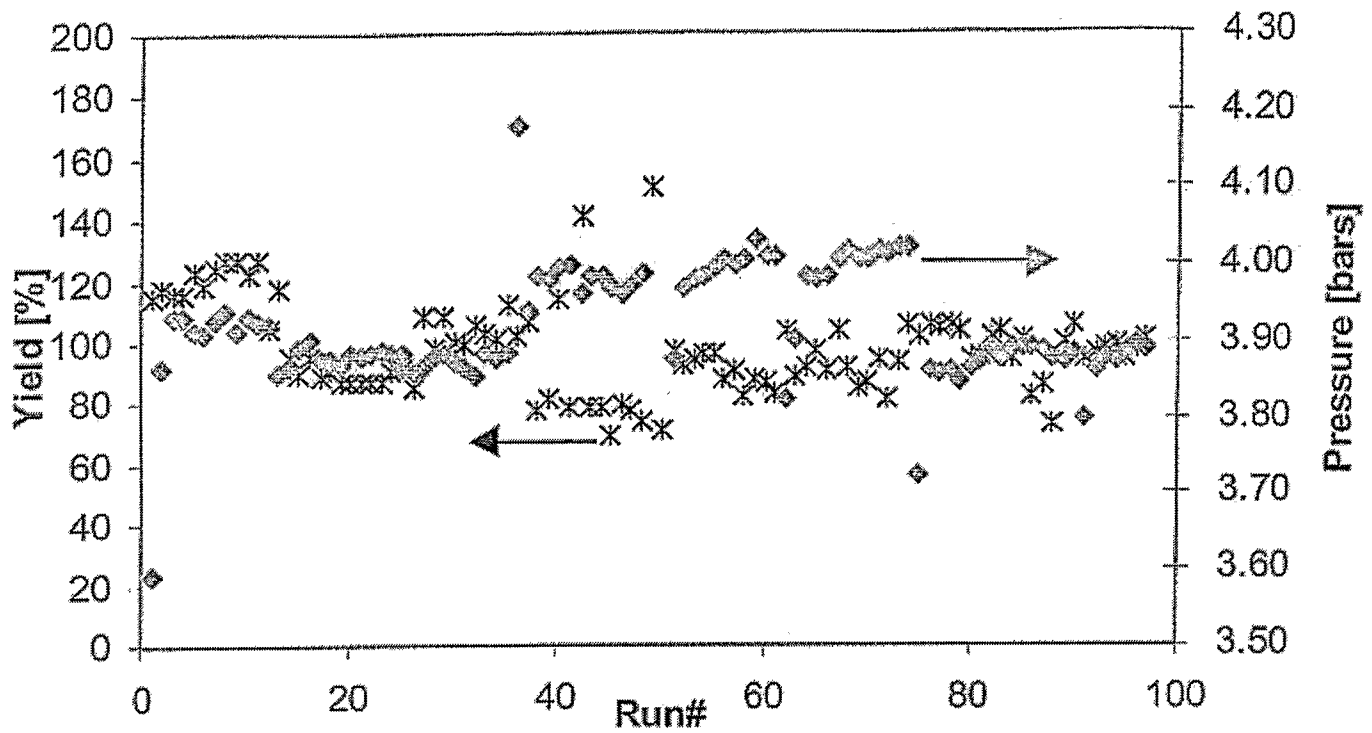


Figure 15: Example for performance of inventive device B. Yield and pressure drop over close to 100 consecutive adsorption/desorption cycles with convective adsorber (target protein: genetically engineered blood coagulation Factor FVIII variant; convective adsorber: commercial adsorber, Mustang Q, Pall Corporation).

16/17

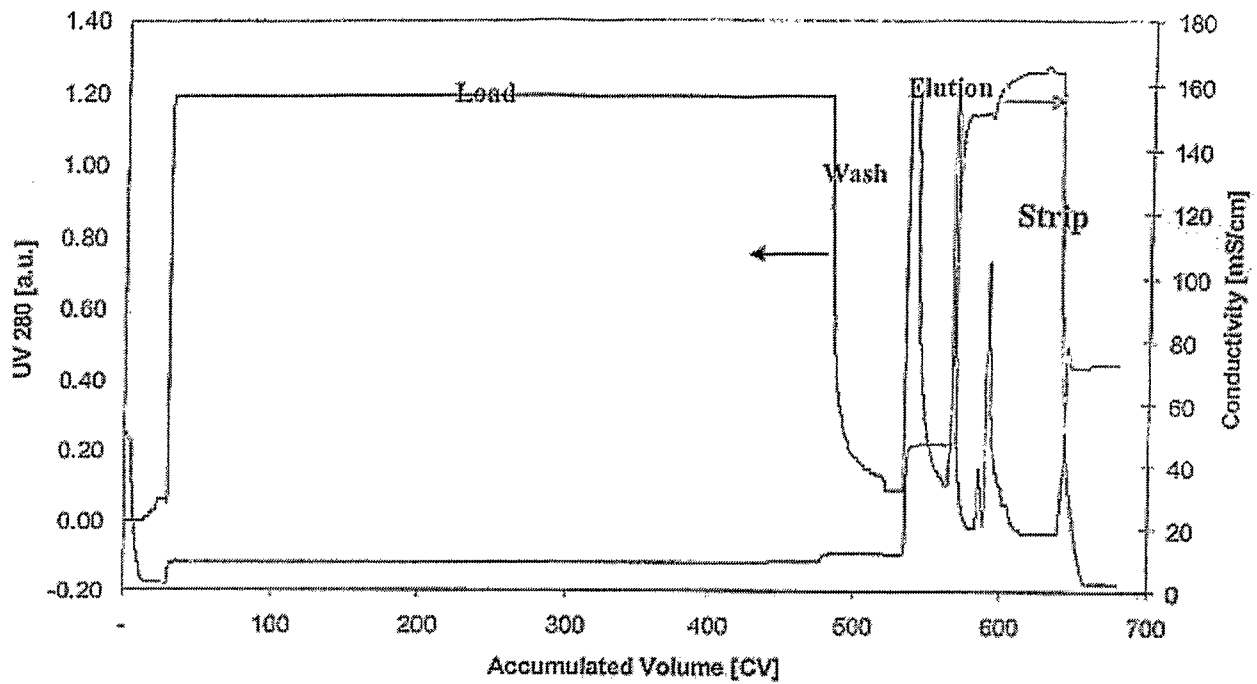


Figure 16: Example for performance of inventive device B. UV and conductivity profile during a typical adsorption/desorption cycle with convective adsorber (target protein: genetically engineered blood coagulation Factor FVIII variant; convective adsorber: commercial adsorber, Mustang Q, Pall Corporation).

17/17

Figure 17: Example for performance of inventive device B. SDS-Page gel (silver stained) of Load = clarified harvest continuously leaving the particle removal system (100) and semi-continuously loaded onto the convective adsorber system (400) and typical adsorption/desorption eluate shown. Target protein: genetically engineered blood coagulation Factor FVIII variant; convective adsorber: commercial adsorber, Mustang Q, Pall Corporation). Eluate was diluted back to load concentration before run on gel.

