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(54) **Title**: METHOD OF PREPARING A THERAPEUTIC PROTEIN FORMULATION AND ANTIBODY FORMULATION PRODUCED BY SUCH A METHOD

(57) **Abstract**: The invention relates to a method of preparing a protein formulation including a therapeutic protein, the method comprising the steps of: providing a solution comprising said protein; concentrating the protein in the solution by a first ultra-filtration step; diafiltering the solution with a diafiltration buffer including at least one first excipient, whereby a retentate is obtained comprising the protein and the first excipient; further concentrating the protein in the retentate by a second ultra-filtration step; and adding at least one final excipient, whereby the protein formulation with a desired protein concentration is obtained. According to the invention, the method further comprises, before the second ultra- filtration step, adding a second excipient to the retentate obtained from the diafiltration step. The invention is also directed to antibody formulations produced by the foregoing method.

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METHOD OF PREPARING A THERAPEUTIC PROTEIN FORMULATION AND ANTIBODY FORMULATION PRODUCED BY SUCH A METHOD

Technical field

The present invention relates to a method of preparation of a protein formulation including excipients and at least one therapeutic protein.

The invention is of particular interest in the field of antibody formulations intended for a therapeutic use and is also directed to an antibody formulation produced by the method.

Background of the invention

The invention is more particularly related to methods sequentially comprising:

- providing a solution comprising said protein;
- concentrating the protein in the solution by a first ultra-filtration step;
- diafiltering the solution thus obtained with a diafiltration buffer including at least one first excipient, whereby a retentate is obtained comprising the protein and the first excipient;
- further concentrating the protein in the retentate by a second ultra-filtration step in an ultra-filtration equipment;
- adding at least one final excipient, whereby the protein formulation with a desired protein concentration and including said first and final excipients is obtained.

In general, the final protein formulations for therapeutic antibodies include at least an amino-acid, such as histidine, which is added during the diafiltration step, and a sugar acting as a stabilizer, such as trehalose. The trehalose is commonly added with the other excipients in the final addition step.

In conventional methods applied to therapeutic antibodies, the above steps are performed with a protein solution, once purified by a number of purification steps usually including a virus retaining filtration as the last purification step. The protein solution (or "product") is concentrated by the first ultra-filtration step, from a concentration of approximately 5 to 20 g/l to a concentration of about 40 to 100 g/l (depending on the protein). Then the concentrated product is diafiltered in a diafiltration buffer, such as histidine. In some instances, the diafiltration buffer may be another standard buffer such as acetate, tris or phosphate. The diafiltration buffer is chosen based on the final protein formulation as well as on any offset that is required due to the Donnan effect. The Donnan effect occurs as the product is concentrated and results in the exclusion of certain charged buffer species, e.g. histidine. The diafiltration buffer is therefore usually adjusted to a higher buffer concentration and a lower pH than are specified for the protein formulation. Once the diafiltration is complete, the product goes through the second ultra-filtration step for concentration to approximately 50% above the desired protein concentration for the final protein formulation. Then the product is removed from the ultra-filtration system and the system is rinsed to recover additional product. With a final concentration of more than 50% above the desired concentration in the protein formulation, all of the rinse can be added back to the product to maximize recovery, without excessively diluting the product. Then the excipients are added (sugar, surfactant, chelator, etc.) as a concentrated solution, usually with a dilution ratio of approximately 4, meaning that 1 unitary volume of the concentrated excipient solution is added to 3 unitary volumes of the product. The dilution ratio of 4 is based on the maximum solubility of the sugar component of the excipient solution, which is usually the limiting factor. If necessary, the product is then further diluted with formulation buffer for adjustment to the final desired concentration.

Such conventional methods may therefore not be applicable when it is desired to obtain a highly concentrated protein in the final formulation, and even more when the protein is of particularly high viscosity.

For example, in the case of a therapeutic antibody formulation with a desired final concentration of 150 g/l, the viscosity of the molecule precludes concentrating to the targeted value of 50% above the desired final concentration.

It is an aim of the invention to provide a method of preparation of a protein formulation that may be applied to highly viscous and highly concentrated proteins.

It is a further aim of the invention that the method may be implemented at a manufacturing scale, without negatively affecting the overall yield of the manufacturing process and without incurring extra costs due to an excessive waste of certain excipients. In particular, it is an aim to keep the use of the sugar components, which are particularly costly, at a similar level as the conventional methods.

It is still a further aim to preserve the stability of the protein over all the steps of the method and to protect the protein from aggregation.

Summary

According to a first aspect of the present invention, there is provided a method of the above type further comprising, before the second ultra-filtration step, adding a second excipient to the retentate obtained from the diafiltration step.

By moving the addition of a second excipient, in particular the trehalose (or more generally the sugar), to post-diafiltration, the remaining excipients can be added at a much higher concentration in a subsequent step, thus generating a lower dilution of the product. This in turn means that the maximal required concentration can be brought to only about 10% (in some instances between 5 to 15 %) above the final desired concentration, as compared to the value of about 50% for the conventional methods. This 10% value is obtainable with standard ultra-filtration equipments, even with higher molecule viscosity. This also allows recovering product from a rinse and thus allows obtaining a 90% yield of the ultra-filtration/dia-filtration process.

Also, adding the second excipient (the sugar) before the final concentration protects the protein from aggregation.

According to preferred embodiments of the invention:

- the method further includes, after step (e) and before step (f), rinsing the ultra-filtration equipment with a rinse buffer, whereby the recovery of the protein is enhanced;
- the rinse buffer comprises the first and the second excipients at concentrations substantially equal to, respectively, the concentrations of the first and of the second excipients in the protein formulation;
- the first excipient is an amino-acid, preferably histidine;
- the first excipient in the protein formulation has a concentration of between 16 and 24 mM, preferably of between 17 and 23 mM, most preferably of about 20 mM;
- the second excipient is a sugar, preferably a disaccharide;
- the final excipients include a surfactant, preferably polysorbate 80;
- the final excipients include a chelating agent, preferably EDTA;
- the protein formulation has a protein concentration of between 110 and 165 g/l;
- the protein is an antibody.

In a first preferred embodiment:

- the antibody is an anti-PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) antibody;
- the anti-PCSK9 antibody is selected from the group consisting of bococizumab, evolocumab (REPATHA™), alirocumab (PRALUENT™), REGN728, 31H4, 11F1, 12H11, 8A1, 8A3, 3C4, 300N, 1D05, LGT209, RG7652, and LY3015014;
- the anti-PCSK9 antibody comprises a heavy chain variable region (VH) comprising complementarity determining region one CDR1, CDR2, and CDR3 of the amino acid sequence shown in SEQ ID NO: 1; and a light chain

variable region (VL) comprising CDR1, CDR2, and CDR3 of the amino acid sequence shown in SEQ ID NO: 2; or alternatively the anti-PCSK9 antibody comprises a VH CDR1 having the amino acid sequence shown in SEQ ID NO: 3, 4, or 5, a VH CDR2 having the amino acid sequence shown in SEQ ID NO: 6 or 7, a VH CDR3 having the amino acid sequence shown in SEQ ID NO: 8, a VL CDR1 having the amino acid sequence shown in SEQ ID NO: 9, a VL CDR2 having the amino acid sequence shown in SEQ ID NO:10, and a VL CDR3 having the amino acid sequence shown in SEQ ID NO: 11;

- the protein formulation has a protein concentration of between 135 and 165 g/l, preferably of between 142 and 158 g/l, most preferably of about 150 g/l;
- the second excipient in the protein formulation is trehalose at a concentration of between 67.2 and 100.8 g/l, preferably of between 71.4 and 96.6 g/l, most preferably of about 84 g/l;
- the final excipients include polysorbate 80 which, in the protein formulation, has a concentration of between 0.16 and 0.24 g/l, preferably of between 0.17 and 0.23 g/l, most preferably of about 0.2 g/l;
- the final excipients include EDTA which, in the protein formulation, has a concentration of between 0.04 and 0.06 g/l, preferably of between 0.0425 and 0.0575 g/l, most preferably of about 0.05 g/l;
- the protein formulation has a pH of between 5.2 and 5.8, preferably of about 5.5;
- the solution provided in step (a) has a protein concentration of between 5 and 20 g/l;
- the protein is concentrated to between 80 and 120 g/l, preferably to between 90 and 110 g/l, and most preferably to about 100 g/l, by the first ultra-filtration step;
- the protein is concentrated to between 143 and 173 g/l, preferably to between 150 and 166 g/l, and most preferably to about 158 g/l, by the second ultra-filtration step;
- the first excipient in the diafiltration buffer has a concentration higher than the concentration of the first excipient in the protein formulation, said concentration of the first excipient in the diafiltration buffer being preferably of between 29.75 and 40.25 mM, most preferably of about 35 mM;

- the diafiltration buffer has a pH of between 5.1 and 5.5, preferably about 5.3;
- adding the second excipient to the retentate obtained from the diafiltration step is achieved by adding a first additive solution to the retentate, said first additive solution comprising the second excipient at a concentration of between 340 and 460 g/l, preferably of between 380 and 420 g/l, most preferably of about 400 g/l;
- the first additive solution comprises the first excipient at a concentration lower than the concentration of the first excipient in the diafiltration buffer and higher than the concentration of the first excipient in the protein formulation, said concentration of the first excipient in the first additive solution being preferably of between 25.5 and 34.5 mM, most preferably of about 30 mM;
- the first additive solution further comprises a final excipient;
- the first additive solution comprises about 30 mM histidine and about 400 g/l trehalose;
- adding the first additive solution to the retentate is performed at a dilution ratio of about 4.15, whereby one volume of the first additive solution is added to approximately 3.15 fold the same volume of the retentate;
- adding the final excipients includes the step of adding a second additive solution to the solution obtained from the second ultra-filtration step, said second additive solution comprising the second excipient at a concentration lower than the concentration of the second excipient in the first additive solution and higher than the concentration of the second excipient in the protein formulation;
- the second additive solution comprises the first excipient at a concentration substantially equal to the concentration of the first excipient in the protein formulation;
- the second additive solution comprises about 20 mM histidine, about 84 g/l trehalose, about 1 g/l EDTA and about 4 g/l polysorbate 80;
- adding the second additive solution is performed at a dilution ratio of about 20, whereby one volume of the second additive solution is added to approximately 19 fold the same volume of to the solution obtained from the second ultra-filtration step.

In a second preferred embodiment:

- the antibody is an anti-IL7R antibody;
- preferably, the anti-IL-7R antibody comprises a heavy chain variable region (VH) comprising complementarity determining region one CDR1, CDR2, and CDR3 of the amino acid sequence shown in SEQ ID NO: 13 (examples of the sequences of such CDRs are SEQ ID NOs. 17, 18 and 19 respectively); and a light chain variable region (VL) comprising CDR1, CDR2, and CDR3 of the amino acid sequence shown in SEQ ID NO: 14 (examples of the sequences of such CDRs are SEQ ID Nos. 20, 21 and 22 respectively);
- more preferably, the VH region of the anti-IL-7R antibody comprises the amino acid sequence shown in SEQ ID NO. 13, and the VL region of the anti-IL-7R antibody comprises the amino acid sequence shown in SEQ ID NO. 14;
- even more preferably, the heavy chain of the anti IL-7R antibody comprises the amino acid sequence shown in SEQ ID NO. 15 and a light chain of the anti IL-17 antibody has the amino acid sequence shown in SEQ ID NO. 16;
- the protein formulation has a protein concentration of between 110 and 130 g/l, preferably of about 120 g/l;
- the second excipient in the protein formulation is sucrose at a concentration of between 42 and 58 g/l, preferably of about 50 g/l;
- the final excipients include polysorbate 80 which, in the protein formulation, has a concentration of between 0.017 and 0.023 g/l, preferably of about 0.02 g/l;
- the final excipients include EDTA which, in the protein formulation, has a concentration of between 0.42 and 0.58 g/l, preferably of about 0.5 g/l;
- the final excipients include arginine which, in the protein formulation, has a concentration of between 85 and 115 mM, preferably of about 100 mM;
- the protein formulation has a pH of between 6.5 and 7.5, preferably of about 7.0;
- the solution provided in step (a) has a protein concentration of between 2.6 and 3.4 g/l, preferably of about 3 g/l;
- the protein is concentrated to between 36 and 54 g/l, preferably to between 40 and 50 g/l, and most preferably to about 45 g/l, by the first ultra-filtration step;

- the protein is concentrated to between 170 and 210 g/l, preferably to about 190 g/l, by the second ultra-filtration step;
- the first excipient in the diafiltration buffer has a concentration higher than the concentration of the first excipient in the protein formulation, said concentration of the first excipient in the diafiltration buffer being preferably of between 19 and 25 mM, most preferably of about 22 mM;
- the diafiltration buffer includes arginine at a concentration of between 95 and 125 mM, preferably of about 110 mM;
- the diafiltration buffer has a pH of between 6.5 and 7.5, preferably about 7.0;
- adding the second excipient to the retentate obtained from the diafiltration step is achieved by adding a first additive solution to the retentate, said first additive solution comprising the second excipient at a concentration of between 230 and 320 g/l, preferably of about 275 g/l;
- the first additive solution comprises the first excipient at a concentration substantially equal to the concentration of the first excipient in the diafiltration buffer and higher than the concentration of the first excipient in the protein formulation, said concentration of the first excipient in the first additive solution being preferably of between 19 and 25 mM, most preferably of about 22 mM;
- the first additive solution further comprises a final excipient;
- the first additive solution comprises about 22 mM histidine, 110 mM arginine and about 275 g/l sucrose, at a pH of about 7.0;
- adding the first additive solution to the retentate is performed at a dilution ratio of about 5, whereby one volume of the first additive solution is added to approximately 4 fold the same volume of the retentate;
- adding the final excipients includes the step of adding a second additive solution to the solution obtained from the second ultra-filtration step, said second additive solution comprising EDTA and polysorbate 80;
- adding the second additive solution is performed at a dilution ratio of about 20, whereby one volume of the second additive solution is added to approximately 19 fold the same volume of to the solution obtained from the second ultra-filtration step.

According to a second aspect of the invention, there is provided an antibody formulation produced by the foregoing method.

In a preferred embodiment, the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody, and
- from 16 mM to 24 mM, preferably about 20 mM, of histidine buffer.

In another preferred embodiment, the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody, and
- from 67.2 mg/ml to 100.8 mg/ml, preferably about 84 mg/ml, of trehalose.

In another preferred embodiment, the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody, and
- from 0.16 mg/ml to 0.24 mg/ml, preferably about 0.2 mg/ml, of polysorbate.

In another preferred embodiment, the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody,
- from 16 mM to 24 mM, preferably about 20 mM, of histidine buffer, and
- from 67.2 mg/ml to 100.8 mg/ml, preferably about 84 mg/ml, of trehalose.

In another preferred embodiment, the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody,
- from 16 mM to 24 mM, preferably about 20 mM, of histidine buffer, and
- from 0.16 mg/ml to 0.24 mg/ml, preferably about 0.2 mg/ml, of polysorbate.

In another preferred embodiment, the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody,

- from 67.2 mg/ml to 100.8 mg/ml, preferably about 84 mg/ml, of trehalose, and
- from 0.16 mg/ml to 0.24 mg/ml, preferably about 0.2 mg/ml, of polysorbate.

In a still preferred embodiment, the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody,
- from 16 mM to 24 mM, preferably about 20 mM, of histidine buffer,
- from 67.2 mg/ml to 100.8 mg/ml, preferably about 84 mg/ml, of trehalose, and
- from 0.16 mg/ml to 0.24 mg/ml, preferably about 0.2 mg/ml, of polysorbate.

In some embodiments, the antibody formulation has a pH of between 5.2 and 5.8, preferably about 5.5.

In another preferred embodiment, the protein formulation comprises:

- from 110 g/l to 130 g/l, preferably about 120 g/l, of the anti-IL-7R antibody;
- from 17 mM to 23 mM, preferably about 20 mM, of histidine;
- from 42 g/l to 58 g/l, preferably about 50 g/l, of sucrose; and
- from 0.017 g/l to 0.023 g/l, preferably about 0.02 g/l, of polysorbate

and has a pH of between 6.5 and 7.5, preferably about 7.0.

