

5 Claims

1. A method for extracting a protein of interest from a precipitate, comprising:
 - a. mixing the precipitate with a liquid in a first tank (1) to form a suspension having a first dilution factor;
 - 10 b. feeding the suspension into a first filtration unit (5) comprising a dynamic filter element adapted to produce a first retentate and a first permeate enriched with the protein of interest;
 - c. diluting the suspension in the first tank (1) by adding liquid to a second dilution factor, optionally by streaming the first retentate (13) into the first tank; and
 - 15 d. recovering the first permeate enriched with the protein of interest in a second tank (7).
2. The method of claim 1 further comprising:
 - e. subjecting the first permeate in the second tank (7) to a continuous concentration process in a second filtration unit comprising a cross flow filter element, thereby
 - 20 producing a second retentate enriched with the protein of interest and a second permeate depleted of the protein of interest;
 - f. optionally diluting the suspension in the first tank (1) by streaming the second permeate to the first tank (1), thereby diluting the suspension to the second dilution factor; and
 - 25 g. either returning the second retentate enriched with the protein of interest to the second tank (7) and/or collecting the second retentate enriched with the protein of interest.
3. An industrial scaled method for extracting a protein of interest in high yield from a precipitate, comprising:
 - 30 a. mixing the precipitate with a liquid in a first tank to form a suspension having a first dilution factor;
 - b. continuously feeding the suspension into a first filtration unit comprising a rotational cross flow filter element comprising a filter disc having a ceramic membrane with an average pore size between 5 nm and 5000 nm, the filter element adapted to produce
 - 35 a first retentate, and a first permeate enriched with the protein of interest;
 - c. diluting the suspension in the first tank by adding liquid to a second dilution factor in part by streaming the first retentate into the first tank;

- d. recovering the first permeate enriched with the protein of interest in a second tank;
and
 - e. subjecting the first permeate in the second tank to a continuous concentration process
in a second filtration unit comprising a cross flow filter element, thereby producing a
5 second retentate enriched with the protein of interest and a second permeate depleted
of the protein of interest;
 - f. optionally diluting the suspension in the first tank by continuously streaming the
second permeate to the first tank, thereby diluting the suspension to the second
dilution factor; and
 - 10 g. either returning the second retentate enriched with the protein of interest to the second
tank and/or collecting the second retentate enriched with the protein of interest.
4. The method according to claim 2 or claim 3, wherein the first permeate is continuously fed
into the concentration process.
- 15 5. The method according to claim 2 or claim 3, wherein the concentration process is an
ultrafiltration performed in the second filtration unit.
6. The method according to any one of the preceding claims, wherein the first permeate is
20 collected in the second tank, and once the suspension of the first tank is completely
filtrated, the first permeate from the second tank is subjected to the continuous
concentration process.
7. The method according to any one of the preceding claims, wherein either the steps b) to
25 c) or steps b) to f) are repeated until either the second dilution factor or a predetermined
value of protein concentration of the suspension in the first tank has been achieved.
8. The method according to any one of the preceding claims, wherein the first dilution factor
is at least 3 (1:3; parts of protein-comprising composition:total), preferably between 1 to
30 10, preferably between 3 to 9, preferably between 3 to 5, preferably about 3, 5, 6, 9 or 10.
9. The method according to any one of the preceding claims, wherein the predetermined
second dilution factor (weight of protein-comprising precipitate to weight of total dilution)
is between 6 and 70, between 10 and 70, between 20 and 50, preferably between 30 and
35 40, about 10, about 20, about 30, about 40, about 50, about 60, or about 70 (1:70; parts
precipitate:total).

