Claims

1. A method for extracting a protein of interest from a precipitate, comprising:

a) mixing the precipitate comprising the protein of interest with a liquid in a first tank to form a first suspension having a first dilution factor;

b) feeding the first suspension into a first filtration unit comprising a dynamic filter element adapted to produce a first retentate depleted of the protein of interest and a first permeate enriched with the protein of interest;

c) diluting the first suspension in the first tank by adding liquid to a second dilution factor~~, optionally by streaming the first retentate into the first tank~~;

d) recovering the first permeate enriched with the protein of interest in a second tank;

e) precipitating one or more impurities in the first permeate enriched with the protein of interest to produce a second suspension; and

f) removing the precipitated impurities from the second suspension to produce a solution containing the protein of interest.

2. The method of claim 1, wherein step c) comprises diluting the first suspension in the first tank by streaming the first retentate into the first tank.

3~~2~~. The method of claim 1 or claim 2, wherein the method comprises the step of concentrating the first permeate enriched with the protein of interest prior to the step of precipitating the one or more impurities in the first permeate.

4~~3~~. The method of any one of claims 1 to 3~~or 2~~, wherein removing the precipitated impurities from the second suspension comprises:

a) feeding the second suspension into a second filtration unit comprising a dynamic filter element adapted to produce a second retentate containing the one or more precipitated impurities and a second permeate enriched with the protein of interest; and

b) ~~optionally streaming the second retentate into the tank comprising the second suspension;~~

~~c)~~ recovering the second permeate enriched with the protein of interest in a further tank.

5. The method of claim 4, wherein the method further comprises step ia) streaming the second retentate into the tank comprising the second suspension.

64. The method of claim 4 or claim 53, wherein removing the precipitated impurities from the second suspension further comprises:

a) subjecting the second permeate in the further tank to a continuous concentration process in a third filtration unit comprising a cross flow filter element, thereby producing a third retentate enriched with the protein of interest and a third permeate depleted of the protein of interest;

b) optionally diluting the suspension in the tank comprising the second suspension by streaming the third permeate to the tank comprising the second suspension, thereby diluting the suspension to a third dilution factor; and

c) either returning the third retentate enriched with the protein of interest to the third tank and/or collecting the third retentate enriched with the protein of interest.

7. The method of claim 6, wherein the method further comprises step 1a) diluting the suspension in the tank comprising the second suspension by streaming the third permeate to the tank comprising the second suspension, thereby diluting the suspension to a third dilution factor.

85. A method for extracting a protein of interest from a precipitate, comprising:

a) mixing the precipitate with a liquid in a first tank to form a first suspension having a first dilution factor;

b) feeding the first suspension into a first filtration unit comprising a dynamic filter element adapted to produce a first retentate depleted of the protein of interest and a first permeate enriched with the protein of interest;

c) diluting the first suspension in the first tank by adding liquid to a second dilution factor, optionally by streaming the first retentate into the first tank;

d) recovering the first permeate enriched with the protein of interest in a second tank;

d1) subjecting the first permeate in the second tank to a continuous concentration process in a second filtration unit comprising a cross flow filter element, adapted to

produce a second retentate, enriched with the protein of interest and a second permeate, depleted of the protein of interest;

~~d2) optionally diluting the first suspension in the first tank by streaming the second permeate to the first tank, thereby diluting the suspension to the second dilution factor;~~ and

d2~~3~~) 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;

e) precipitating one or more impurities from the second retentate enriched with the protein of interest to produce a second suspension; and

f) removing the precipitated impurities from the second suspension to produce a solution containing the protein of interest.

9. The method of claim 8, wherein the method additionally comprises step d2a) diluting the first suspension in the first tank by streaming the second permeate to the first tank, thereby diluting the suspension to the second dilution factor.

10~~6~~. The method of claim 8 or claim 9~~5~~, wherein removing the precipitated impurities from the second suspension comprises:

a) feeding the second suspension into a third filtration unit comprising a dynamic filter element adapted to produce a third retentate containing the one or more precipitated impurities and a third permeate enriched with the protein of interest; and

b) ~~optionally streaming the third retentate into the tank comprising the second suspension to a third dilution factor;~~

~~c).~~ recovering the third permeate enriched with the protein of interest in a further tank.