SEQ ID NO: 1 to 12 referred to in the foregoing are described in the table below:

1 (VH)	qvqlvqsgae vkkpgasvkv sckasgytft syymhwvrqa pgqglewmge ispfggrtny nekfksrvtm trdtststvy melsslrsed tavyycarer plyasdlwgq gttvtvss
2 (VL)	diqmtqspss lsasvgdrvt itcrasqgis salawyqqkp gkapklliys asyrytgvps rfsgsgsgtd ftftisslqp ediatyycqq ryslwrtfgq gtkleik
3 (VH – CDR1)	SYYMH
4 (VH – CDR1)	GYTFTSY
5 (VH-CDR1)	GYTFTSYYMH
6 (VH-CDR2)	EISPFGGRTNYNEKFKS
7 (VH-CDR2)	ISPFGGR
8 (VH-CDR3)	ERPLYASDL
9 (VL-CDR1)	RASQGISSALA
10 (VL-CDR2)	SASYRYT

11 (VL-CDR3)	QQRYSLWRT
12 (PCSK9 human)	MGTVSSRRSW WPLPLLLLLL LLLGPAGARA QEDEDGDYEE LVLALRSEED GLAEAPEHGT TATFHRCAKD PWRLPGTYVV VLKEETHLSQ SERTARRLQA QAARRGYLTK ILHVFHGLLP GFLVKMSGDL LELALKLPHV DYIEEDSSVF AQSIPWNLER ITPPRYRADE YQPPDGGSLVEVYLLDTSIQ SDHREIEGRV MVTDFENVPE EDGTRFHRQA SKCDSHGTHL AGVVSGRDAG VAKGASMRSL RVLNCQGKGT VSGTLIGLEF IRKSQLVQPV GPLVVLLPLA GGYSRVLNAA CQRLARAGVV LVTAAGNFRD DACLYSPASA PEVITVGATN AQDQPVTLGT LGTNFGRCVD LFAPGEDIIG ASSDCSTCFV SQSGTSQAAA HVAGIAAMML SAEPELTLAE LRQRLIHFSAKDVINEAWFP EDQRVLTPNL VAALPPSTHG AGWQLFCRTV WSAHSGPTRM ATAVARCAPD EELLSCSSFS RSGKRRGERM EAQGGKLVCR AHNAFGGEGV YAIARCCLLP QANCSVHTAPPAEASMGTRV HCHQQGHVLT GCSSHWEVED LGTHKPPVLR PRGQPNQCVG HREASIHASC CHAPGLECKV KEHGIPAPQE QVTVACEEGW TLTGCSALPG TSHVLGAYAV DNTCVVRSRDVSTTGSTSEG AVTAVAICCR SRHLAQASQE LQ

SEQ ID Nos. 13-16 in the forgoing are described in the table below

13 (VH)	EVQLVESGGGLVKPGGSLRLSCAASGFTFDDSVMHWVRQAPGKGLEWVSLV GWDGFFTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQGDYM GNNWGQGTLVTVSS
14 (VL)	NFML TQPHSVSESPGKTVTISCTRSSGSIDSSYVQWYQQRPGSSPTTVI YEDDQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDFHH LVFGGGTKLTVL
15	EVQLVESGGGLVKPGGSLRLSCAASGFTFDDSVMHWVRQAPGKGLEW VSLVGWDGFFTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC ARQGDYMGNNWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FL YSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
16	NFML TQPHSVSESPGKTVTISCTRSSGSIDSSYVQWYQQRPGSSPTTVI YEDDQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDFHH LVFGGGTKLTVLQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVT VAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPTECS
17 (VH-CDR1)	DSVMH
18 (VH-CDR2)	LVGWDGFFTYYADSVKG
19 (VH-CDR3)	QGDYMGNN
20 (VL-CDR1)	TRSSGSIDSSYVQ

21 (VL-CDR2)	EDDQRPS
22 (VL-CDR3)	QSYDFHHLV

Detailed description

The following definitions will be used in the present description and claims:

- the term “protein formulation” designates the final product including the protein of interest and excipients. When referring to proteins intended for a therapeutic use, the term “Drug Substance” may be used instead of “protein formulation” and the protein of interest may be designated by the term “active ingredient” or “product”. The “excipients” are defined by all the constituents of the “protein formulation”, which are not the “protein” or “active ingredient”. The excipients typically include protein stabilizers, surfactants, amino-acids e.g. contributing to protein stabilization, etc…;
- in connection with the dia-filtration step, the term “retentate” refers to the solution retained on the retentate side of the membrane and containing the molecules that are too large to pass through the membrane, such as the protein of interest. The retentate is the solution that is transferred to the subsequent part of the ultra-filtration / dia-filtration system. The other solution circulated on the other side (the permeate side) of the membrane in the dia-filtration part of the system is referred to as the “dia-filtration buffer” (or “basal buffer”);
- the term “concentrated pool” designates the solution directly obtained from the final ultra-filtration step;
- the term “final excipients” designates the excipients that are added to the “concentrated pool” after the final ultra-filtration step i.e. after the final concentration step;
- the term “nx spike”, with n a numeral value, designates a solution of excipients that is added to a certain volume of the protein-containing solution, with a dilution ratio equal to n, which means that one volume of the solution of

excipients is added to n-1 fold the same volume of the protein-containing solution. For example a 4x spike is a solution that is added according to the ratio: 1 volume of the spike for 3 volumes of the protein-containing solution;

- unless stated otherwise, the terms "approximately", "about" or "substantially" associated with a numeral value mean within a range of ± 5% of said value;
- "viscosity," as used herein, may be "absolute viscosity" or "kinematic viscosity." "Absolute viscosity," sometimes called dynamic or simple viscosity, is a quantity that describes a fluid's resistance to flow. "Kinematic viscosity" is the quotient of absolute viscosity and fluid density. Kinematic viscosity is frequently reported when characterizing the resistive flow of a fluid using a capillary viscometer. When two fluids of equal volume are placed in identical capillary viscometers and allowed to flow by gravity, a viscous fluid takes longer than a less viscous fluid to flow through the capillary. If one fluid takes 200 seconds to complete its flow and another fluid takes 400 seconds, the second fluid is twice as viscous as the first on a kinematic viscosity scale. If both fluids have equal density, the second fluid is twice as viscous as the first on an absolute viscosity scale. The dimensions of kinematic viscosity are L^2/T where L represents length and T represents time. The SI units of kinematic viscosity are m^2/s. Commonly, kinematic viscosity is expressed in centistokes, cSt, which is equivalent to mm^2/s. The dimensions of absolute viscosity are M/L/T, where M represents mass and L and T represent length and time, respectively. The SI units of absolute viscosity are Pa·s, which is equivalent to kg/m/s. The absolute viscosity is commonly expressed in units of centiPoise, cP, which is equivalent to milliPascal-second, mPa·s. In the context of the invention, an antibody is deemed to be of high viscosity if its viscosity is at least 20 cP.

For conciseness, the acronyms "UF", "DF" and "UF/DF" (or "UFDF") may be used across the description and should be understood as follows: "UF" means "ultra-filtration, "DF" means "dia-filtration" and "UF/DF" (or "UFDF") means "ultra-filtration / dia-filtration". The method of the invention, which is defined as a method of preparation of a protein formulation, may be referred to as a UFDF method.

The invention will now be further illustrated by the following Examples, each in connection with a specific therapeutic monoclonal antibody and a specific formulation of this monoclonal antibody. The Examples are provided for illustrative purpose only and should not be construed as limiting the scope of the invention.

A – Example 1

In illustrative Example 1, the protein of interest is bococizumab, a PCSK9-targeting monoclonal antibody that specifically binds to PCSK9 (Proprotein Convertase Subtilisin Kexin type 9), e.g. SEQ ID NO: 12 or Uniprot Accession Number Q8NBP7. The method has been designed to achieve a targeted product concentration of 150 g/l in the Drug Substance, with the Drug Substance including the following excipients at a pH of 5.5:

- histidine at a 20 mM concentration,
- trehalose at a 84 g/l concentration, and
- PS80 (PolySorbate 80) at a 0.2 g/l concentration.

It is deemed acceptable that the above requirements are achieved with a tolerance of ± 8 g/l in the protein concentration, of ± 15% in the excipients concentration and of ± 0.2 in the pH value.

In terms of yield, the method is required to achieve a product recovery of more than 90%.

Experiments have been conducted for defining preferred operating modes and establishing that the method of the invention is suitable for achieving the above requirements (while conventional methods are not). Some of these experiments are presented in the following part of the description.

A.1 Materials

The starting material used for experiments was a fully purified bococizumab solution that had been processed through a MabSelect® column to remove excipient

components prior to use. After MabSelect® purification, the eluate was adjusted to pH 5.0 by acetic acid, resulting in a product concentration of 17.09 g/l.

Ultra-filtration / Dia-filtration Device

All experiments were performed using a GE Crossflow® system (300 ml reservoir) fitted with Pellicon® 3 (30 KDa, C-screen, 88 cm^2) regenerated cellulose membranes or Sartocon® (30 KDa E-channel 200 cm^2) regenerated cellulose membranes. TransMembrane Pressure (TMP) was maintained at approximately 14-22 psi with P_{Feed} less than 55 psi. Unless otherwise specified, all rinses were generated by recirculating rinse buffer for at least15 minutes, then concentrating to the minimal working volume of the system.

A.2 Experimental Design and Results

Determination of Trehalose Solubility

An initial experiment was completed to evaluate the limit of the trehalose solubility in 30 mM histidine pH 5.35 solution (the histidine concentration and pH are adjusted from the final specifications to account for the exclusion of the histidine ion as the protein concentration increases). To obtain the 150 g/l final Drug Substance target, the minimal concentration of the concentrated pool would need to be 180 g/l with a 6x trehalose /EDTA/PS80 spike, or 187.5 g/l with a 5x trehalose/EDTA/PS80 spike. At the trehalose concentration required to provide a 6x spike (~500 g/l), the trehalose did not dissolve (particulates were still present) at room temperature (22 °C) after extended stirring and had to be heated to 30 °C to dissolve. The solution was filtered through a 0.22 µm Pall Acrodisc® syringe filter under 15 psi pressure without re-precipitating at room temperature.

However, this manufacturing method may be difficult to scale up, therefore the maximum practical concentration for the trehalose spike may be capped at 5x (~420 g/l trehalose).

Accordingly, in a preferred process, the trehalose concentration of the spike solution may be about 400 g/l.

Process Development

A first experiment was designed to test the histidine concentration needed in the dia-filtration solution, to check the histidine concentration in the dia-filtered solution at different protein concentrations (76.6 g/l and 114 g/l), and to generate material for density measurement. The starting material was concentrated to 76.6 g/l using a 200 cm^2 Sartocon® E-channel membrane at a load capacity of 345 g/m^2, and then dia-filtered with 35 mM histidine, pH 5.26 buffer. The flux of the dia-filtration was 17 LMH (liters/m^2/hour) at 300 LMH feed flowrate and 22 psi TMP. The material was then further concentrated to 213 g/l (data not shown) and samples of both the diafiltered pool and final concentrated material were analyzed for histidine and trehalose concentration (see Table 1).

A second experiment was performed to determine if diafiltered material containing trehalose resulted in a lower final concentration versus material without trehalose in the diafiltration buffer. The starting material was concentrated to 114 g/L and diafiltered with 35 mM histidine, pH 5.26 buffer. The diafiltration flux was 10 LMH under the operational conditions described in Table 2, Experiment 2A. The diafiltered solution was concentrated to 184.9 g/L at < 55 psi of feed pressure and 22 psi of TMP. The concentrated material was drained from the reservoir and combined with the 35 mM histidine, pH 5.26 rinse solution to achieve a concentration of 153.7 g/L. The pool was spiked with 4x trehalose excipient buffer (30 mM histidine, 400 g/L trehalose, pH 5.4) to achieve a final protein concentration of 114 g/L. The spiked solution was then concentrated to 202.4 g/L under the operational conditions described in Table 2, Experiment 2B. The concentration step was stopped at 15 LMH feed flow rate due to pump limitations.

Table 1 shows that both the histidine and trehalose concentrations in all concentrated samples were within 10% of the final target specification, 20 mM histidine and 84 g/L trehalose.

This information provides an acceptable operating range of the diafiltration concentration from 75-114 g/L, within which the final excipient concentrations meet concentration specifications.

Table 1. Initial Evaluation Excipient Concentration Results

Sample Name	Concentration (g/L)	Histidine (mM)	Trehalose (g/L)
Diafiltration Exp 1	76.6	29.81	Not Tested
Concentration Exp 1	213	19.63	
Load Exp 2B	114	26.19	83.82
Concentration Exp 2B	202.4	18.67	76.37
Diafiltration Buffer	N/A	34.34	Not Tested

Table 2. Initial Evaluation Process Data

UFDF System and Load Charge	706263-18-Exp2A	706263-18-Exp2B
Membrane Type	Sartocon E-channel	
Membrane Area, m2	0.02	0.02
UF Protein Challenge (g/m^2)	572	490
Concentration 1		
Load Volume (ml)	338.2	NA
Load Concentration (g/L)	33.8	
Concentration 1 (g/L)	114	
Concentration 1 Process Time (hr)	Not recorded	
Diafiltration		
Diafiltration Buffer	35 mM Histidine pH 5.26	NA
Diavolumes (TOV)	8	
Feed Flow Rate (LMH)	300	
Average Permeate Flux (LMH)	10	
Average TMP (psi)	22	
Diafiltration Process Time (hr)	4	
Concentration 2		
Spike Solution	NA	30 mM Histidine, 400 g/L trehalose pH 5.4
Post Spike Concentration (g/L)	NA	114
Average TMP (psi)	22	22
Final Flow Rate (LMH)	Not recorded	15
Product Volume (ml)	48.1	Not recorded
Product Concentration (g/L)	184.9	202.4
Concentration 2 Process Time (hr)*	Not recorded	~1
Retentate pH	5.50	5.51
Rinse		
Rinse Buffer	35 mM Histidine pH 5.26	20 mM Histidine, 84 g/L trehalose pH 5.5
Rinse Volume (ml)	26	Not recorded
Rinse Concentration (g/L)	58.7	54.5
Rinse pH	Not recorded	5.52

* The actual time was not recorded or could not be retrieved, it is based on a calculation of the flux and volume processed.

Additional experimentation was performed to evaluate changes in histidine and trehalose concentration as a function of protein concentration at end of the concentration 2 step. The starting material was concentrated to 105.9 g/l and dia-filtered with 35 mM histidine, pH 5.29 buffer using a 200 cm^2 Sartocon® E-channel membrane. The flux of the dia-filtration was 12 LMH at a feed flow rate of 300 LMH and 22 psi TMP. The dia-filtered material was then spiked with 4x trehalose

excipient solution. The spike solution was added directly into the reservoir and mixed for 15 minutes, then the material was concentrated to 172, 188 and 209 g/l final concentration (see Table 3). As shown in Table 4, the histidine concentration dropped as the protein concentration increased, but all values were within 10% of the target concentration of 20 mM histidine, 84 g/L trehalose.

Table 3. Additional Development Process Data

UFDF System and Load Charge	Notebook: 706263-20
Membrane Type	Sartocon Slice E-channel
Membrane Area, m2	0.02
UF Protein Challenge (g/m^2)	477
Concentration 1	
Load Volume (ml)	558.1
Load Concentration (g/L)	17.09
Final Concentration (g/L)	105.9
Diafiltration	
Diafiltration Buffer	35 mM Histidine pH 5.29
Diavolumes (TOV)	8
Feed Flow Rate (LMH)	300
Average TMP (psi)	22
Average Permeate Flux (LMH)	12
Diafiltration Time (hr)	3
Concentration 2	
Spike Solution	30 mM Histidine, 400 g/L trehalose pH 5.22
Post Spike Concentration (g/L)	78.6
Solution pH (At Spike)	5.38
Average TMP (psi)	22
Process Time (hr)*	~1
Product volume (ml)	32.1
Product Concentration (g/L)	209
Retentate pH	5.53
Yield Recovery (%)	76.6
Rinse	
Rinse Buffer	20 mM Histidine, 84 g/L trehalose pH 5.5
Rinse Volume (ml)	31.6
Rinse Concentration (g/L)	53.2
Rinse pH	5.48
Rinse Recovery (%)	19.2

* The actual time was not recorded or could not be retrieved, it is based on a calculation of the flux and volume processed.

Table 4. Additional Development Excipient Concentration Results

Sample Name	Concentration (g/L)	Histidine (mM)	Trehalose (g/L)
Diafiltration Pool	109.4	27.81	Not Tested
Concentration Load	78.6	27.57	94.02
Concentration 1	172	20.15	84.65
Concentration 2	188	19.47	84.24
Concentration 3	209	18.11	81.86
Spike Buffer	N/A	27.83	392.88

The method was scaled up to the 500 L pilot scale (Lot 12P126J603-MV-B): see Table 5 for process details. 517 g of Capto Adhere® purified material was concentrated to 107 g/l using a 0.5 m^2 Millipore® V-screen membrane, and then dia-filtered with 35 mM histidine, pH 5.29 buffer, a feed flow rate of 1000 LMH and a feed pressure of 40 psi. The retentate was then spiked with 4x trehalose solution (30 mM histidine, 400 g/l trehalose, pH 5.22), which was added directly into the reservoir taking into account the system hold-up volume. The spiked material was then concentrated to 202 g/l, and the concentrated product removed from the system. The skid was rinsed with 20 mM histidine, 84 g/l trehalose, pH 5.50 buffer, and the rinse added to the concentrated material. The measured concentration of the final combined solution was 160 g/l with an overall yield of 97.1%.

Table 6 summarizes the excipient concentration and product quality results for the experiment, which shows that the final combined pool levels were within 10% of the aforementioned targeted concentrations, without any significant effect on product quality as measured by SEC when compared to past final UF values.

Table 5. Pilot Scale Process Data

UFDF System and Load Charge	12P120J603-MV-B
Equipment	Millipore System
Membrane Type	Millipore 30K V screen RC
Membrane Area, m2	0.5
UF Protein Challenge (g/m^2)	1142
Concentration 1	
Load Volume (L)	7.5
Load Concentration (g/L)	67.85
Process Time (minutes)	18
Final Concentration (g/L)	107
Diafiltration	
Diafiltration Buffer	35 mM histidine pH 5.3
Diavolumes (TOV)	8
Feed Flow Rate (LMH)	~1000
Average Flux (LMH)	20
Diafiltration Time (hours)	3.75
Concentration 2	
Spike Solution	30 mM Histidine, 400 g/L trehalose pH 5.22
Average TMP (psi)	<28
Final flow Rate (LMH)	108
Process Volume (L)	2.2
Process Time (hr)	1
Product Concentration (g/L)	202
Product pH	5.44
Yield Recovery (%)	85.7
Rinse	
Rinse Buffer	20 mM histidine, 84 g/L trehalose pH 5.5
Rinse Volume (L)	1.1
Rinse concentration (g/L)	53.7
Rinse pH	5.55
Rinse Recovery (%)	11.4

Table 6. Pilot Scale Excipient and Product Quality Results

Sample Name	Concentration (mg/ml)	Histidine (mM)	Trehalose (g/L)	Total HMMS	Monomer	Total LMMS
Diafiltered Pool	107	28.28	N/A	1.0	99.0	<0.1%
Post-Spike Pool	78.6	28.04	90.35	0.8	99.2	<0.1%
Concentration 2 Pool	202	21.57	88.21	0.9	99.1	<0.1%
Rinse Pool	53.7	18.94	80.47	0.7	99.2	<0.1%
Final Pool	160	21.28	88.25	1.2	98.8	<0.1%
DF Buffer	N/A	34.80	N/A	N/A		
Excipient Buffer		31.52	413.75			
Rinse Buffer		19.93	83.26			

Evaluation of Dia-filtration process

Protein Density and Viscosity at Different Concentrations

Figure 1 plots the viscosity of bococizumab versus product concentration in (i) 20 mM histidine, pH 5.5 and (ii) 20 mM histidine, 84 g/l trehalose, pH 5.5. The graph shows that at approximately 175 g/l the viscosity reaches the 30 cP value, which is considered the cutoff for viable UFDF processing at large scale.