10. The method according to any one of claims 8 to 10, wherein the predetermined value of protein concentration is less than 0.1 g/L, preferably between about 0.001 to 0.1 g/L.
11. The method according to any one of the preceding claims, wherein the precipitate is an intermediate product of an alcohol fractionation process, preferably of blood plasma, more preferably of human blood plasma.
12. The method according to claim 11, wherein the intermediate product is selected from the group consisting of Cohn Fraction I (Fr I), Cohn Fraction II+III (Fr II+III), Cohn Fraction I+II+III (Fr I+II+III), or Kistler/Nitschmann Precipitate A (KN A), or a combination of KN A and one or more of Fr I, Fr II+III and Fr I+II+III.
13. The method according to any one of claims 1 to 10, wherein the precipitate is a culture supernatant or a fermentation product.
14. The method according to any one of the preceding claims, wherein the protein of interest is an immunoglobulin (Ig), preferably immunoglobulin G such as from human plasma or a recombinantly produced immunoglobulin G.
15. The method according to any one of the preceding claims, wherein the first filtration unit comprises a pressure vessel.
16. The method according to any one of the preceding claims, wherein the dynamic filter element is a dynamic cross flow filter element, preferably a rotational cross flow filter element.
17. The method according to claim 16, wherein the rotating speed of the rotational cross-filter element is between about 600 rpm and about 1200 rpm, preferably between about 800 rpm and about 1200 rpm, preferably about 800 rpm, about 1000 rpm or about 1200 rpm.
18. The method according to any one of claims 15 to 17, wherein the first filtration unit is equipped with rotating filter discs comprising ceramic membranes and optionally baffles for turbulence mixing of the content of the first filtration unit, preferably the tangential speed of the disks is between about 1 to 7 m/sec.

19. The method according to any one of the preceding claims, wherein the temperature in the first filtration unit is controlled, preferably between 2 °C and 25 °C, more preferably at about 2 °C to 10 °C.
- 5 20. The method according to any one of the preceding claims, wherein the dynamic filter element or the cross-flow filter element comprises a filtration membrane having an average pore size of between 5 nm to 5000 nm, preferably between 5 nm to 2000 nm, between 5 nm to 500 nm, between 5 nm to 200 nm, more preferably between 7 nm to 100 nm, even more preferably between 7 nm to 80 nm.
- 10 21. The method according to any one of the preceding claims, wherein the precipitate comprises a filter aid.
22. The method according to claim 21, wherein the filter aid is removed prior to the first
15 filtration.
23. The method according to any one of the preceding claims, wherein a second tank (7) is provided to receive the first permeate (14) and/or the second retentate (16), wherein the flow velocity of the first permeate and the second permeate are controlled such that a
20 substantially constant product volume is maintained in the second tank (7).
24. The method according to any one of the preceding claims, wherein the transmembrane pressure is from 0.1 bar to 2.5 bar, preferable from 0.2 bar to 2.4 bar, preferably from 0.4 bar to 2.0 bar, most preferably from 0.4 bar to 1.5 bar.
- 25 25. The method according to any one of the preceding claims, wherein the extraction process is further assisted by regulating the flow rate and/or the residence time of the suspension in the first filtration unit and/or the flow rate of the first retentate and/or the flow rate of the first permeate.
- 30 26. The method according to any one of the preceding claims, wherein the suspension has a pH of between about 3.0 and 9.0, preferably between about 4.0 and 7.0, between about 4.0 to 6.0, between about 4.0 to 5.0, between about 4.3 to 4.9, between about 4.4 to 4.8, more preferably about 5.0.

27. The method according to any one of the preceding claims, wherein the precipitate has a total protein concentration of about 0.5 to 6.5 % w/v, more preferably about 1.0 to 4.0 % w/v, still more preferably about 1.5 to 3.0 % w/v, most preferably about 1.8 to 2.5 % w/v, e.g. about 2.0 % w/v.
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28. The method according to any one of the preceding claims, wherein the liquid comprises a buffer comprising one or more of sodium acetate, sodium phosphate, and citric acid.
29. The method according to any one of the preceding claims, wherein the precipitate is added
10 to the first tank in the form of a suspension, pellet or preferably a paste.
30. The method according to any one of the preceding claims, wherein the product of the method is subjected to further processing including one or more of chromatography steps, virus inactivation steps, concentration and formulation such that the end product is suitable
15 for administration to a subject.
31. A closed system (100) for extracting a protein of interest from a precipitate, comprising
- a. a first tank adapted to contain the precipitate in the form of a suspension having a first dilution factor;
- 20 b. a first filtration unit comprising a dynamic filter element, in connection with the first tank for receiving the suspension and the filter element adapted to produce a first permeate enriched with the protein of interest and a first retentate depleted of the protein of interest, wherein the first filtration unit is adapted to return the first retentate to the first tank;
- 25 c. a second tank in connection with the first filtration unit for recovering the first permeate enriched with the protein of interest;
- d. a second filtration unit for concentrating the first permeate in the second tank, adapted to produce a second retentate enriched with the protein of interest and a second permeate depleted in the protein of interest, wherein the second unit is adapted to
30 return the second retentate to the second tank and/or the second permeate to the first tank.
32. The closed system (100) according to claim 31, wherein the dynamic filter element is a rotating filter element comprising one or more filter discs.