11. The method of claim 10, wherein the method further comprises step ia) streaming the third retentate into the tank comprising the second suspension to a third dilution factor.

12~~7~~. The method of claim 10 or claim 11~~6~~, wherein removing the precipitated impurities from the second suspension further comprises:

a) subjecting the third permeate in the further tank to a continuous concentration process in a fourth filtration unit comprising a cross flow filter element, thereby producing

a fourth retentate enriched with the protein of interest and a fourth permeate depleted of the protein of interest; and

b) optionally diluting the suspension in the tank comprising the second suspension by streaming the fourth permeate to the tank comprising the second suspension, thereby diluting the suspension to the third dilution factor; and

c) either returning the fourth retentate enriched with the protein of interest to the further tank and/or collecting the fourth retentate enriched with the protein of interest.

13. The method of claim 12, wherein the method further comprises step ia) diluting the suspension in the tank comprising the second suspension by streaming the fourth permeate to the tank comprising the second suspension, thereby diluting the suspension to the third dilution factor.

148. An industrial scale method for extracting a protein of interest in high yield from a precipitate, comprising:

a) mixing the precipitate with a liquid in a first tank to form a suspension having a first dilution factor;

b) feeding the first 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 a first retentate depleted of the protein of interest, and a first permeate enriched with the protein of interest;

c) diluting the first 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 second retentate enriched with the protein of interest and a second permeate depleted of the protein of interest;

f) optionally diluting the first 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

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;

gh) precipitating one or more impurities in the second retentate enriched with the protein of interest to produce a second suspension; and

hi) removing the precipitated impurities from the second suspension to produce a solution containing the protein of interest.

15. The method of claim 14, wherein the method further comprises step e1) diluting the first suspension in the first tank by continuously streaming the second permeate to the first tank, thereby diluting the suspension to the second dilution factor.

169. The method of claim 14 or claim 158 wherein the precipitated impurities are removed from the second suspension by feeding the second suspension into a third filtration unit comprising a dynamic filter element adapted to produce a third retentate containing the one or more precipitated impurities and a third permeate enriched with the protein of interest; optionally streaming the third retentate into the tank comprising the second suspension; and recovering the third filtrate enriched with the protein of interest in a further tank.

17. The method of claim 16, wherein after feeding the second suspension into a third filtration unit comprising a dynamic filter element adapted to produce a third retentate containing the one or more precipitated impurities and a third permeate enriched with the protein of interest, the third retentate is streamed into the tank comprising the second suspension.

1810. The method of claim 16 or claim 179, wherein removing the precipitated impurities from the second suspension further comprises subjecting the third permeate in the further tank to a continuous concentration process in a fourth filtration unit comprising a cross flow filter element, thereby producing a fourth retentate enriched with the protein of interest and a fourth permeate depleted of the protein of interest; optionally diluting the suspension in the tank comprising the second suspension by streaming the fourth permeate to the tank comprising the second suspension, thereby diluting the suspension to a third dilution factor; and either returning the fourth retentate enriched with the protein of interest to the further tank and/or collecting the fourth retentate enriched with the protein of interest.

19. The method of claim 18, wherein after the fourth retentate enriched with the protein of interest and a fourth permeate depleted of the protein of interest are produced, the method further comprises diluting the suspension in the tank comprising the second suspension by streaming the fourth permeate to the tank comprising the second suspension, thereby diluting the suspension to a third dilution factor.

2011. The method of any one of claims 14 8 to 190, wherein the first retentate and the second permeate are continuously streamed into the first tank to dilute the suspension to the second dilution factor, and/or the third retentate and fourth permeate are continuously streamed into the tank comprising the second suspension, in order to dilute the second suspension to a third dilution factor.

2112. The method of any one of claims 148 to 2011, wherein the filtration unit adapted to produce a permeate enriched for the protein of interest comprises more than one hollow shaft adapted to collect the permeate, each shaft connected to at least one filter disc comprising a ceramic membrane.