Densities of bococizumab in (i) 20 mM histidine, pH 5.5 and (ii) 20 mM histidine, 84 g/l trehalose, pH 5.5 solutions were measured and are shown in Figure 2 and Figure 3. The data shows that the density is slightly less in histidine buffer as compared to histidine/trehalose buffer, which is as expected.

Based on the experiments above, it was found that the targeted concentrations in the Drug Substance could be achieved at a manufacturing scale by with a DF buffer containing histidine, without trehalose.

The results from the experiments showed not only that the method of the invention resulted in acceptable yield, protein and excipient final concentrations, but also that it required lower protein concentration prior to the excipient spike, as compared to conventional methods (158 g/L versus 188 g/L). Such a lower protein concentration

is easier to achieve on a regular basis as the process is scaled up. In addition, the method of the invention is advantageous over the conventional methods due to the better cost-of-goods profile achieved by removing trehalose from the diafiltration buffer.

It has been found that the UFDF process utilizing a Millipore® C-screen membrane, as an alternative to the Millipore® V-screen membrane, consistently resulted in a concentration greater than 175 g/l, which was sufficient to allow addition of the wash pool (rinse) while still remaining above the 158 g/l needed prior to a 20x excipient spike. To ensure that the process would work with a trehalose spike, the process utilizing a C-Screen membrane was evaluated both at the laboratory and pilot scale.

The process was evaluated at the laboratory scale using Millipore® PLCTK C-Screen cassettes, utilizing the ultrafiltration (UF) run conditions outlined in Table 7.

For the TFF (Tangential flow Filtration) equipment, the lab scale process was performed employing a feed flow rate range of 30-300 LMH at an achievable pressure limit of approximately 50-55 psi as the operational limits. The upper feed flow rate of 300 LMH, where most of the process will occur, has a principle impact on process time, where reduced feed flow results in lower process flux which increases process pump time. The lower feed flow rate of 30 LMH is critical to the final concentration achievable, due to the increased viscosity increasing the pressure drop through the retentate channels, therefore lower flow rates enable pumping of more viscous solutions.

During the lab scale process run in the presence of approximately 84 g/l Trehalose a final concentration of 177 g/l was achieved in the final retentate pool with a feed flow rate of 30 LMH and feed pressure of 50 psi. The wash fractions from the lab scale runs were measured separately for yield as displayed in Table 8.

Table 7. Laboratory Scale Run Conditions

Step	Solution	Feed Pressure (psig)	Retentate Pressure (psig)	Target
Equilibration	10 mM Histidine, 50 mM NaCl, pH 6.4	20 (± 2)	10 (± 2)	± 0.2 pH Units
Concentration 1	VRF Product Pool	34 (± 6)	16 (± 6)	600-1000 g/m^2
Diafiltration	35 mM Histidine pH 5.3	34 (± 6)	16 (± 6)	> 7 TOV
Concentration 2	Diafiltration Pool	35 (± 20)	10 (± 10)	20-30% over DS Target
Buffer Flush	20 mM Histidine, 84 g/L Trehalose, pH 5.5	30 (± 20)	10 (± 10)	Concentration Dependent

Table 8. Results of Laboratory Scale Development Experiments

Experiment	Load Challenge (g/m^2)	Conc. 1 Flux (LMH) & Pressures (P_{feed}/P_{ret})	Diafiltration Flux (LMH) & Pressures (P_{feed}/P_{ret})	Conc. 2 Flux (LMH) & Pressures (P_{feed}/P_{ret})	% Yield Ret / Flush
No Trehalose	354*	90-20 (35/15)	25-42 (35/15)	35-4 (50/0)	85 /13
With Trehalose	354*	20-90 (35/15)	25-42 (35/15)	45-4 (50/0)	85 /13

*Lower load challenge is employed at lab scale due to material limitations and process cycling time and represents a worst case yield recovery option.

The laboratory scale process flux profile may be seen in Figure 4, where the vertical line indicates the starting point for reducing the feed flow rate to keep the feed pressure below ~50 psi with an open retentate (zero psi), where a reduction in process flux occurs due to reducing the cross flow rate. Figure 5 shows the process feed channel pressure drop and the feed flow rate during the final concentration. In the lab scale system, the flow is manually adjusted by reducing the feed pump rate as the feed pressure approaches ~50 psi.

Pilot Scale Confirmation Batches

The UFDF process with the C-screen membrane was performed at the 500 L scale to confirm that the final concentration targets could be achieved. The process is

shown in Table 9 and the process data for the 3 lots performed in the pilot facility is shown in Table 10.

The results show that the process achieved high recoveries and that concentrations met the intermediate and final targets. In addition, the excipient concentrations for lot 13P120J604 were measured at 21.2 mM histidine, 85.4 g/l trehalose, and 0.051 g/l EDTA, which are within the target specifications of ± 15%.

Table 9. UFDF Process Parameters for Pilot Scale Manufacturing

Parameter	Details	Category
Membrane	Millipore PLCTK (30 kDa cellulose) C-Screen	
Membrane Surface Area	500–1350 g/m^2	Control Limit
Operating Temperature	18-25 °C	Control Limit
Equilibration Buffer	10 mM Histidine, 50 mM NaCl, pH 6.4	
Pre-filter	≤3000 L/m^2 for a 0.2 um filter	Target
Diafiltration Buffer	35 mM Histidine pH 5.3	
Dilution Buffer	30 mM Histidine, 400 g/L Trehalose, pH 5.4	
Final UF Flush Buffer	20 mM Histidine, 84 g/L Trehalose, pH 5.5	
Filter Conditioning / Equilibration	≥ 10 L/m^2	Target Range
Maximum Inlet & Retentate Pressure	≤ 80 psig	Control Limit
Concentration 1 Inlet Pressure	Setpoint 35 psig, target range 22-55 psig	Target Range
Concentration 1 Retentate Pressure	Setpoint 15 psig, target range 0-40 psig	Target Range
Target Crossflow Rate	0–10 L/min/m^2	Target Range
Permeate Flux	0–50 LMH	Expected
Diafiltration Inlet Pressure	Setpoint 40 psig, target range 22-55 psig	Target Range
Diafiltration Retentate Pressure	Setpoint 15 psig, target range 0-40 psig	Target Range
Diafiltration Concentration	70-90 g/L based on tank volume of 350-450 L	Target
	70–110 g/L	Target Range
Diafiltration Volume	Minimum 8 TOV's	Target Range
Permeate pH and Conductivity (Diafiltration End)	After 8 TOVs verify pH is 5.5 ± 0.20 units. Continue diafiltration until target is met.	Control Limit
4X Trehalose Buffer Addition	Add 30 mM Histidine, 400 g/L Trehalose pH 5.4 after DF at 1:3 ratio	Control Limit
Concentration 2 Inlet Pressure	Setpoint 22-55 psig, target range 20-60 psig	Target Range
Concentration 2 Retentate Pressure	Setpoint 0 psig, target range 0-40 psig	Target Range
Retentate Concentration	170-190 g/L (per material balance in tank)	Target
	> 158 g/L (actual retentate concentration)	Control Limit
Buffer Flush	minimal volume recirculated rinse	
UF Pool Concentration Target	158 ± 10 g/L before 20X EDTA/PS80 Spike	Control Limit
UF Pool Density	Density calculation: 0.0004 * Concentration + 1.0126	
Post-filter	≤450 L/m^2 for a 0.2 um Filter	Target
Process Notes	Diafiltration Concentration based on 32-42 kg in 350 – 450 L retentate tank volume Add 4X trehalose buffer directly into tank after diafiltration. Addition at 1:3 ratio of total volume (volume in tank + system hold volume). Circulate for 10 minutes and concentrate to >158 mg/ml.	

Table 10. UFDF Process Data for 3 Pilot Scale Batches

Batch #	UNITS	13P120J604	13P120J605	13P120J606
UF Filter Type		30kD Millipore C-screen	30kD Millipore C-screen	30kD Millipore C-screen
UF Total Area	m2	1,14	1,14	1,14
Load Volume	L	68.9L / 30.56L	81.53L / 26.57L	85.2L / 32.0L
Load Concentration	g/L	11.03 g/L / 10.9 g/L	11.04 g/L / 11.22 g/L	8.79 g/L / 11.51 g/L
UF Protein Challenge	g/m2	959	950	982
UF Volumetric Challenge	l/m2	87,2	95	103
Concentration 1 Start Time	AM/PM	08:21	08:21	09:08
Concentration 1 End Time	AM/PM	11:38	12:07	15:02
Concentration 1 Permeate Volume	L	86,66	97,66	107,16
Concentration 1 Feed Pressure	psig	35	35	35
Concentration 1 Retentate Pressure	psig	15	15	15
Concentration 1 Permeate Pressure	psig	0	0	0
Concentration 1 End Retentate volume	L	12,8	10,4	10,2
Concentration 1 Average Flux	L/m2/h	23,1	22,7	15,9
Concentration 1 Average TMP	psig	25	25	25
Diafiltration Start Time	AM/PM	11:49	12:14	10:05
Diafiltration End Time	AM/PM	14:45	16:22	13:39
Diafiltration Permeate Volume	L	109	82,76	103
DiaVolumes		8,0	8,0	8,0
Diafiltration Feed Pressure	psig	40	40	40
Diafiltration Retentate Pressure	psig	15	15	15
Diafiltration Permeate Pressure	psig	0	0	0
Diafiltration Average Flux	L/m2/h	32,6	18,8	25,3
Diafiltration Average TMP	psig	27,5	27,5	27,5
Concentration 2 Start Time	AM/PM	15:15	16:45	14:02
Concentration 2 End Time	AM/PM	15:55	17:27	14:45
Concentration 2 Feed Pressure	psig	25 – 55	25 – 55	25 – 55
Concentration 2 Retentate Pressure	psig	0 - 2	0 - 2	0 – 2
Concentration 2 Permeate Pressure	psig	0	0	0
Concentration 2 Permeate Volume	L	7,1	7,9	6,8
Concentration 2 Average Flux	L/m2/h	9,3	9,9	8,3

Concentration 2 Average TMP (psig)		20	20	20
Product Pool Volume	L	6,433	6,337	7,175
Product Pool Concentration	g/L	158,6	157,5	156,6
Recovery	%	95	92	99
Wash Pool Volume	L	833	915	790
Wash Pool Concentration	g/L	62,88	63,20	54,28
Wash Pool Grams	g	52	58	43
Wash Pool Ratio	g/m2	5,14%	5,79%	3,82%

A.3 Conclusions

The above-described experiments were able to demonstrate 92-99% step yield while achieving the Drug Substance target.

The Example demonstrates that a UFDF method according to the invention is suitable for the preparation of a highly concentrated (150 g/l) bococizumab Drug Substance with the pH and all excipient concentrations in the acceptable ranges. The same may be achieved with other proteins with the same benefits, especially with proteins having a particularly high viscosity.

B – Example 2

In illustrative Example 2, the protein of interest is antibody C1GM, an IL-7R antagonist monoclonal antibody that specifically binds to IL-7R. The method has been designed to achieve a targeted product concentration of 120 g/l in the Drug Substance, with the Drug Substance including the following excipients at a pH of 7.0:

- histidine at a 20 mM concentration,
- arginine at a 100 mM concentration,
- sucrose at a 50 g/l concentration,
- PS80 (PolySorbate 80) at a 0.02 g/l concentration, and
- EDTA at a 0.5 g/l concentration.

It is deemed acceptable that the above requirements are achieved with a tolerance of ± 10 g/l in the protein concentration, of ± 15% in the excipients concentration and of ± 0.5 in the pH value.

In terms of yield, the method is required to achieve a product recovery of more than 85%.

The starting material used for the experiments described below was a fully purified solution that had been processed through MabSelect® and Q membrane chromatography.

Ultrafiltration/Diafiltration Device

All experiments were performed using a GE Crossflow system (300 mL reservoir) or the Quattroflow™ pump system fitted with Pellicon 3® (30 KDa, C-screen, 88 cm^2) regenerated cellulose membranes. Transmembrane pressure (TMP) was maintained at approximately 14-22 psi with P_{Feed} <55 psi. Unless otherwise specified, all rinses were generated by recirculating rinse buffer for >15 minutes, then concentrating to the minimal working volume of the system.

Analytical Assays

UV-visible spectrophotometry for protein concentration was performed using the Thermo Scientific Nanodrop 2000C™, or Solo VPE™ from C Technologies Inc. The extinction coefficient at 280 nm, as determined experimentally by ARD, is 1.51 $mL*mg^{-1}*cm^{-1}$.

Experiments

Experiment 1

The starting material was spiked with 5% of 2 M NaCl and adjusted to pH 7.0 with 2 M Tris base, concentrated to 50 g/L, diafiltered with 22 mM histidine, 110 mM

arginine pH 7.0, spiked with 5X sucrose buffer (22 mM histidine, 110 mM arginine, 275 g/L sucrose pH 7.0), and concentrated to 146.9 g/L at a feed flow rate of ~34 LMH, as detailed in Table 11. The pH of the concentrated solution was 7.00. The UF system was flushed in a single pass mode (without recirculation) with the

diafiltration solution, resulting in a concentration of 33 g/L. The overall yield was approximately 88%.

Table 11 - Diafiltration and Concentration with Arginine Buffer pH 7.0

Notebook	706263-77
Pump	Quattro Flow
UF Protein Challenge (g/m^2)	348
Concentration 1	
Load Volume (L)	1.36
Load Concentration (g/L)	2.25
Feed Flow Rate (LMH)	Varied
Feed Pressure (psi)	<50
Concentration 1 Time (minutes)	320
Concentration 1 Concentration (g/L)	49.8
Diafiltration	
Diafiltration Buffer	22 mM histidine, 110 mM Arginine, pH 7.0
DiaVolumes (TOV)	8
Feed Flow Rate @ DF (LMH)	300
Feed Pressure @ DF (psi)	40
TMP @ DF (psi)	20
Permeate Flow Rate @ DF (LMH)	11.5-16.5
Diafiltration Time (minutes)	220
Concentration 2	
Spike Solution	22 mM histidine, 110 mM Arginine, 275 g/L sucrose pH 7.0
TMP @ Concentration 2 (psi)	<25
Max Feed Pressure (psi)	50
Flow rate at End (LMH)	34
Concentration 2 Time (minutes)	Not recorded
Final Concentration (g/L)	146.9

Tables 12-14 show the excipient, CGE and SEC assay results. The histidine, arginine, and sucrose concentrations in the final concentrated material were all

within ± 10% of the desired value. There was no new aggregation formed during the UF process, nor any change in the level of fragmentation.

Table 12 - Process Excipient Concentrations for Arginine Buffer pH 7.0

Sample Name	Protein Concentration (g/L)	Arginine (mM)	Histidine (mM)	Sucrose (g/L)
Diafiltrate	50.1	113.7	22.6	N/A
Post-Spike	38.2	110.0	21.9	55.0
Final Concentrate	146.9	108.5	19.8	50.0
System Rinse	33	111.1	22.1	49.9
Diafiltration Buffer	N/A	116.1	23.6	N/A
5X Sucrose Buffer	N/A	87.6	15.8	277.6

Table 13 - nrCGE and rCGE Results for Arginine Buffer pH 7.0 Experiment

Sample	nrCGE			rCGE		
	% IgG	% Fragment	% Other	% HC + LC	% Fragment	% Other
ANTI-IL-7R Reference	97.5	2.5	0	98.7	0.5	0.8
Diafiltrate	96.2	3.8	0	98.8	0.5	0.7
Post-Spike	96.2	3.8	0	99.1	0.3	0.5
Final Concentrate	96.3	3.7	0	98.9	0.5	0.6
System Rinse	96.8	3.2	0	99.1	0.3	0.6

Table 14 - SEC Results for Arginine Buffer pH 7.0 Experiment

Sample Name	% HMMS	% LMMS	% Monomer
Diafilitrate	0.6	0.2	99.2
Post-Spike	0.6	0.2	99.2
Final Concentrate	0.6	0.2	99.2
System Rinse	0.6	0.2	99.2

Experiment 2

The experiment was repeated, as detailed in Table 15, the only difference being the way the spike volume was calculated. The total volume in the UF system prior to the spike was calculated from the overall material balance (the total load divided by the diafiltrate concentration) versus adding the volume in the reservoir plus the system hold-up volume. After the 5X sucrose spike, the protein was concentrated to 183.6 g/L at ~34 LMH feed flow rate and P_{feed} < 50 psi.

Table 15 - Diafiltration and Concentration with Arginine Buffer at pH 7.0

Notebook	706263-78
Pump	Quattro Flow
UF Protein Challenge (g/m^2)	387
Concentration 1	
Load Volume (L)	1.5
Load Concentration (g/L)	2.27
Feed Flow Rate (LMH)	varied
Feed Pressure (psi)	<50
Concentration 1 Time (minutes)	400
Concentration 1 Concentration (g/L)	43
Diafiltration	
Diafiltration Buffer	22 mM histidine, 110 mM Arginine, pH 7.0
DiaVolumes (TOV)	10
Feed Flow Rate @ DF (LMH)	300
Feed Pressure @ DF (psi)	28
TMP @ DF (psi)	20
Permeate Flow Rate @ DF (LMH)	13.4-19.1
Diafiltration Time (minutes)	300
Concentration 2	
Spike Solution	22 mM histidine, 110 mM Arginine, 275 g/L sucrose pH 7.0
TMP @ Concentration 2 (psi)	<25
Max Feed Pressure (psi)	50
Flow Rate at End (LMH)	34
Concentration 2 Time (minutes)	Not recorded
Final Concentration (g/L)	183.6

Table 16 shows that the excipient concentrations, histidine, arginine, and sucrose concentrations in the final material were within ± 10% of the desired target value. The difference in how the spike volume was calculated did not appear to have any significant effect on the final excipient concentrations.

Table 16 - Excipient Concentrations for Arginine pH 7.0 Buffer

Sample Name	Protein Concentration (g/L)	Arginine (mM)	Histidine (mM)	Sucrose (g/L)
Diafiltrate	43.71	112.9	21.8	ND
Post-Spike	36.2	113.1	22.1	55.6
Final Concentrate	182	106.9	19.4	48.5
5X Spike Buffer	N/A	102.1	22.2	266.7

Experiment 3

The experiment was repeated for a third time after the final formulation was nominated, and the results are detailed in Table . After diafiltration, the addition volume of the 5X spike solution was calculated as outlined in Experiment 2. The protein was concentrated to 190 g/L at ~34 LMH feed flow rate and P_{feed} < 50 psi. The UF system was rinsed with 20 mM histidine, 100 mM arginine, 50 g/L sucrose, pH 7.0 in the single pass mode. The protein concentration in the combined retentate and rinse pool was 151 g/L, resulting in an overall yield of approximately 84%.

Table 17 - Diafiltration and Concentration with Arginine Buffer at pH 7.0

Notebook	706263-79
Pump	Quattro Flow
UF Protein Challenge (g/m^2)	500
Concentration 1	
Load Volume (L)	1.96
Load Concentration (g/L)	2.25
Feed Pressure (psi)	<50
Concentration 1 Time (minutes)	Not recorded
Concentration 1 Concentration (g/L)	45.4
Diafiltration	
Diafiltration Buffer	22 mM histidine, 110 mM Arginine, pH 7.0
DiaVolumes (TOV)	8
Feed Flow Rate @ DF (LMH)	450
Feed Pressure @ DF (psi)	30
TMP @ DF (psi)	25
Permeate Flow Rate @ DF (LMH)	18
Diafiltration Time (minutes)	300
Concentration 2A	
Spike Solution	22 mM histidine, 110 mM Arginine, 275 g/L sucrose pH 7.0
TMP @ Concentration 2 (psi)	<25
Max Feed Pressure (psi)	50
Flow Rate at End (LMH)	34
Concentration 2 Time (minutes)	Not recorded
Final Concentration (g/L)	190

Table 18 summarizes the excipient concentrations, showing that the histidine, arginine, and sucrose concentrations were all within ± 10% of the desired value. Table 19 and Table 20 indicate that no additional aggregation or fragmentation was formed during the UFDF process.

Table 18 - Excipient Concentrations for Arginine pH 7.0 Buffer

Samples	Protein Concentration (g/L)	Histidine (mM)	Arginine (mM)	Sucrose (g/L)
Diafiltrate	45.4	22	110.4	N/A
Post-Spike	37.3	22	110.9	55.5
Concentration 2	190	19.5	102	49.7
System Rinse	37.6	20.5	102.2	52.2
Final ANTI-IL-7R Material	151.6	19.9	102.7	48.9
Diafiltration Buffer	N/A	22.2	110.3	N/A
5X Spike Buffer	N/A	22.2	110.4	274.4
Rinse Buffer	N/A	20	99.1	51.8

Table 19 - nrCGE and rCGE Results for Arginine pH 7.0 Buffer

Sample	nrCGE			rCGE		
	% IgG	% Fragment	% Other	% HC + LC	% Fragment	% Other
ANTI-IL-7R Reference	97.1	2.9	<0.3	98.7	0.5	0.8
ANTI-IL-7R diafiltrate	96.2	3.8	<0.3	98.9	0.4	0.7
ANTI-IL-7R spike	96.1	3.9	<0.3	99	0.4	0.7
ANTI-IL-7R concentrate	95.2	4.5	0.3	99	0.3	0.8
ANTI-IL-7R rinse	96.1	3.9	<0.3	99	0.3	0.7
ANTI-IL-7R final	95.3	4.4	0.3	99	0.3	0.7

Table 20 - SEC Results for Arginine pH 7.0 Buffer

Sample Name	% Total HMMS	% Total LMMS	% Monomer
ANTI-IL-7R Std	0.6	0.2	99.1
ANTI-IL-7R Diafiltrate	0.5	0.3	99.3
ANTI-IL-7R Spiked	0.5	0.3	99.3
ANTI-IL-7R Con	0.8	0.3	98.9
ANTI-IL-7R Rinse	0.5	0.3	99.3
ANTI-IL-7R Final	0.7	0.3	99

The process as performed in Experiment 3 above results in acceptable concentration values for all of the excipients and the protein of interest, and does not appear to have an effect on either formation of aggregate or fragmentation. This process will be scaled up to the pilot scale to ensure that it performs as expected.

Pilot Scale UFDF Process

The UF process developed above (Experiment 3) was tested in the Pilot Plant using a Millipore® C-screen regenerated cellulose membrane, and using material purified from a 500 L scale bioreactor.

During the unit operation, detailed in Table 21, 86 L of starting material at a starting product concentration of 2.92 g/L was spiked with 5% of 2 M NaCl, then concentrated to 44.6 g/L. The material was then diafiltered with 22 mM histidine, 110 mM arginine, pH 7.0 at a feed flow rate of approximately 150 LMH and P_{feed} <40 psi. After 8 TOV diafiltration, the retentate was spiked with 22 mM histidine, 110 mM arginine, 275 g/L sucrose pH 7.0, and recirculated for 10 minutes, then concentrated to 191.4 g/L. The concentration process was stopped at 30 LMH permeate flow rate and P_{feed} < 50 psi. The skid was then rinsed with 20 mM histidine, 100 mm histidine, 50 g/L sucrose, pH 7.0 in single pass mode. The overall yield was approximately 87%. The entire UF process took approximately 5 hours to complete.

A UF pool at a concentration of 135.4 g/L was created by mixing the retentate pool, the rinse pool, and additional rinse buffer. The pool was filtered through a Millipore® 05/0.2 um Opticap Express SHC at 59 L/m^2 throughput. A 20X EDTA and PS80 excipient buffer was spiked into the UF pool to produce Drug Substance at a final concentration of 129.4 g/L.

Table 21 - Pilot Scale UF Process Data

UFDF System and Load Charge	
Membrane Type	Millipore 30K C screen RC
Membrane Area, m2	2.28
UF Protein Challenge (g/m^2)	123
Concentration 1	
Load Volume (L)	96
Load Concentration (g/L)	2.923
Concentration 1 Time (minutes)	~120
Concentration 1 Concentration (g/L)	44.6
Diafiltration	
Diafiltration Buffer	22 mM Histidine, 110 mM Arginine pH 7.0
DiaVolumes (TOV)	8
Retentate Flow Rate @ DF (LMH)	131.6
Feed Pressure @ DF (psi)	29
TMP @ DF (psi)	24
Permeate Flow Rate @ DF (LMH)	18.4
Diafiltration Time (minutes)	80
Concentration 2	
Spike Solution	22 mM Histidine, 110 mM Arginine, 275 g/L Sucrose pH 7.0
TMP @ Concentration 2 (psi)	25
Retentate Flow Rate @ End (LMH)	30
Concentration 2 Final Volume (L)	0.738
Concentration 2 Time (minutes)	~30

Concentration 2 Concentration (g/L)	191.4
Concentration 2 Recovery (%)	50.35
Rinse	
Rinse Buffer	20 mM Histidine, 100 mM Arginine, 50 g/L Sucrose pH 7.0
Rinse Final Volume (mL)	1057
Rinse Concentration (g/L)	97.3
Rinse Recovery (%)	36.7

The excipient concentrations are summarized in Table 22, which shows that the histidine, arginine, and sucrose concentrations in the final pool were within ± 15% of the target values. The targeted range during the process development at lab scale was set at ± 10% of the target values for all excipient concentrations, but the acceptance range at large scale was set at ± 15% to allow for latitude during scale-up.

Table 23 and Table 24 summarize the product quality results from the run, which show that no increase in aggregation or fragmentation was detected.

Table 22 - Pilot Scale Run Excipient Concentration Results

Sample Name	Concentration (g/L)	Arginine (mM)	Histidine (mM)	Sucrose (g/L)
Diafiltration Pool	44.6	111.4	22.2	N/A
5X Spiked Pool	32.9	111.2	22.1	46.3
Concentration 2 Retentate	191.4	105.8	20.1	43.9
Final Rinse Pool	97.3	101.8	19.9	46.4
Drug Substance	135.4	103.9	20.2	43.8
UF Buffer	N/A	110.6	22.2	N/A
5X Spike	N/A	112.7	22.5	269.5
Rinse Buffer	N/A	101.0	20.4	46.9

Table 23 - Pilot Scale Run SEC Results

Sample Name	% Total HMMS	% Total LMMS	% Monomer
ANTI-IL-7R Reference	0.6	0.4	99.0
UF Load	0.5	0.4	99.2
Concentration 2 UF Retentate	0.7	0.4	98.9
Final Rinse Pool	0.6	0.3	99.1
Drug Substance	0.6	0.3	99.0

Table 24 - Pilot Scale Run nrCGE Results

Sample	% IgG	% Fragment	% Other
ANTI-IL-7R Reference	96.5	3.1	0.3
UF Load	96.7	3.3	0.0
Diafiltration Pool	97.3	2.7	0.0
Concentration 2 UF Retentate	96.2	3.4	0.3
Final Rinse Pool	97.1	2.9	0.0
Drug Substance	96.5	3.5	0.0

Conclusions

In conclusion, the above-described experiments demonstrate the successful process development of a UFDF process for >120 mg/ml drug substance for the ANTI-IL-7R antibody of interest. The UFDF process includes an initial concentration, a diafiltration, a sucrose spike prior to a final concentration, then spiking with the remaining excipients. The pH and all excipient concentrations in the developed process are in the acceptable ranges.

CLAIMS

1. A method of preparing a protein formulation including excipients and at least one therapeutic protein, the method comprising the sequential steps of:

(a) providing a solution comprising said protein;

(b) concentrating the protein in the solution by a first ultra-filtration step;

(c) diafiltering the solution thus obtained with a diafiltration buffer including at least one first excipient, whereby a retentate is obtained comprising the protein and the first excipient;

(d) adding a second excipient to the retentate obtained from the diafiltration step;

(e) further concentrating the protein in the retentate by a second ultra-filtration step in an ultra-filtration equipment; and

(f) adding at least one final excipient, whereby the protein formulation with a desired protein concentration and including said first and final excipients is obtained.

2. The method of claim 1, further including, after step (e) and before step (f), rinsing the ultra-filtration equipment with a rinse buffer, whereby the recovery of the protein is enhanced.

3. The method of claim 2, wherein the rinse buffer comprises the first and the second excipients at concentrations substantially equal to, respectively, the concentrations of the first and of the second excipients in the protein formulation.

4. The method of any one of claims 1 to 3, wherein the first excipient is an amino-acid, preferably histidine.

5. The method of any one of claims 1 to 4, wherein the first excipient in the protein formulation has a concentration of between 16 and 24 mM, preferably of between 17 and 23 mM, most preferably of about 20 mM.

6. The method of any one of claims 1 to 5, wherein the second excipient is a sugar, preferably a disaccharide.

7. The method of any one of claims 1 to 6, wherein the final excipients include a surfactant, preferably polysorbate 80.

8. The method of any one of claims 1 to 7, wherein the final excipients include a chelating agent, preferably EDTA.

9. The method of any one of claims 1 to 8, wherein the protein formulation has a protein concentration of between 110 and 165 g/l.

10. The method of anyone of claims 1 to 9, wherein the protein is an antibody.

11. The method of claim 10, wherein the antibody is an anti-PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) antibody.

12. The method of claim 11, wherein the anti-PCSK9 antibody is selected from the group consisting of bococizumab, evolocumab (REPATHA™), alirocumab (PRALUENT™), REGN728, 31H4, 11F1, 12H11, 8A1, 8A3, 3C4, 300N, 1D05, LGT209, RG7652, and LY3015014.

13. The method of claim 11 or 12, wherein the anti-PCSK9 antibody comprises a heavy chain variable region (VH) comprising complementarity determining region one CDR1, CDR2, and CDR3 of the amino acid sequence shown in SEQ ID NO: 1; and a light chain variable region (VL) comprising CDR1, CDR2, and CDR3 of the amino acid sequence shown in SEQ ID NO: 2.

14. The method of claim 11 or 12, wherein the anti-PCSK9 antibody comprises a VH CDR1 having the amino acid sequence shown in SEQ ID NO: 3, 4, or 5, a VH CDR2 having the amino acid sequence shown in SEQ ID NO: 6 or 7, a VH CDR3 having the amino acid sequence shown in SEQ ID NO: 8, a VL CDR1 having the amino acid sequence shown in SEQ ID NO: 9, a VL CDR2 having the amino acid sequence shown in SEQ ID NO:10, and a VL CDR3 having the amino acid sequence shown in SEQ ID NO: 11.

15. The method of any one of claims 11 to 14, wherein the protein formulation has a protein concentration of between 135 and 165 g/l, preferably of between 142 and 158 g/l, most preferably of about 150 g/l.

16. The method of any one of claims 11 to 15, wherein the second excipient in the protein formulation is trehalose at a concentration of between 67.2 and 100.8 g/l, preferably of between 71.4 and 96.6 g/l, most preferably of about 84 g/l.

17. The method of any one of claims 11 to 16, wherein the final excipients include polysorbate 80 which, in the protein formulation, has a concentration of between 0.16 and 0.24 g/l, preferably of between 0.17 and 0.23 g/l, most preferably of about 0.2 g/l.

18. The method of any one of claims 11 to 17, wherein the final excipients include EDTA which, in the protein formulation, has a concentration of between 0.04 and 0.06 g/l, preferably of between 0.0425 and 0.0575 g/l, most preferably of about 0.05 g/l.

19. The method of any one of claims 11 to 18, wherein the protein formulation has a pH of between 5.2 and 5.8, preferably of about 5.5.

20. The method of any one of claims 11 to 19, wherein the solution provided in step (a) has a protein concentration of between 5 and 20 g/l.

21. The method of any one of claims 11 to 20, wherein the protein is concentrated to between 80 and 120 g/l, preferably to between 90 and 110 g/l, and most preferably to about 100 g/l, by the first ultra-filtration step.

22. The method of any one of claims 11 to 21, wherein the protein is concentrated to between 143 and 173 g/l, preferably to between 150 and 166 g/l, and most preferably to about 158 g/l, by the second ultra-filtration step.

23. The method of any one of claims 11 to 22, wherein the first excipient in the diafiltration buffer has a concentration higher than the concentration of the first

excipient in the protein formulation, said concentration of the first excipient in the diafiltration buffer being preferably of between 29.75 and 40.25 mM, most preferably of about 35 mM.

24. The method of any one of claims 11 to 23, wherein the diafiltration buffer has a pH of between 5.1 and 5.5, preferably about 5.3.

25. The method of any one of claims 11 to 24, wherein adding the second excipient to the retentate obtained from the diafiltration step is achieved by adding a first additive solution to the retentate, said first additive solution comprising the second excipient at a concentration of between 340 and 460 g/l, preferably of between 380 and 420 g/l, most preferably of about 400 g/l.

26. The method of claim 25, wherein the first additive solution comprises the first excipient at a concentration lower than the concentration of the first excipient in the diafiltration buffer and higher than the concentration of the first excipient in the protein formulation, said concentration of the first excipient in the first additive solution being preferably of between 25.5 and 34.5 mM, most preferably of about 30 mM.

27. The method of claim 25 or 26, wherein the first additive solution further comprises a final excipient.

28. The method of claim 27, wherein the first additive solution comprises about 30 mM histidine and about 400 g/l trehalose.

29. The method of any one of claims 25 to 28, wherein adding the first additive solution to the retentate is performed at a dilution ratio of about 4.15, whereby one volume of the first additive solution is added to approximately 3.15 fold the same volume of the retentate.

30. The method of any one of claims 25 to 29, wherein adding the final excipients includes the step of adding a second additive solution to the solution obtained from

the second ultra-filtration step, said second additive solution comprising the second excipient at a concentration lower than the concentration of the second excipient in the first additive solution and higher than the concentration of the second excipient in the protein formulation.

31. The method of claim 30, wherein the second additive solution comprises the first excipient at a concentration substantially equal to the concentration of the first excipient in the protein formulation.

32. The method of claim 31, wherein the second additive solution comprises about 20 mM histidine, about 84 g/l trehalose, about 1 g/l EDTA and about 4 g/l polysorbate 80.

33. The method of any one of claims 30 to 32, wherein adding the second additive solution is performed at a dilution ratio of about 20, whereby one volume of the second additive solution is added to approximately 19 fold the same volume of to the solution obtained from the second ultra-filtration step.

34. The method of claim 10, wherein the antibody is an anti-IL-7R antibody.

35. The method of claim 10, wherein the antibody has a VH region comprising the amino acid sequence shown in SEQ ID NO 13, and VL region comprising the amino acid sequence shown in SEQ ID NO 14.

36. The method of claims 34 or 35, wherein the protein formulation has a protein concentration of between 110 and 130 g/l, preferably of about 120 g/l.

37. The method of any one of claims 34 to 36, wherein the second excipient in the protein formulation is sucrose at a concentration of between 42 and 58 g/l, preferably of about 50 g/l.

38. The method of any one of claims 34 to 37, wherein the final excipients include polysorbate 80 which, in the protein formulation, has a concentration of between 0.017 and 0.023 g/l, preferably of about 0.02 g/l.

39. The method of any one of claims 34 to 38, wherein the final excipients include EDTA which, in the protein formulation, has a concentration of between 0.42 and 0.58 g/l, preferably of about 0.5 g/l.

40. The method of any one of claims 34 to 39, wherein the final excipients include arginine which, in the protein formulation, has a concentration of between 85 and 115 mM, preferably of about 100 mM.