2213. The method of any one of claims 148 to 2112 wherein, the filtration unit for performing the concentration process comprises a dynamic cross flow filter element.

2314. The method of any one of the preceding claims wherein precipitating the one or more impurities from the permeate or retentate enriched with the protein of interest is by altering the solvation potential of the solvent.

2145. The method of claim 2134, wherein altering the solvation potential of the solvent comprises lowering the solubility of the one or more impurities by addition of a reagent and/or modulating the pH or conductivity of the solution.

2516. The method of claim 2145, wherein the reagent for precipitating the one or more impurities is an ionisable organic additive.

2167. The method of claim 2156, wherein the ionisable organic additive is a fatty acid or a salt, or ester thereof.

2178. The method of claim 2167, wherein the fatty acid comprises a general structural formula of CH3(CH2)nCOOH, preferably wherein the fatty acid is a C 7 to C 10 carboxylic acid or a salt, or ester thereof.

2189. The method of claim 2178, wherein the fatty acid is selected from the group consisting of: enanthic (heptanoic) acid, caprylic (octanoic) acid, octenoic acid,

pelargonic (nonanoic) acid, nonenoic acid, or capric (decanoic) acid or a salt, or ester thereof.

~~20. The method of claim 19, wherein the fatty acid is caprylic (octanoic) acid, or a salt, or ester thereof.~~

29~~21~~. The method of claim 28~~20~~, wherein the fatty acid is caprylic (octanoic) acid and is added to a final concentration of about 0.1 g/g total protein, about 0.25 g/g total protein, about 0.5 g/g total protein, about 0.75 g/g total protein, about 1.0 g/g total protein, about 1.5 g/g total protein, about 2.0 g/g total protein, about 2.5 g/g total protein, about 3.0 g/g total protein, about 3.5 g/g total protein, or about 4.0 g/g total protein, preferably, wherein the amount of fatty acid, preferably caprylic (octanoic) acid, is about 0.275 g/g total protein, about 0.280 g/g total protein, about 0.285 g/g total protein, about 0.290 g/g total protein, about 0.300 g/g total protein or about 0.325 g/g total protein.

30~~22~~. The method of claim 29~~21~~, wherein the caprylic acid is added to and incubated with the permeate or retentate enriched for the protein of interest, at a temperature of between about 22°C to about 40°C, preferably at a temperature of between about 25°C to about 38°C, preferably about 27°C to about 37°C, more preferably about 32°C.

31~~23~~. The method of any one of claims 1 to 2~~1~~5~~6~~, wherein the reagent for precipitating the one or more impurities from the permeate or retentate enriched with the protein of interest is a nonionic organic polymer.

32~~24~~. The method of claim 31~~23~~, wherein the non-ionic organic polymer is selected from the group consisting of: polyethylene glycol (PEG), polypropylene glycol, polyvinylpyrrolidone, dextran, cellulose, and other polymers.

33~~25~~. The method of any one of claims 1 to 32~~24~~, wherein the precipitate is an insoluble solid that comprises the protein of interest.

34~~26~~. The method of claim 33~~25~~, wherein the insoluble solid is in the form of a pellet or a paste.

35~~27~~. The method of any one of claims 1 to 34~~26~~, wherein the precipitate comprising the protein of interest is an intermediate product of an alcohol fractionation process.

36~~28~~. The method of claim 35~~27~~, wherein the alcohol fractionation is of human blood plasma.

~~29. The method of claim 28, wherein the precipitate is a Cohn fraction.~~

~~30. The method of claim 29, wherein the plasma fraction 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), Cohn Fraction II (Fr II), Cohn Fraction III (Fr III), Cohn Fraction IV (Fr IV), Cohn Fraction V (Fr V), Kistler/Nitschmann Precipitate A (KN A), Kistler/Nitschmann Precipitate B (KN B), and Kistler/Nitschmann Precipitate C (KN C), or combinations of one or more thereof.~~

3~~1~~. The method of any one of claims 1 to 36~~26~~, wherein the precipitate is a lyophilisate and/or crystalized solid form comprising the protein of interest.