41. The method of any one of claims 34 to 40, wherein the protein formulation has a pH of between 6.5 and 7.5, preferably of about 7.0.

42. The method of any one of claims 34 to 41, wherein the solution provided in step (a) has a protein concentration of between 2.6 and 3.4 g/l, preferably of about 3 g/l.

43. The method of any one of claims 34 to 42, wherein the protein is concentrated to between 36 and 54 g/l, preferably to between 40 and 50 g/l, and most preferably to about 45 g/l, by the first ultra-filtration step.

44. The method of any one of claims 34 to 43, wherein the protein is concentrated to between 170 and 210 g/l, preferably to about 190 g/l, by the second ultra-filtration step.

45. The method of any one of claims 34 to 44, wherein the first excipient in the diafiltration buffer has a concentration higher than the concentration of the first excipient in the protein formulation, said concentration of the first excipient in the diafiltration buffer being preferably of between 19 and 25 mM, most preferably of about 22 mM.

46. The method of any one of claims 34 to 45, wherein the diafiltration buffer includes arginine at a concentration of between 95 and 125 mM, preferably of about 110 mM.

47. The method of any one of claims 34 to 46, wherein the diafiltration buffer has a pH of between 6.5 and 7.5, preferably about 7.0.

48. The method of any one of claims 34 to 47, wherein adding the second excipient to the retentate obtained from the diafiltration step is achieved by adding a first additive solution to the retentate, said first additive solution comprising the second excipient at a concentration of between 230 and 320 g/l, preferably of about 275 g/l.

49. The method of claim 48, wherein the first additive solution comprises the first excipient at a concentration substantially equal to the concentration of the first excipient in the diafiltration buffer and higher than the concentration of the first excipient in the protein formulation, said concentration of the first excipient in the first additive solution being preferably of between 19 and 25 mM, most preferably of about 22 mM.

50. The method of claim 48 or 49, wherein the first additive solution further comprises a final excipient.

51. The method of claim 50, wherein the first additive solution comprises about 22 mM histidine, 110 mM arginine and about 275 g/l sucrose, at a pH of about 7.0.

52. The method of any one of claims 34 to 51, wherein adding the first additive solution to the retentate is performed at a dilution ratio of about 5, whereby one volume of the first additive solution is added to approximately 4 fold the same volume of the retentate.

53. The method of any one of claims 34 to 52, wherein adding the final excipients includes the step of adding a second additive solution to the solution obtained from

the second ultra-filtration step, said second additive solution comprising EDTA and polysorbate 80.

54. The method of claim 53, wherein adding the second additive solution is performed at a dilution ratio of about 20, whereby one volume of the second additive solution is added to approximately 19 fold the same volume of to the solution obtained from the second ultra-filtration step.

55. A formulation of an antibody having a high viscosity produced by the method of any one of claims 1 to 54.

56. A formulation of an antibody having a high viscosity produced by the method of any one of claims 11 to 33, wherein the protein formulation comprises:

- from 135 mg/ml to 165 mg/ml, preferably about 150 mg/ml, of the anti-PCSK9 antibody;
- from 16 mM to 24 mM, preferably about 20 mM, of histidine;
- from 67.2 mg/ml to 100.8 mg/ml, preferably about 84 mg/ml, of trehalose; and
- from 0.16 mg/ml to 0.24 mg/ml, preferably about 0.2 mg/ml, of polysorbate

and has a pH of between 5.2 and 5.8, preferably about 5.5.

57. A formulation of an antibody having a high viscosity produced by the method of any one of claims 34 to 54, wherein the protein formulation comprises:

- from 110 g/l to 130 g/l, preferably about 120 g/l, of the anti-IL-7R antibody;
- from 17 mM to 23 mM, preferably about 20 mM, of histidine;
- from 42 g/l to 58 g/l, preferably about 50 g/l, of sucrose; and
- from 0.017 g/l to 0.023 g/l, preferably about 0.02 g/l, of polysorbate

and has a pH of between 6.5 and 7.5, preferably about 7.0.

FIG. 1

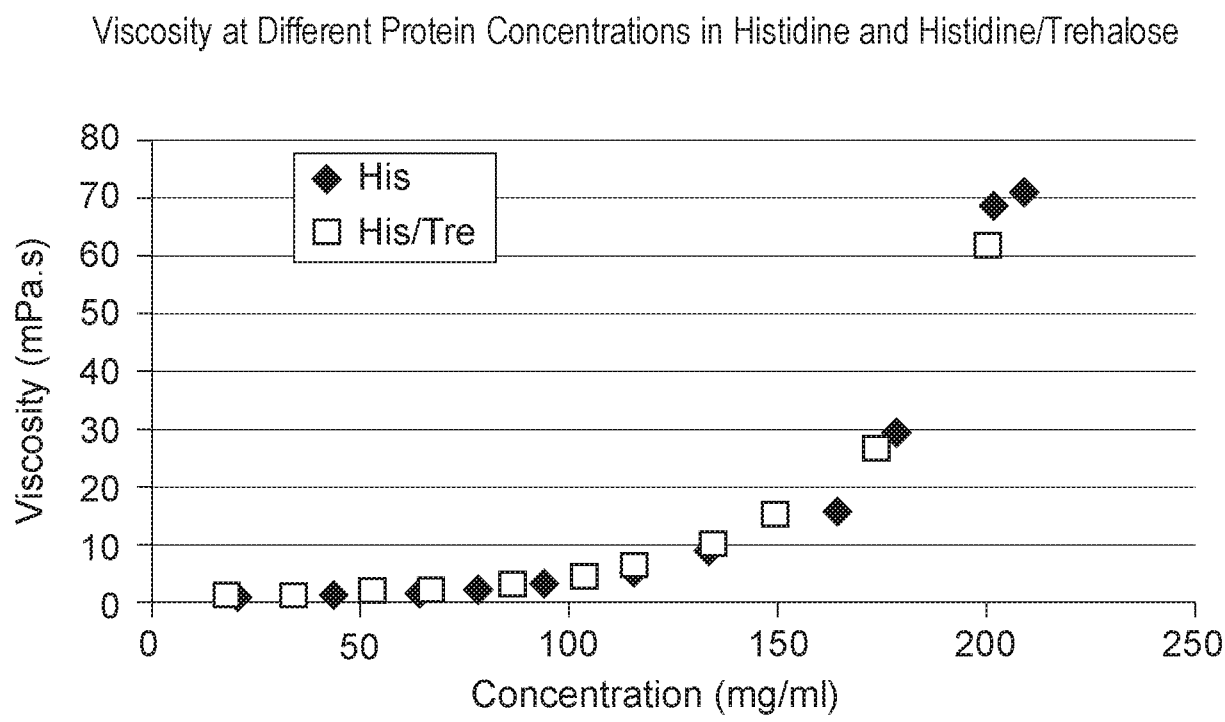

Viscosity at Different Protein Concentrations in Histidine and Histidine/Trehalose
His
His/Tre
Viscosity (mPa.s)
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Concentration (mg/ml)

FIG. 2

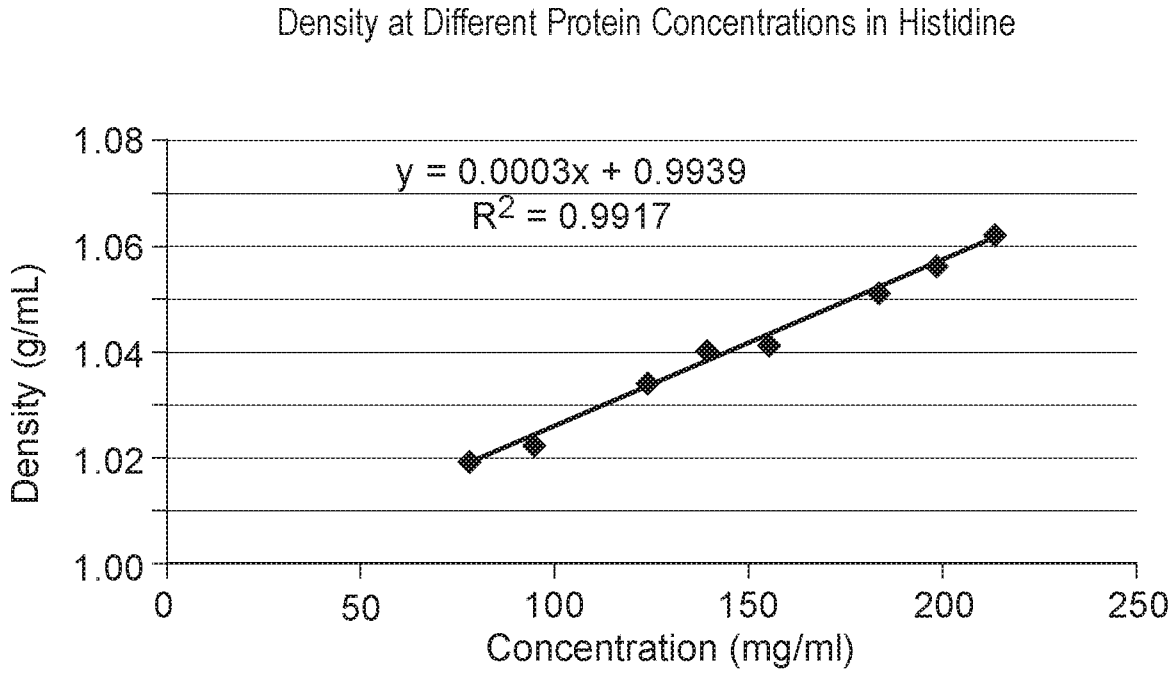

Density at Different Protein Concentrations in Histidine
y = 0.0003x + 0.9939
R2 = 0.9917
Density (g/mL)
1.08
1.06
1.04
1.02
1.00
0
50
100
150
200
250
Concentration (mg/ml)

FIG. 3

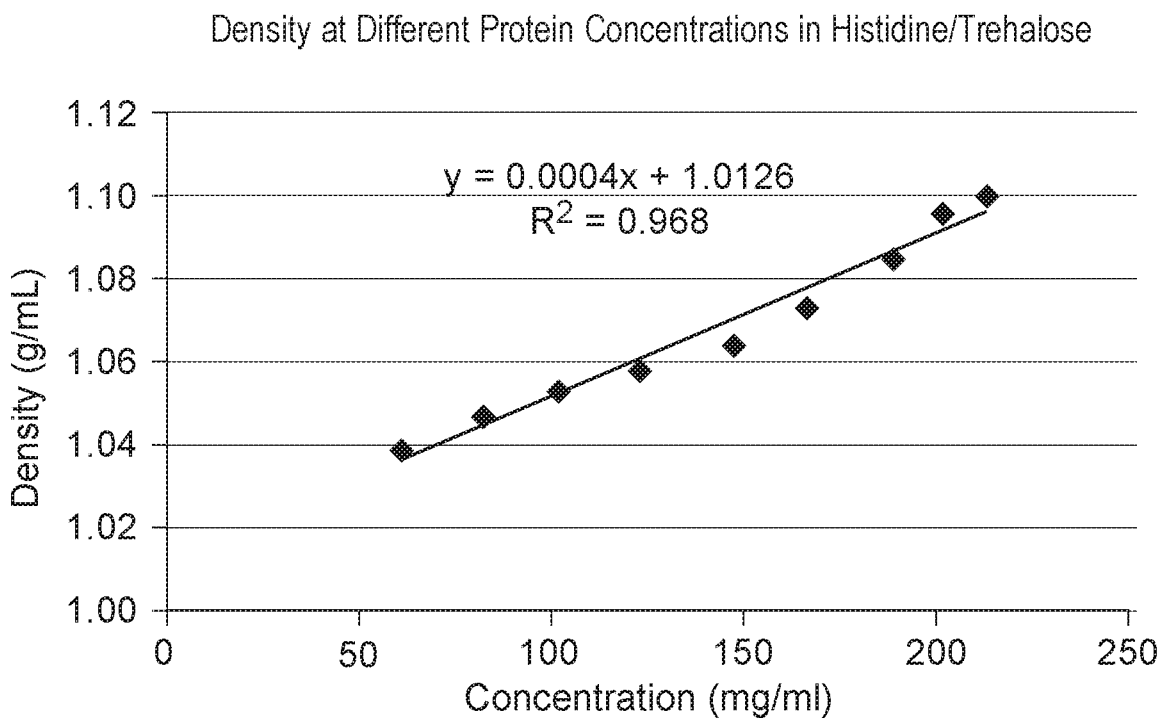

Density at Different Protein Concentrations in Histidine/Trehalose
y = 0.0004x + 1.0126
R2 = 0.968
Density (g/mL)
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0
50
100
150
200
250
Concentration (mg/ml)

FIG. 4

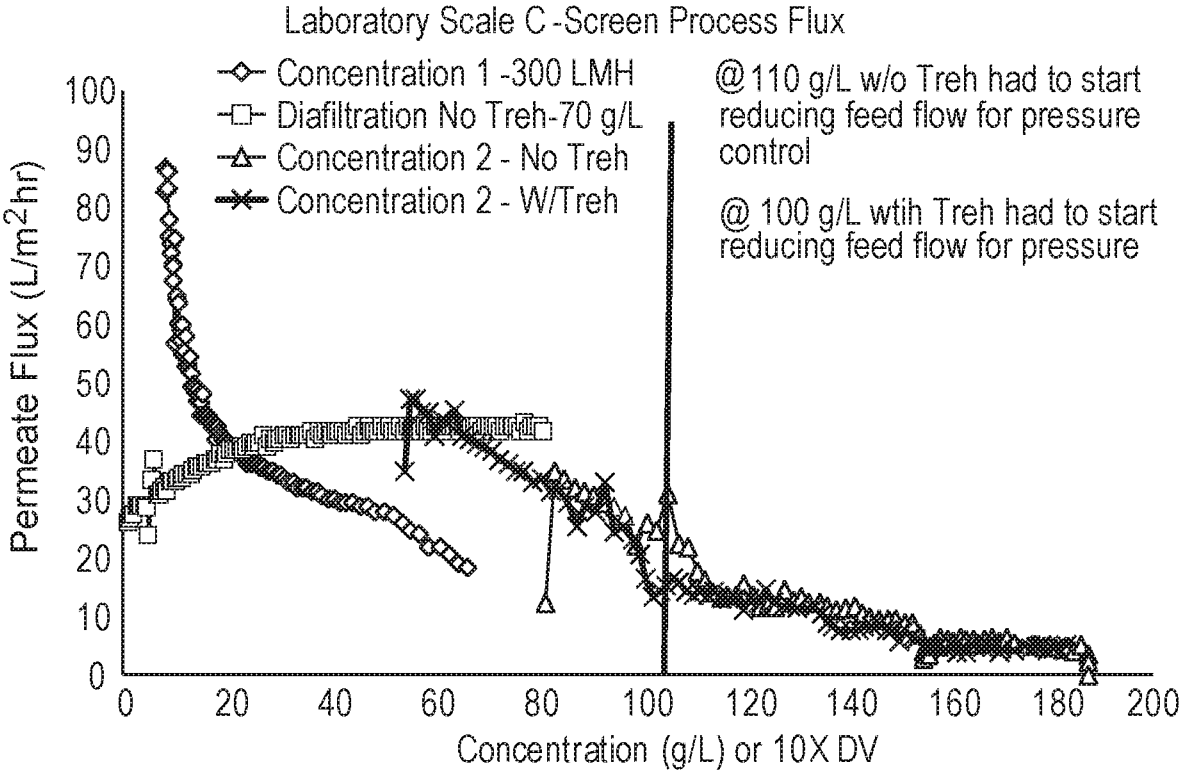
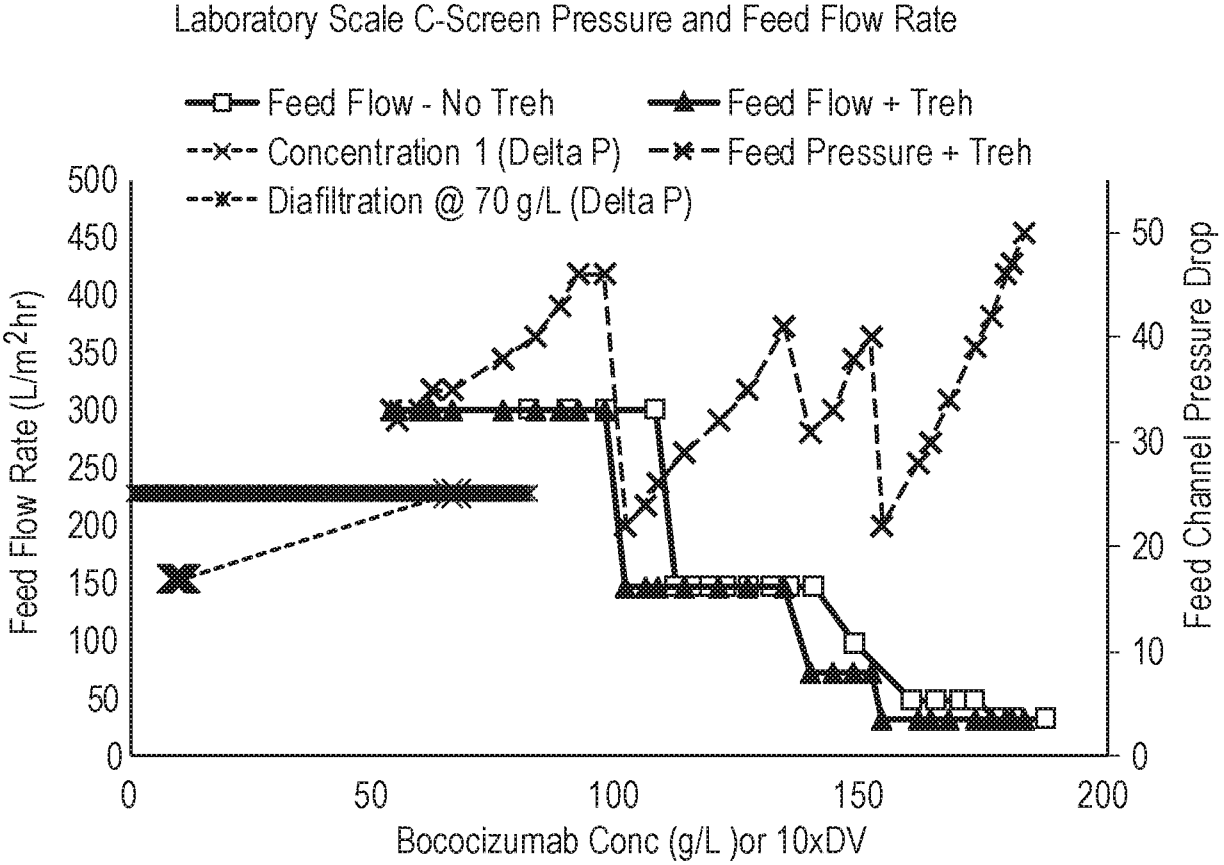

Laboratory Scale C-Screen Process Flux
Concentration 1 -300 LMH
Diafiltration No Treh-70 g/L
Concentration 2 - No Treh
Concentration 2 - W/Treh
@110 g/L w/o Treh had to start reducing feed flow for pressure control
@ 100 g/L wtih Treh had to start reducing feed flow for pressure
Permeate Flux (L/m2hr)
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
200
Concentration (g/L) or 10X DV

FIG. 5


Laboratory Scale C-Screen Pressure and Feed Flow Rate
Feed Flow - No Treh
Feed Flow + Treh
Concentration 1 (Delta P)
Feed Pressure + Treh
Diafiltration @ 70 g/L (Delta P)
Feed Flow Rate (L/m2hr)
Feed Channel Pressure Drop
Bococizumab Conc (g/L)or 10xDV
0
50
100
150
200
250
300
350
400
450
500
10
20
30
40

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/055355

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K47/18 A61K47/26 G01N33/68
ADD.

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)
A61K G01N C07K

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 practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/168491 A1 (NOVARTIS AG [CH]; GIFFARD MARION [CH]) 13 December 2012 (2012-12-13)	55,56
Y	[0096]; claims 1-53	11-33

X	WO 2008/071394 A1 (HOFFMANN LA ROCHE [CH]; GOLDBACH PIERRE [FR]; MAHLER HANNS-CHRISTIAN [) 19 June 2008 (2008-06-19)	1-10
Y	page 25, paragraph 6 - page 26, paragraph 1 page 18, paragraph 5 - page 19, paragraph 1	11-54

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 November 2016	14/11/2016

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, Fax: (+31-70) 340-3016	Klee, Barbara

INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). 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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X	US 2006/051347 A1 (WINTER CHARLES M [US]) 9 March 2006 (2006-03-09)	1-10
Y	[0004], [0071-0081], [0154-0160]); claims 1, 29	11-55,57

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/055355

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/055355

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G01N 33/68(2006.01)

权利要求书4页 说明书25页
序列表11页 附图3页

(54)发明名称

制备治疗用蛋白质制剂的方法和由这种方法生产的抗体制剂

(57)摘要

本发明涉及制备包含治疗用蛋白质的蛋白质制剂的方法，所述方法包括步骤：提供包含所述蛋白质的溶液；由第一超滤步骤浓缩所述溶液中的所述蛋白质；用包含至少一种第一赋形剂的渗滤缓冲液渗滤所述溶液，由此获得包含所述蛋白质和所述第一赋形剂的保留物；由第二超滤步骤进一步浓缩保留物中的所述蛋白质；和添加至少一种最终赋形剂，由此获得具有所需蛋白质浓度的蛋白质制剂。根据本发明，所述方法进一步包括在第二超滤步骤之前，将第二赋形剂添加到从渗滤步骤获得的保留物中。本发明还涉及通过前述方法生产的抗体制剂。

CN 108025072 A

1.制备包含赋形剂和至少一种治疗用蛋白质的蛋白质制剂的方法,所述方法包括按次序的步骤:

(a)提供包含所述蛋白质的溶液;

(b)由第一超滤步骤浓缩所述溶液中的所述蛋白质;

(c)用包含至少一种第一赋形剂的渗滤缓冲液渗滤如此获得的溶液,由此获得包含所述蛋白质和所述第一赋形剂的保留物;

(d)将第二赋形剂添加到由渗滤步骤获得的保留物中;

(e)由在超滤设备中的第二超滤步骤进一步浓缩保留物中的所述蛋白质;和

(f)添加至少一种最终赋形剂,由此获得具有所需蛋白质浓度并包含所述第一和最终赋形剂的蛋白质制剂。

2.权利要求1的方法,其在步骤(e)之后且在步骤(f)之前进一步包括用漂洗缓冲液漂洗超滤设备,由此增强蛋白质的回收。

3.权利要求2的方法,其中所述漂洗缓冲液包含浓度基本上分别等于蛋白质制剂中第一和第二赋形剂浓度的第一和第二赋形剂。

4.权利要求1-3任一项的方法,其中所述第一赋形剂是氨基酸,优选组氨酸。

5.权利要求1-4任一项的方法,其中所述蛋白质制剂中的第一赋形剂具有16-24 mM,优选17-23 mM,最优选约20 mM的浓度。

6.权利要求1-5任一项的方法,其中所述第二赋形剂是糖,优选二糖。

7.权利要求1-6任一项的方法,其中所述最终赋形剂包括表面活性剂,优选聚山梨醇酯80。

8.权利要求1-7任一项的方法,其中所述最终赋形剂包括螯合剂,优选EDTA。

9.权利要求1-8任一项的方法,其中所述蛋白质制剂具有110-165 g/l的蛋白质浓度。

10.权利要求1-9任一项的方法,其中所述蛋白质是抗体。

11.权利要求10的方法,其中所述抗体是抗-PCSK9(蛋白质原转变酶枯草杆菌蛋白酶Kexin 9型)抗体。

12.权利要求11的方法,其中所述抗-PCSK9抗体选自:博科昔单抗、依伏库单抗(REPATHA™)、阿利库单抗(PRALUENT™)、REGN728、31H4、11F1、12H11、8A1、8A3、3C4、300N、1D05、LGT209、RG7652和LY3015014。

13.权利要求11或12的方法,其中所述抗-PCSK9抗体包含重链可变区(VH),所述重链可变区(VH)包含SEQ ID NO:1中所示氨基酸序列的互补性决定区一CDR1、CDR2和CDR3;和轻链可变区(VL),所述轻链可变区(VL)包含SEQ ID NO:2中所示氨基酸序列的CDR1、CDR2和CDR3。

14.权利要求11或12的方法,其中所述抗-PCSK9抗体包含具有SEQ ID NO:3、4或5中所示氨基酸序列的VH CDR1,具有SEQ ID NO:6或7中所示氨基酸序列的VH CDR2,VH CDR3具有SEQ ID NO:8中所示氨基酸序列的VH CDR3,具有SEQ ID NO:9中所示氨基酸序列的VL CDR1,具有SEQ ID NO:10中所示氨基酸序列的VL CDR2和具有SEQ ID NO:11中所示氨基酸序列的VL CDR3。

15.权利要求11-14任一项的方法,其中所述蛋白质制剂具有135-165 g/l,优选142-158 g/l,最优选约150 g/l的蛋白质浓度。

16.权利要求11-15任一项的方法，其中所述蛋白质制剂中的第二赋形剂是浓度为67.2-100.8 g/l，优选71.4-96.6 g/l，最优选约84 g/l的海藻糖。

17.权利要求11-16任一项的方法，其中所述最终赋形剂包括聚山梨醇酯80，其在蛋白质制剂中的浓度为0.16-0.24 g/l，优选为0.17-0.23 g/l，最优选为约0.2 g/l。

18.权利要求11-17任一项的方法，其中所述最终赋形剂包括EDTA，其在蛋白质制剂中的浓度为0.04至0.06 g/l，优选0.0425至0.0575 g/l，最优选约0.05 g/l。

19.权利要求11-18任一项的方法，其中所述蛋白质制剂具有5.2-5.8，优选约5.5的pH。

20.权利要求11-19任一项的方法，其中所述步骤(a)中提供的溶液具有5-20 g/l的蛋白质浓度。

21.权利要求11-20任一项的方法，其中通过第一超滤步骤将所述蛋白质浓缩至80-120 g/l，优选至90-110 g/l，且最优选至约100 g/l。

22.权利要求11-21任一项的方法，其中通过第二超滤步骤将所述蛋白质浓缩至143-173 g/l，优选至150-166 g/l，且最优选至约158 g/l。

23.权利要求11-22任一项的方法，其中所述渗滤缓冲液中的第一赋形剂的浓度高于所述蛋白质制剂中第一赋形剂的浓度，所述渗滤缓冲液中第一赋形剂的浓度优选为29.75-40.25 mM，最优选约35 mM。

24.权利要求11-23任一项的方法，其中所述渗滤缓冲液具有5.1-5.5，优选约5.3的pH。

25.权利要求11-24任一项的方法，其中将所述第二赋形剂添加到从渗滤步骤获得的保留物中是通过将第一添加剂溶液添加到保留物中来实现的，所述第一添加剂溶液包含浓度为340-460 g/l，优选380-420 g/l，最优选约400 g/l的第二赋形剂。

26.权利要求25的方法，其中所述第一添加剂溶液包含第一赋形剂，其浓度低于所述渗滤缓冲液中第一赋形剂的浓度并高于所述蛋白质制剂中第一赋形剂的浓度，所述第一添加剂溶液中第一赋形剂的浓度为优选25.5-34.5 mM，最优选约30 mM。

27.权利要求25或26的方法，其中所述第一添加剂溶液还包含最终赋形剂。

28.权利要求27的方法，其中所述第一添加剂溶液包含约30 mM组氨酸和约400 g/l海藻糖。

29.权利要求25-28任一项的方法，其中将所述第一添加剂溶液添加到保留物中是以约4.15的稀释比进行的，由此将一体积的所述第一添加剂溶液添加到约3.15倍相同体积的保留物中。

30.权利要求25-29任一项的方法，其中添加最终赋形剂包括将第二添加剂溶液添加到由第二超滤步骤获得的溶液中的步骤，所述第二添加剂溶液包含浓度低于所述第一添加剂溶液中第二赋形剂的浓度并且高于所述蛋白质制剂中第二赋形剂的浓度的第二赋形剂。

31.权利要求30的方法，其中所述第二添加剂溶液包含浓度基本上等于所述蛋白质制剂中第一赋形剂浓度的第一赋形剂。

32.权利要求31的方法，其中所述第二添加剂溶液包含约20 mM组氨酸，约84 g/l海藻糖，约1 g/l EDTA和约4 g/l聚山梨醇酯80。

33.权利要求30-32任一项的方法，其中添加第二添加剂溶液以约20的稀释比进行，由此将一体积的第二添加剂溶液添加到约19倍相同体积的从第二超滤步骤获得的溶液中。

34.权利要求10的方法，其中所述抗体是抗-IL-7R抗体。

35.权利要求10的方法，其中所述抗体具有包含SEQ ID NO. 13中所示的氨基酸序列的VH区和包含SEQ ID NO. 14中所示的氨基酸序列的VL区。

36.权利要求34或35的方法，其中所述蛋白质制剂具有110-130 g/l，优选约120 g/l的蛋白质浓度。

37.权利要求34-36任一项的方法，其中所述蛋白质制剂中的第二赋形剂是浓度为42-58 g/l，优选约50 g/l的蔗糖。

38.权利要求34-37任一项的方法，其中所述最终赋形剂包括聚山梨醇酯80，其在蛋白质制剂中的浓度为0.017-0.023 g/l，优选约0.02 g/l。

39.权利要求34-38任一项的方法，其中所述最终赋形剂包括EDTA，其在蛋白质制剂中的浓度为0.42-0.58 g/l，优选约0.5 g/l。

40.权利要求34-39任一项的方法，其中所述最终赋形剂包括精氨酸，其在蛋白质制剂中的浓度为85-115 mM，优选约100 mM。

41.权利要求34-40任一项的方法，其中所述蛋白质制剂具有6.5至7.5，优选约7.0的pH。

42.权利要求34-41任一项的方法，其中所述步骤(a)中提供的溶液具有2.6-3.4 g/l，优选约3 g/l的蛋白质浓度。

43.权利要求34-42任一项的方法，其中通过第一超滤步骤将所述蛋白质浓缩至36-54 g/l，优选至40-50 g/l，且最优选至约45 g/l。

44.权利要求34-43任一项的方法，其中通过第二超滤步骤将所述蛋白质浓缩至170-210 g/l，优选至约190 g/l。

45.权利要求34-44任一项的方法，其中所述渗滤缓冲液中的第一赋形剂的浓度高于所述蛋白质制剂中第一赋形剂的浓度，所述渗滤缓冲液中第一赋形剂的浓度优选为19-25 mM，最优选约22 mM。

46.权利要求34-45任一项的方法，其中所述渗滤缓冲液包含浓度为95-125 mM，优选约110 mM的精氨酸。

47.权利要求34-46任一项的方法，其中所述渗滤缓冲液具有6.5-7.5，优选约7.0的pH。

48.权利要求34-47任一项的方法，其中将所述第二赋形剂添加到从渗滤步骤获得的保留物中是通过将第一添加剂溶液添加到保留物中来实现的，所述第一添加剂溶液包含浓度为230-320 g/l，优选约275 g/l的第二赋形剂。

49.权利要求48的方法，其中所述第一添加剂溶液包含第一赋形剂，其浓度基本上等于所述渗滤缓冲液中第一赋形剂的浓度并且高于所述蛋白质制剂中第一赋形剂的浓度，所述第一添加剂溶液中第一赋形剂的浓度为优选19-25 mM，最优选约22 mM。

50.权利要求48或49的方法，其中所述第一添加剂溶液还包含最终赋形剂。

51.权利要求50的方法，其中所述第一添加剂溶液在约7.0的pH包含约22 mM组氨酸，110 mM精氨酸和约275 g/l蔗糖。

52.权利要求34-51任一项的方法，其中将所述第一添加剂溶液添加到保留物中以约5的稀释比进行，由此将一体积的所述第一添加剂溶液添加到约4倍相同体积的保留物中。

53.权利要求34-52任一项的方法，其中添加最终赋形剂包括将第二添加剂溶液添加到从第二超滤步骤获得的溶液中的步骤，所述第二添加剂溶液包含EDTA和聚山梨醇酯80。

54.权利要求53的方法,其中添加第二添加剂溶液以约20的稀释比进行,由此将一体积的所述第二添加剂溶液添加到约19倍相同体积的从第二超滤步骤获得的溶液中。

55.由权利要求1-54任一项的方法生产的具有高粘度的抗体制剂。

56.由权利要求11-33任一项的方法生产的具有高粘度的抗体制剂,其中所述蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体;

· 16 mM-24 mM,优选约20 mM的组氨酸;

· 67.2 mg/ml-100.8 mg/ml,优选约84 mg/ml的海藻糖;和

· 0.16 mg/ml-0.24 mg/ml,优选约0.2 mg/ml聚山梨醇酯

并具有5.2-5.8,优选约5.5的pH。

57.由权利要求34-54任一项的方法生产的具有高粘度的抗体制剂,其中所述蛋白质制剂包含:

· 110 g/l-130 g/l,优选约120 g/l的抗-IL-7R抗体;

· 17 mM-23 mM,优选约20 mM的组氨酸;

· 42 g/l-58 g/l,优选约50 g/l的蔗糖;和

· 0.017 g/l-0.023 g/l,优选约0.02 g/l聚山梨醇酯

并具有6.5-7.5,优选约7.0的pH。

制备治疗用蛋白质制剂的方法和由这种方法生产的抗体制剂

技术领域

[0001] 本发明涉及制备包含赋形剂和至少一种治疗用蛋白质的蛋白质制剂的方法。

[0002] 本发明在准备用作治疗用途的抗体制剂领域中特别重要，并且也涉及由所述方法生产的抗体制剂。

[0003] 发明背景

本发明更具体地涉及按次序包括以下的方法：

· 提供包含所述蛋白质的溶液；

· 由第一超滤步骤浓缩所述溶液中的所述蛋白质；

· 用包含至少一种第一赋形剂的渗滤缓冲液渗滤如此获得的溶液，由此获得包含所述蛋白质和所述第一赋形剂的保留物(retentate)；

· 由在超滤设备中的第二超滤步骤进一步浓缩保留物中的所述蛋白质；

· 添加至少一种最终赋形剂，由此获得具有所需蛋白质浓度并包含所述第一和最终赋形剂的蛋白质制剂。

[0004] 通常，用于治疗用抗体的最终蛋白质制剂至少包含在渗滤步骤期间添加的氨基酸，例如组氨酸，和充当稳定剂的糖，例如海藻糖。海藻糖通常在最终添加步骤中与其他赋形剂一起添加。

[0005] 在应用于治疗用抗体的常规方法中，上述步骤用在由许多纯化步骤纯化的情况下的蛋白质溶液进行，所述许多纯化步骤通常包括病毒保留过滤作为最后的纯化步骤。通过第一超滤步骤从约5至20 g/l的浓度至约40至100 g/l的浓度(取决于蛋白质)浓缩蛋白质溶液(或“产物”)。然后将浓缩产物在渗滤缓冲液例如组氨酸中渗滤。在一些情况下，渗滤缓冲液可以是另一种标准缓冲液，如乙酸盐、三羟甲基氨基甲烷或磷酸盐。基于最终蛋白质制剂以及由于唐南(Donnan)效应而需要的任何补偿来选择渗滤缓冲液。唐南效应在浓缩产物时发生并导致排除某些带电荷的缓冲种类，例如，组氨酸。因此通常将渗滤缓冲液调整至比对于蛋白质制剂规定的更高的缓冲液浓度和更低的pH。一旦渗滤完成，产物就通过第二超滤步骤浓缩至高于最终蛋白质制剂所需蛋白质浓度的约50％。然后将产物从超滤系统中取出并漂洗系统以回收额外的产物。如果最终浓度比蛋白质制剂中的所需浓度高不止50％，那么可在不过度稀释产物的情况下将所有漂洗液(rinse)加回到产物中以使回收达到最大。然后以浓缩溶液的形式添加赋形剂(糖、表面活性剂、螯合剂等)，通常稀释比为约4，这意味着将1单位体积的浓缩赋形剂溶液添加到3个单位体积的产物中。稀释比4基于赋形剂溶液中糖组分的最大溶解度，其通常是限制因素。如有必要，那么然后将产物用制剂缓冲液(formulation buffer)进一步稀释以调整至最终所需浓度。