~~32. The method of any one of claims 1 to 26 and 31, wherein the protein-comprising precipitate is obtained from a culture supernatant, a fermentation product or a material derived from a milk or whey fraction.~~

3~~8~~3. The method of any one of claims 1 to 3~~7~~2, wherein the protein of interest is an immunoglobulin, preferably human immunoglobulin G (IgG).

39~~34~~. The method of any one of claims 1 to 3~~8~~3, wherein the first suspension is produced by mixing the protein-comprising precipitate with a liquid, thereby providing the starting composition with the first dilution factor.

40~~35~~. The method of any one of claims 1 to 39~~34~~, wherein the first suspension is continuously fed into the first filtration unit, preferably wherein first suspension is continuously fed into the first filtration unit until the first suspension has been diluted to at least the second dilution factor.

41~~36~~. The method of any one of claims 1 to 40~~35~~, wherein the second suspension is continuously fed into a further filtration unit, preferably wherein the second suspension is continuously fed into the further filtration unit until the second suspension has been diluted to at least the third dilution factor.

42~~37~~. The method of any one of claims 1 to 41~~36~~, wherein the filtration unit comprising a dynamic filter element adapted to produce a first retentate and a first permeate enriched with the protein of interest, comprises a rotational cross flow filter element.

43~~38~~. The method of any one of claims 1 to 42~~37~~, wherein the dynamic filter element is a dynamic cross flow filter element, preferably a rotational cross flow filter element, preferably wherein at least the dynamic filter element in the first filter unit is a rotational cross flow filter element.

4439. The method of any one of claims 1 to 4338, wherein one or more of the filter elements comprise a ceramic membrane.

4540. The method of any one of claims 1 to 4439, wherein the dynamic filter element comprises a filter membrane having an average pore size in the range of greater than or equal to 5 nm to less than or equal to 2 µm.

461. The method of any one of claims 1 to 450, wherein one or more of the filtration units comprise a pressure vessel, preferably wherein at least the first filtration unit comprises a pressure vessel.

472. The method of any one of claims 1 to 461, wherein the temperature in one or more of the filtration units is controlled to a temperature between 2 °C and 25 °C.

483. The method of any one of claims 1 to 4742, wherein the transmembrane filtration pressure in one or more of the filtration units is from 0.1 to 2.5 bar.

4944. The method of any one of claims 1 to 483, wherein the method comprises regulating the flow rate and/or the residence time of the suspension or the solution into the filter units and/or the flow rate of the retentate/raffinate comprising impurities/precipitant and/or the flow rate of the first permeate/filtrate enriched for the protein of interest.

5045. The method of any one of claims 1 to 4944, wherein the method comprises periodic backflushing intervals, wherein the flow of liquid through the filtration units is reversed at regular intervals and for a defined period of time.

5146. The method of claim 5045, wherein the period of time between backflushing intervals is increased as the turbidity and protein concentration of the liquid in the filtration units decreases.

5247. The method of claims 5045 or 5146, wherein the duration of the backflushing interval is less than the duration of the filtration interval, preferably, wherein the duration of the backflushing interval is at least one quarter, one eighth, one tenth, one sixteenth or smaller than the duration of the filtration interval.

5348. The method of any one of claims 1 to 5247, wherein the method recovers at least 75%, at least 80%, at least 85%, at least 90%, at least 95 %, or typically at least 98 % of the protein of interest in the protein-comprising precipitate.

5449. The method of any one of claims 1 to 5348, wherein the filter element for performing the concentration process comprises a filtration membrane having an average pore size suitable for ultrafiltration and/or diafiltration.

~~50. The method of claim 49, wherein the pore size is between 2 nm to 200 nm, preferably between 3 to 50 nm.~~

551. The method of any one of claims 1 to 540, wherein the filter element for performing the concentration process is a rotational cross flow filter element adapted for performing a continuous concentration process.