[0006] 因此，当期望在最终制剂中获得高度浓缩的蛋白质时，这种常规方法可能不适用，并且当蛋白质具有特别高的粘度时甚至更不适用。

[0007] 例如，在治疗用抗体制剂具有150 g/l的所需最终浓度的情况下，分子的粘度阻碍浓缩至比所需最终浓度高50％的目标值。

[0008] 本发明的目的是提供一种制备蛋白质制剂的方法，其可以应用于高粘性和高度浓

缩的蛋白质。

[0009] 本发明的另一个目的是该方法可以在不消极影响制造方法的总得率并且不由于某些赋形剂的过量浪费而招致额外成本的情况下在制造规模上实施。特别地，目的是将糖组分的使用保持在与常规方法类似的水平，所述糖组分是特别昂贵的。

[0010] 保持蛋白质在该方法的所有步骤中的稳定性并保护蛋白质免于聚集是更进一步的目的。

[0011] 概述

根据本发明的第一方面，提供了上述类型的方法，其进一步包括在第二超滤步骤之前，将第二赋形剂添加到由渗滤步骤获得的保留物中。

[0012] 通过将第二赋形剂，特别是海藻糖(或更一般的糖)的添加移动到渗滤后，剩余的赋形剂可以在后来的步骤中以更高的浓度添加，从而产生产物的更低稀释度。这本身又意味着与常规方法的约50％的值相比，可以使最大所需浓度仅仅为高于最终所需浓度的约10％(在一些情况下在5至15％之间)。即使在分子粘度更高的情况下，用标准超滤设备也可获得这个10％的值。这也允许从漂洗液回收产物，并从而允许获得超滤/渗滤方法的90％得率。

[0013] 同样，在最终浓缩前添加第二赋形剂(糖)保护蛋白质免于聚集。

[0014] 根据本发明的优选实施方案：

- 所述方法在步骤(e)之后且在步骤(f)之前进一步包括用漂洗缓冲液漂洗超滤设备，由此增强蛋白质的回收；

- 所述漂洗缓冲液包含浓度基本上分别等于蛋白质制剂中第一和第二赋形剂浓度的第一和第二赋形剂；

- 所述第一赋形剂是氨基酸，优选组氨酸；

- 所述蛋白质制剂中的第一赋形剂具有16-24 mM，优选17-23 mM，最优选约20 mM的浓度；

- 所述第二赋形剂是糖，优选二糖；

- 所述最终赋形剂包括表面活性剂，优选聚山梨醇酯80；

- 所述最终赋形剂包括螯合剂，优选EDTA；

- 所述蛋白质制剂具有110-165 g/l的蛋白质浓度；

- 所述蛋白质是抗体。

[0015] 在第一优选实施方案中：

- 所述抗体是抗-PCSK9(蛋白质原转变酶枯草杆菌蛋白酶Kexin 9型(Proprotein Convertase Subtilisin Kexin type 9))抗体；

- 所述抗-PCSK9抗体选自：博科昔单抗(bococizumab)、依伏库单抗(evolocumab)(REPATHA™)、阿利库单抗(alirocumab)(PRALUENT™)、REGN728、31H4、11F1、12H11、8A1、8A3、3C4、300N、1D05、LGT209、RG7652和LY3015014；

- 所述抗-PCSK9抗体包含重链可变区(VH)，所述重链可变区(VH)包含SEQ ID NO:1中所示氨基酸序列的互补性决定区一CDR1、CDR2和CDR3；和轻链可变区(VL)，所述轻链可变区(VL)包含SEQ ID NO:2中所示氨基酸序列的CDR1、CDR2和CDR3；或者，所述抗-PCSK9抗体包含具有SEQ ID NO:3、4或5中所示氨基酸序列的VH CDR1，具有SEQ ID NO:6或7中所示氨基

酸序列的VH CDR2,VH CDR3具有SEQ ID NO:8中所示氨基酸序列的VH CDR3,具有SEQ ID NO:9中所示氨基酸序列的VL CDR1,具有SEQ ID NO:10中所示氨基酸序列的VL CDR2和具有SEQ ID NO:11中所示氨基酸序列的VL CDR3;

- 所述蛋白质制剂具有135-165 g/l,优选142-158 g/l,最优选约150 g/l的蛋白质浓度;

- 所述蛋白质制剂中的第二赋形剂是浓度为67.2-100.8 g/l,优选71.4-96.6 g/l,最优选约84 g/l的海藻糖;

- 所述最终赋形剂包括聚山梨醇酯80,其在蛋白质制剂中的浓度为0.16-0.24 g/l,优选为0.17-0.23 g/l,最优选为约0.2 g/l;

- 所述最终赋形剂包括EDTA,其在蛋白质制剂中的浓度为0.04至0.06 g/l,优选0.0425至0.0575 g/l,最优选约0.05 g/l;

- 所述蛋白质制剂具有5.2-5.8,优选约5.5的pH;

- 所述步骤(a)中提供的溶液具有5-20 g/l的蛋白质浓度;

- 通过第一超滤步骤将所述蛋白质浓缩至80-120 g/l,优选至90-110 g/l,且最优选至约100 g/l;

- 通过第二超滤步骤将所述蛋白质浓缩至143-173 g/l,优选至150-166 g/l,且最优选至约158 g/l;

- 所述渗滤缓冲液中的第一赋形剂的浓度高于所述蛋白质制剂中第一赋形剂的浓度,所述渗滤缓冲液中第一赋形剂的浓度优选为29.75-40.25 mM,最优选约35 mM;

- 所述渗滤缓冲液具有5.1-5.5,优选约5.3的pH;

- 将所述第二赋形剂添加到从渗滤步骤获得的保留物中是通过将第一添加剂溶液添加到保留物中来实现的,所述第一添加剂溶液包含浓度为340-460 g/l,优选380-420 g/l,最优选约400 g/l的第二赋形剂;

- 所述第一添加剂溶液包含第一赋形剂,其浓度低于所述渗滤缓冲液中第一赋形剂的浓度并高于所述蛋白质制剂中第一赋形剂的浓度,所述第一添加剂溶液中第一赋形剂的浓度为优选25.5-34.5 mM,最优选约30 mM;

- 所述第一添加剂溶液还包含最终赋形剂;

- 所述第一添加剂溶液包含约30 mM组氨酸和约400 g/l海藻糖;

- 将所述第一添加剂溶液添加到保留物中是以约4.15的稀释比进行的,由此将一体积的所述第一添加剂溶液添加到约3.15倍相同体积的保留物中;

- 添加最终赋形剂包括将第二添加剂溶液添加到由第二超滤步骤获得的溶液中的步骤,所述第二添加剂溶液包含浓度低于所述第一添加剂溶液中第二赋形剂的浓度并且高于所述蛋白质制剂中第二赋形剂的浓度的第二赋形剂;

- 所述第二添加剂溶液包含浓度基本上等于所述蛋白质制剂中第一赋形剂浓度的第一赋形剂;

- 所述第二添加剂溶液包含约20 mM组氨酸,约84 g/l海藻糖,约1 g/l EDTA和约4 g/l聚山梨醇酯80;

- 添加第二添加剂溶液以约20的稀释比进行,由此将一体积的第二添加剂溶液添加到约19倍相同体积的从第二超滤步骤获得的溶液中。

[0016] 在第二优选实施方案中：

- 所述抗体是抗-IL7R抗体；

- 优选地，所述抗-IL-7R抗体包含重链可变区(VH)，所述重链可变区(VH)包含SEQ ID NO:13中所示氨基酸序列的互补性决定区一CDR1、CDR2和CDR3(这种CDRs的序列的实例分别为SEQ ID NO.17、18和19)；和轻链可变区(VL)，所述轻链可变区(VL)包含SEQ ID NO:14中所示氨基酸序列的CDR1、CDR2和CDR3(这种CDRs的序列的实例分别为SEQ ID Nos. 20、21和22)；

- 更优选地，所述抗-IL-7R抗体的VH区包含SEQ ID NO. 13中所示的氨基酸序列，且所述抗-IL-7R抗体的VL区包含SEQ ID NO. 14中所示的氨基酸序列；

- 甚至更优选地，所述抗-IL-7R抗体的重链包含SEQ ID NO. 15中所示的氨基酸序列，且所述抗-IL-17抗体的轻链具有SEQ ID NO. 16中所示的氨基酸序列；

- 所述蛋白质制剂具有110-130 g/l，优选约120 g/l的蛋白质浓度；

- 所述蛋白质制剂中的第二赋形剂是浓度为42-58 g/l，优选约50 g/l的蔗糖；

- 所述最终赋形剂包括聚山梨醇酯80，其在蛋白质制剂中的浓度为0.017-0.023 g/l，优选约0.02 g/l；

- 所述最终赋形剂包括EDTA，其在蛋白质制剂中的浓度为0.42-0.58 g/l，优选约0.5 g/l；

- 所述最终赋形剂包括精氨酸，其在蛋白质制剂中的浓度为85-115 mM，优选约100 mM；

- 所述蛋白质制剂具有6.5至7.5，优选约7.0的pH；

- 所述步骤(a)中提供的溶液具有2.6-3.4 g/l，优选约3 g/l的蛋白质浓度；

- 通过第一超滤步骤将所述蛋白质浓缩至36-54 g/l，优选至40-50 g/l，且最优选至约45 g/l；

- 通过第二超滤步骤将所述蛋白质浓缩至170-210 g/l，优选至约190 g/l；

- 所述渗滤缓冲液中的第一赋形剂的浓度高于所述蛋白质制剂中第一赋形剂的浓度，所述渗滤缓冲液中第一赋形剂的浓度优选为19-25 mM，最优选约22 mM；

- 所述渗滤缓冲液包含浓度为95-125 mM，优选约110 mM的精氨酸；

- 所述渗滤缓冲液具有6.5-7.5，优选约7.0的pH；

- 将所述第二赋形剂添加到从渗滤步骤获得的保留物中是通过将第一添加剂溶液添加到保留物中来实现的，所述第一添加剂溶液包含浓度为230-320 g/l，优选约275 g/l的第二赋形剂；

- 所述第一添加剂溶液包含第一赋形剂，其浓度基本上等于所述渗滤缓冲液中第一赋形剂的浓度并且高于所述蛋白质制剂中第一赋形剂的浓度，所述第一添加剂溶液中第一赋形剂的浓度为优选19-25 mM，最优选约22 mM；

- 所述第一添加剂溶液还包含最终赋形剂；

- 所述第一添加剂溶液在约7.0的pH包含约22 mM组氨酸，110 mM精氨酸和约275 g/l蔗糖；

- 将所述第一添加剂溶液添加到保留物中以约5的稀释比进行，由此将一体积的所述第一添加剂溶液添加到约4倍相同体积的保留物中；

- 添加最终赋形剂包括将第二添加剂溶液添加到从第二超滤步骤获得的溶液中的步骤,所述第二添加剂溶液包含EDTA和聚山梨醇酯80;

- 添加第二添加剂溶液以约20的稀释比进行,由此将一体积的所述第二添加剂溶液添加到约19倍相同体积的从第二超滤步骤获得的溶液中。

[0017] 根据本发明的第二方面,提供了由前述方法生产的抗体制剂。

[0018] 在优选的实施方案中,蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体,和

· 16 mM-24 mM,优选约20 mM的组氨酸缓冲液。

[0019] 在另一个优选实施方案中,蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体,和

· 67.2 mg/ml-100.8 mg/ml,优选约84 mg/ml的海藻糖。

[0020] 在另一个优选实施方案中,蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体,和

· 0.16 mg/ml-0.24 mg/ml,优选约0.2 mg/ml聚山梨醇酯。

[0021] 在另一个优选实施方案中,蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体,

· 16 mM-24 mM,优选约20 mM的组氨酸缓冲液,和

· 67.2 mg/ml-100.8 mg/ml,优选约84 mg/ml的海藻糖。

[0022] 在另一个优选实施方案中,蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体,

· 16 mM-24 mM,优选约20 mM组氨酸缓冲液,和

· 0.16 mg/ml-0.24 mg/ml,优选约0.2 mg/ml聚山梨醇酯。

[0023] 在另一个优选实施方案中,蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体

· 67.2 mg/ml-100.8 mg/ml,优选约84 mg/ml的海藻糖,和

· 0.16 mg/ml-0.24 mg/ml,优选约0.2 mg/ml聚山梨醇酯。

[0024] 在更优选的实施方案中,蛋白质制剂包含:

· 135 mg/ml-165 mg/ml,优选约150 mg/ml的抗-PCSK9抗体,

· 16 mM-24 mM,优选约20 mM的组氨酸缓冲液,

· 67.2 mg/ml-100.8 mg/ml,优选约84 mg/ml海藻糖,和

· 0.16 mg/ml-0.24 mg/ml,优选约0.2 mg/ml聚山梨醇酯。

[0025] 在一些实施方案中,抗体制剂具有5.2-5.8,优选约5.5的pH。

[0026] 在另一个优选实施方案中,蛋白质制剂包含:

· 110 g/l-130 g/l,优选约120 g/l的抗-IL-7R抗体;

· 17 mM-23 mM,优选约20 mM的组氨酸;

· 42 g/l-58 g/l,优选约50 g/l的蔗糖;和

· 0.017 g/l-0.023 g/l,优选约0.02 g/l聚山梨醇酯

并具有6.5-7.5,优选约7.0的pH。

[0027] 在下面的表中描述了在前面提到的SEQ ID NO:1至12:

1 (VH)	qvqlvqsgae vkkpgasvkv sckasgytft syymhwvrqa pgqglewmge ispfggrtny nekfksrvtm trdtststvy melsslrsed tavyycarer plyasdlwgq gttvtvss
2 (VL)	diqmtqspss lsasvgdrvt itcrasqgis salawyqqkp gkapklliys asyrytgvps rfsgsgsgtd ftftisslqp ediatyycqq ryslwrtfgq gtkleik
3 (VH – CDR1)	SYYMH
4 (VH – CDR1)	GYTFTSY
5 (VH-CDR1)	GYTFTSYYMH
6 (VH-CDR2)	EISPFGGRTNYNEKFKS
7 (VH-CDR2)	ISPFGGR
8 (VH-CDR3)	ERPLYASDL
9 (VL-CDR1)	RASQGISSALA
10 (VL-CDR2)	SASYRYT
11 (VL-CDR3)	QQRYSLWRT
12 (PCSK9 人)	MGTVSSRRSW WPLPLLLLLL LLLGPAGARA QEDEDGDYEE LVLALRSEED GLAEAPEHGT TATFHRCAKD PWRLPGTYVV VLKEETHLSQ SERTARRLQA QAARRGYLTK ILHVFHGLLP GFLVKMSGDL LELALKLPHV DYIEEDSSVF AQSIPWNLER ITPPRYRADE YQPPDGGSLV EVYLLDTSIQ SDHREIEGRV MVTDFENVPE EDGTRFHRQA SKCDSHGTHL AGVVSGRDAG VAKGASMRSL RVLNCQGKGT VSGTLIGLEF IRKSQLVQPV GPLVVLLPLA GGYSRVLNAA CQRLARAGVV LVTAAGNFRD DACLYSPASA PEVITVGATN AQDQPVTLGT LGTNFGRCVD LFAPGEDIIG ASSDCSTCFV SQSGTSQAAA HVAGIAAMML SAEPELTLAE LRQRLIHFSAKDVINEAWFP EDQRVLTPNL VAALPPSTHG AGWQLFCRTV WSAHSGPTRM ATAVARCAPD EELLSCSSFS RSGKRRGERM EAQGGKLVCR AHNAFGGEGV YAIARCCLLP QANCSVHTAPPAEASMGTRV HCHQQGHVLT GCSSHWEVED LGTHKPPVLR PRGQPNQCVG HREASIHASC CHAPGLECKV KEHGIPAPQE QVTVACEEGW TLTGCSALPG TSHVLGAYAV DNTCVVRSRDVSTTGSTSEG AVTAVAICCR SRHLAQASQE LQ

前面的SEQ ID No.13-16在下表中描述

13 (VH)	EVQLVESGGGLVKPGGSLRLSCAASGFTFDDSVMHWVRQAPGKGLEWVSLV GWDGFFTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQGDYM GNNWGQGTLVTVSS
14 (VL)	NFML TQPHSVSESPGKTVTISCTRSSGSIDSSYVQWYQQRPGSSPTTVI YEDDQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDFHH LVFGGGTKLTVL
15	EVQLVESGGGLVKPGGSLRLSCAASGFTFDDSVMHWVRQAPGKGLEW VSLVGWDGFFTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC ARQGDYMGNNWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FL YSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
16	NFML TQPHSVSESPGKTVTISCTRSSGSIDSSYVQWYQQRPGSSPTTVI YEDDQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDFHH LVFGGGTKLTVLQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVT VAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPTECS
17 (VH-CDR1)	DSVMH
18 (VH-CDR2)	LVGWDGFFTYYADSVKG
19 (VH-CDR3)	QGDYMGNN
20 (VL-CDR1)	TRSSGSIDSSYVQ
21 (VL-CDR2)	EDDQRPS
22 (VL-CDR3)	QSYDFHHLV