562. The method of any one of claims 1 to 551, wherein the filter element for performing the concentration process comprises a membrane with a molecular weight cutoff less than the molecular weight of the protein of interest, preferably, wherein the membrane cutoff is at least 3 fold lower than the molecular weight of the protein of interest so that the protein of interest is retained in the retetate.

~~53. The method of claim 52, wherein the membrane has a molecular weight cut of value of less than 50 kDa, preferably less than 30 kDa, more preferably less than 10 kDa or most preferably less than 5 kDa.~~

574. The method of any one of claims 1 to 563, wherein the filter element for performing the concentration process comprises an ultrafiltration device comprising a membrane in the form of a polymer membrane, such as polyethersulfone or regenerated cellulose.

~~55. The method of any one of claims 1 to 54, wherein the filter element for performing the concentration process comprises a ceramic disc.~~

586. The method of any one of claims 1 to 575, wherein the product of the method is subjected to further processing, including one or more chromatography steps, virus inactivation steps, concentration and formulation steps such that the end product is suitable for administration to a subject.

~~57. The method of any one of claims 1 to 56, wherein the precipitate comprises a filter aid.~~

~~58. A method for precipitating one or more impurities from a solution containing a protein of interest, the method comprising~~

a) providing a solution containing a protein of interest and one or more impurities, wherein the solution has a conductivity of 6 to 9 mS/cm, pH 4.7 to 5.2 and a total protein concentration of 15 to 17 g/L; and
b) precipitating one or more impurities by adding a fatty acid to the solution, wherein the fatty acid is added at an amount of about 0.1g to about 0.4g fatty acid / g of total protein.

59. A method for removing one or more impurities from a solution containing a protein of interest, the method comprising
    a) providing a solution containing a protein of interest and one or more impurities, wherein the solution has a conductivity of 6 to 9 mS/cm, pH 4.7 to 5.2 and a total protein concentration of 15 to 17 g/L;
    b) precipitating one or more impurities by adding a fatty acid to the solution, wherein the fatty acid is added at an amount of about 0.1g to about 0.4g fatty acid / g of total protein;
    c) removing the one or more precipitated impurities.

60. The method of claim 58 or 59, wherein the fatty acid comprises a general structural formula of CH3(CH2)nCOOH.

61. The method of claim 60, wherein the fatty acid is a C 7 to C 10 carboxylic acid, preferably, wherein the fatty acid comprises enanthic (heptanoic) acid, caprylic (octanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, or capric (decanoic) acid or a salt or ester thereof.

62. The method of claim 61, wherein the fatty acid is caprylic (octanoic) acid or a salt or ester thereof.

63. The method of any one of claims 58 to 62, wherein the caprylic (octanoic) acid is added at a concentration of about 0.1 g/g total protein, about 0.25 g/g total protein, about 0.5 g/g total protein, about 0.75 g/g total protein, about 1.0 g/g total protein, about 1.5 g/g total protein, about 2.0 g/g total protein, about 2.5 g/g total protein, about 3.0 g/g total protein, about 3.5 g/g total protein, or about 4.0 g/g total protein, preferably, wherein the amount of fatty acid, preferably caprylic (octanoic) acid, is added at a concentration of about 0.275 g/g total protein, about 0.280 g/g total protein, about 0.285 g/g total protein, about 0.290 g/g total protein, about 0.300 g/g total protein or about 0.325 g/g total protein.

64. The method of claim 63, wherein the caprylic acid is added to and incubated with the permeate or retentate enriched for the protein of interest, at a temperature of between about 22°C to about 40°C, preferably at a temperature of between about 25°C to about 38°C, more preferably about 32°C.

65. The method of any one of claims 58 to 64, wherein the solution is a fraction from a plasma fractionation process.

66. The method of any one of claims 58 to 64, wherein the solution is obtained or obtainable from steps a. to d. (optionally including steps d1. to d.3) according to claim 5.

67. The method of any one of claims 58 to 66, wherein the solution does not contain filter aid.

68. The method of any one of claims 58 to 67 wherein the protein of interest is an immunoglobulin, preferably human immunoglobulin G (IgG) such as immunoglobulin G from human plasma or a recombinantly produced immunoglobulin G.