[0028] 详述

以下定义将用于本说明书和权利要求书中：

- 术语“蛋白质制剂”指最终产物，其包含所考虑的蛋白质和赋形剂。当提及准备用作治疗用途的蛋白质时，可以使用术语“药物物质”代替“蛋白质制剂”，并且所考虑的蛋白质可以由术语“活性成分”或“产物”表示。“赋形剂”由“蛋白质制剂”的所有组分定义，它们不是“蛋白质”或“活性成分”。赋形剂一般包括蛋白质稳定剂、表面活性剂、例如有助于蛋白质稳定化的氨基酸等……；

- 关于渗滤步骤，术语“保留物”是指保留在膜的保留物侧并含有太大而不能穿过膜的分子如所考虑的蛋白质的溶液。保留物是转移到超滤/渗滤系统的后来部分的溶液。另一种在系统的渗滤部分中的膜的另一侧(透过物(permeate)侧)循环的溶液称为“渗滤缓冲液”(或“基础缓冲液”)；

- 术语“浓缩池”表示从最终的超滤步骤直接获得的溶液；

- 术语“最终赋形剂”表示在最终超滤步骤之后，即在最终浓缩步骤之后添加到“浓缩池”中的赋形剂；

- n为数值的术语“nx掺料”，表示以等于n的稀释比添加到一定体积的含蛋白质溶液中的赋形剂溶液，其意味着一体积的赋形剂溶液添加到n-1倍相同体积的含蛋白质溶液中。例如，4x掺料是根据以下比例添加的溶液：对于3体积含蛋白质溶液，1体积掺料；

- 除非另有说明，与数值有关的术语“大约”、“约”或“基本”意味着在所述值的±5％的范围内；

- 如本文所用的，“粘度”可以是“绝对粘度”或“运动粘度”。“绝对粘度”，有时称为动态或简单粘度，是描述流体对流动阻力的量。“运动粘度”是绝对粘度和流体密度的商。当使用毛细管粘度计来表征流体的有阻力流动时，常常报告运动粘度。当等体积的两种流体放置在相同的毛细管粘度计中并且允许其通过重力流动时，粘性流体流过毛细管花费的时间比较低粘性的流体长。如果一种流体需要200秒来完成其流动，而另一种流体需要400秒，那么第二种流体的粘性就运动粘度规模来说是第一种的2倍。如果两种流体具有相同的密度，那么第二种流体的粘性就绝对粘度规模来说是第一种的2倍。运动粘度的量纲是L^2/T，其中L代表长度，且T代表时间。运动粘度的SI单位是m^2/s。通常，运动粘度以厘沲，cSt表示，其等于 mm^2/s。绝对粘度的量纲是M/L/T，其中M代表质量，且L和T分别代表长度和时间。绝对粘度的SI单位是Pa·s，其等于kg/m/s。绝对粘度通常以单位厘泊，cP表示，其等于毫帕-秒，mPa·s。在本发明的背景下，如果抗体的粘度至少为20 cP，那么认为所述抗体具有高粘度。

[0029] 为了简明起见，可以遍及说明书使用首字母缩略词“UF”、“DF”和“UF/DF”(或“UFDF”)，并且其应理解如下：“UF”意指“超滤”，“DF”意指“渗滤”且“UF/DF”(或“UFDF”)意指“超滤/渗滤”。被定义为制备蛋白质制剂的方法的本发明的方法可以被称为UFDF方法。

[0030] 现在将通过以下实施例进一步说明本发明，每个实施例都与特定的治疗用单克隆抗体和该单克隆抗体的特定制剂相关。这些实施例仅仅是为了说明起见而提供的，且不应该被解释为限制本发明的范围。

[0031] A-实施例1

在说明性实施例1中，所考虑的蛋白质是博科昔单抗，一种靶向PCSK9的单克隆抗体，其特异性结合PCSK9(蛋白质原转变酶枯草杆菌蛋白酶Kexin 9型)，例如SEQ ID NO:12或Uniprot登录号Q8NBP7。该方法已被设计为在药物物质中实现150 g/l的目标产物浓度，其中药物物质在5.5的pH包含以下赋形剂：

- 浓度为20 mM的组氨酸，
- 浓度为84 g/l的海藻糖，和
- 浓度为0.2 g/l的PS80(聚山梨醇酯80)。

[0032] 在蛋白质浓度中±8 g/l、赋形剂浓度中±15％和pH值中±0.2的容差的情况下实现上述要求被认为是可以接受的。

[0033] 就得率而论，需要该方法达到超过90％的产物回收。

[0034] 已经进行了实验以确定优选的操作模式并确定本发明的方法适合实现上述要求(而常规方法不适合)。这些实验中的一些在说明书的下面部分中给出。

[0035] *A.1 材料*

用于实验的原材料是完全纯化的博科昔单抗溶液，其在使用前已通过MabSelect®柱处理以去除赋形剂组分。MabSelect®纯化后，用乙酸将洗出物调整至pH 5.0，从而导致17.09 g/l的产物浓度。

[0036] *超滤/渗滤设备*

所有实验均使用装配Pellicon® 3(30KDa，C-滤网，88 cm^2)再生纤维素膜或Sartocon®(30KDa E-通道200 cm^2)再生纤维素膜的GE Crossflow®系统(300 ml储器)进行。跨膜压力(TransMembrane Pressure)(TMP)保持在大约14-22 psi，$P_{进料}$小于55 psi。除非另有说明，否则所有漂洗液都是通过将漂洗缓冲液再循环至少15分钟，然后浓缩至系统的最小工

作体积产生的。

[0037] *A.2 实验设计和结果*

海藻糖溶解度的测定

完成了初始实验以评价30 mM组氨酸pH 5.35溶液中海藻糖溶解度的范围(从最终规格调整组氨酸浓度和pH以计及随着蛋白质浓度增加组氨酸离子的排除)。为了获得150 g/l的最终药物物质靶,浓缩池的最小浓度将需要为具有6x海藻糖/EDTA/PS80掺料的180 g/l或具有5x海藻糖/EDTA/PS80掺料的187.5 g/l。在提供6x掺料所需的海藻糖浓度(～500 g/l),在延长的搅拌后,海藻糖在室温(22℃)不溶解(颗粒仍然存在),且必须加热至30℃以溶解。在没有在室温再沉淀的情况下将溶液在15 psi压力下通过0.22 μm Pall Acrodisc®针筒式过滤器过滤。

[0038] 然而,这种制造方法可能难以按比例扩大,因此海藻糖掺料最大实际浓度可能被限制在5x(～420 g/l海藻糖)。

[0039] 因此,在优选的方法中,掺料溶液的海藻糖浓度可以是约400 g/l。

[0040] *工艺开发*

设计第一个实验以测试渗滤溶液中所需的组氨酸浓度,以检查在不同蛋白质浓度(76.6 g/l和114 g/l)在渗滤的溶液中的组氨酸浓度,且以产生用于密度测量的材料。使用载荷量为345 g/m^2的200 cm^2 Sartocon® E-通道膜将原材料浓缩至76.6 g/l,然后用35 mM组氨酸,pH 5.26缓冲液进行渗滤。在300 LMH进料流率和22 psi TMP,渗滤通量为17 LMH(升/平方米/小时)。然后将材料进一步浓缩至213 g/l(数据未显示),并分析渗滤的池和最终浓缩的材料两者的样品的组氨酸和海藻糖浓度(参见表1)。

[0041] 进行第二个实验以确定与在渗滤缓冲液中没有海藻糖的材料相比,含有海藻糖的渗滤的材料是否导致更低的最终浓度。将原材料浓缩至114 g/L并用35 mM组氨酸,pH 5.26缓冲液渗滤。在表2,实验2A中描述的操作条件下,渗滤通量为10 LMH。在<55 psi的进料压力和22 psi的TMP将渗滤的溶液浓缩至184.9 g/L。将浓缩的材料从储器中排出并与35 mM组氨酸,pH 5.26漂洗溶液合并以达到153.7 g/L的浓度。所述池用4x海藻糖赋形剂缓冲液(30 mM组氨酸,400 g/L海藻糖,pH 5.4)掺料以达到114 g/L的最终蛋白质浓度。然后在表2,实验2B中描述的操作条件下将掺料的溶液浓缩至202.4 g/L。由于泵的限制,浓缩步骤在15 LMH进料流率停止。

[0042] 表1显示所有浓缩的样品中的组氨酸和海藻糖浓度两者均在最终目标规格,20 mM组氨酸和84 g/L海藻糖的10%以内。

[0043] 该信息提供了75-114 g/L的渗滤浓度的可接受的操作范围,其中最终赋形剂浓度满足浓度规格。

[0044] 表1. 初始评价赋形剂浓度结果

样品名称	浓度 (g/L)	组氨酸 (mM)	海藻糖 (g/L)
渗滤实验 1	76.6	29.81	未测试
浓缩实验 1	213	19.63	
加样实验 2B	114	26.19	83.82
浓缩实验 2B	202.4	18.67	76.37
渗滤缓冲液	N/A	34.34	未测试

[0045] 表2．初始评价工艺数据

UFDF 系统和加样负荷	706263-18-Exp2A	706263-18-Exp2B
膜类型	Sartocon	E-通道
膜面积, m2	0.02	0.02
UF 蛋白质挑战（protein challenge） (g/m^2)	572	490
浓缩 1		
加样体积 (ml)	338.2	NA
加样浓度 (g/L)	33.8	
浓缩 1 (g/L)	114	
浓缩 1 处理时间 (hr)	未记录	
渗滤		
渗滤缓冲液	35 mM 组氨酸 pH 5.26	NA
置换体积（Diavolumes）(TOV)	8	
进料流率 (LMH)	300	
平均透过物通量 (LMH)	10	
平均 TMP (psi)	22	
渗滤处理时间 (hr)	4	
浓缩 2		
掺料溶液	NA	30 mM 组氨酸, 400 g/L 海藻糖 pH 5.4
掺料后浓度 (g/L)	NA	114
平均 TMP (psi)	22	22
最终流率 (LMH)	未记录	15
产物体积 (ml)	48.1	未记录
产物浓度 (g/L)	184.9	202.4
浓缩 2 处理时间 (hr)*	未记录	~1
保留物 pH	5.50	5.51
漂洗		
漂洗缓冲液	35 mM 组氨酸 pH 5.26	20 mM 组氨酸, 84 g/L 海藻糖 pH 5.5
漂洗体积 (ml)	26	未记录
漂洗浓度 (g/L)	58.7	54.5
漂洗 pH	未记录	5.52

*实际时间未记录或不能找回，它基于对处理的通量和体积的计算。

[0046] 进行另外的实验以评价浓缩2步骤结束时组氨酸和海藻糖浓度随蛋白质浓度的变化。将原材料浓缩至105.9 g/l并使用200 cm^2 Sartocon® E-通道膜用35 mM组氨酸，pH 5.29缓冲液进行渗滤。在300 LMH的进料流率和22 psi TMP，渗滤通量为12 LMH。渗滤的材料然后用4×海藻糖赋形剂溶液掺料。将掺料的溶液直接添加到储器中并混合15分钟，然后将材料浓缩至172、188和209 g/l最终浓度(参见表3)。如表4所示，随着蛋白质浓度的增加，组氨酸浓度下降，但所有值都在20 mM组氨酸，84 g/L海藻糖的目标浓度的10％以内。

[0047] 表3. 额外的开发工艺数据

UFDF 系统和加样负荷	笔记簿: 706263-20
膜类型	Sartocon Slice E-通道
膜面积, m2	0.02
UF 蛋白质挑战(g/m^2)	477
浓缩 1	
加样体积(ml)	558.1
加样浓度(g/L)	17.09
最终浓度(g/L)	105.9
渗滤	
渗滤缓冲液	35 mM 组氨酸 pH 5.29
置换体积 (TOV)	8
进料流率(LMH)	300
平均 TMP (psi)	22
平均透过物通量(LMH)	12
渗滤时间(hr)	3
浓缩 2	
掺料溶液	30 mM 组氨酸, 400 g/L 海藻糖 pH 5.22
掺料后浓度(g/L)	78.6
溶液 pH(在掺料时)	5.38
平均 TMP (psi)	22
处理时间(hr)*	~1
产物体积(ml)	32.1
产物浓度(g/L)	209
保留物 pH	5.53
得率回收(%)	76.6
漂洗	
漂洗缓冲液	20 mM 组氨酸, 84 g/L 海藻糖 pH 5.5
漂洗体积(ml)	31.6
漂洗浓度(g/L)	53.2
漂洗 pH	5.48
漂洗回收(%)	19.2

*实际时间未记录或不能找回，它基于对处理的通量和体积的计算。

[0048] 表4. 额外的开发赋形剂浓度结果

样品名称	浓度 (g/L)	组氨酸 (mM)	海藻糖 (g/L)
渗滤池	109.4	27.81	未测试

浓缩加样	78.6	27.57	94.02
浓缩1	172	20.15	84.65
浓缩2	188	19.47	84.24
浓缩3	209	18.11	81.86
掺料缓冲液	N/A	27.83	392.88

[0049] 将该方法按比例扩大至500 L中试规模(批号12P126J603-MV-B):工艺细节见表5。使用0.5 m^2 Millipore® V-滤网膜将517 g Capto Adhere®纯化的材料浓缩至107 g/l,然后用35 mM组氨酸,pH 5.29缓冲液、1000 LMH的进料流率和40 psi的进料压力渗滤。保留物然后用4x海藻糖溶液(30 mM组氨酸,400 g/l海藻糖,pH 5.22)掺料,其考虑到系统滞留体积直接添加到储器中。然后将掺料的材料浓缩至202 g/l,并将浓缩的产物从系统取出。用20 mM组氨酸,84 g/l海藻糖,pH 5.50缓冲液漂洗导轨(skid),并将漂洗液添加到浓缩的材料中。最终合并的溶液的测量的浓度为160 g/l,总得率为97.1%。

[0050] 表6总结了实验的赋形剂浓度和产物质量结果,其显示最终合并的池水平在上述目标浓度的10%以内,当与过去的最终UF值比较时对如通过SEC测量的产物质量没有任何显著影响。

[0051] 表5. 中试规模工艺数据

UFDF 系统和加样负荷	12P120J603-MV-B
设备	Millipore 系统
膜类型	Millipore 30K V 滤网 RC
膜面积, m2	0.5
UF 蛋白质挑战(g/m^2)	1142
浓缩 1	
加样体积(L)	7.5
加样浓度(g/L)	67.85
处理时间(分钟)	18
最终浓度(g/L)	107
渗滤	
渗滤缓冲液	35 mM 组氨酸 pH 5.3
置换体积 (TOV)	8
进料流率(LMH)	~1000
平均通量 (LMH)	20
渗滤时间(小时)	3.75
浓缩 2	
掺料溶液	30 mM 组氨酸, 400 g/L 海藻糖 pH 5.22
平均 TMP (psi)	<28
最终流率(LMH)	108
处理体积(L)	2.2
处理时间(hr)	1
产物浓度(g/L)	202
产物 pH	5.44
得率回收(%)	85.7
漂洗	
漂洗缓冲液	20 mM 组氨酸, 84 g/L 海藻糖 pH 5.5
漂洗体积(L)	1.1
漂洗浓度(g/L)	53.7
漂洗 pH	5.55
漂洗回收(%)	11.4

[0052] 表6．中试规模赋形剂和产物质量结果

样品名称	浓度 (mg/ml)	组氨酸 (mM)	海藻糖 (g/L)	总 HMMS	单体	总 LMMS
渗滤的池	107	28.28	N/A	1.0	99.0	<0.1%
掺料后池	78.6	28.04	90.35	0.8	99.2	<0.1%
浓缩 2 池	202	21.57	88.21	0.9	99.1	<0.1%
漂洗池	53.7	18.94	80.47	0.7	99.2	<0.1%
最终池	160	21.28	88.25	1.2	98.8	<0.1%
DF 缓冲液	N/A	34.80	N/A	N/A		
赋形剂缓冲液		31.52	413.75			
漂洗缓冲液		19.93	83.26			

[0053] *渗滤工艺的评价*

不同浓度的蛋白质密度和粘度

图1绘制了(i)20 mM组氨酸,pH 5.5和(ii)20 mM组氨酸,84 g/l海藻糖,pH 5.5中的博科昔单抗的粘度与产物浓度的关系曲线。该图显示,在约175 g/l时,粘度达到30 cP值,这被认为是大规模可行UFDF处理的截止值。

[0054] 测量(i)20 mM组氨酸,pH 5.5和(ii)20 mM组氨酸,84 g/l海藻糖,pH 5.5溶液中的博科昔单抗的密度并示于图2和图3中。数据显示与组氨酸/海藻糖缓冲液相比,组氨酸缓冲液中的密度稍低,这是如所预期的。

[0055] 基于以上实验,发现药物物质中的目标浓度可以通过用含有组氨酸而不含海藻糖的DF缓冲液在制造规模实现。

[0056] 来自实验的结果不仅显示本发明的方法导致可接受的得率、蛋白质和赋形剂终浓度,而且其要求与常规方法相比在赋形剂掺料前较低的蛋白质浓度(158 g/L对188 g/L)。在工艺按比例扩大时,这种较低的蛋白质浓度更容易定期实现。此外,由于通过从渗滤缓冲液中去除海藻糖获得的更好的货物成本分布(cost-of-goods profile),本发明的方法优于常规方法。

[0057] 已经发现,利用Millipore® C-滤网膜作为Millipore® V-滤网膜的替换物的UFDF工艺始终如一地导致大于175 g/l的浓度,其足以允许添加洗涤池(漂洗),同时仍然保持在20x赋形剂掺料之前所需的超过158 g/l。为了确保该工艺在海藻糖掺料的情况下可行得通,在实验室和中试规模都评价了利用C-滤网膜的工艺。

[0058] 利用表7中概述的超滤(UF)运行条件,使用Millipore® PLCTK C-滤网盒(cassette)在实验室规模评价该工艺。

[0059] 对于TFF(切向流过滤)设备,实验室规模工艺采用30-300 LMH的进料流率范围在作为操作限制的可达到的约50-55 psi压力极限进行。300 LMH的上限进料流率(在该情况下大部分工艺将发生)对处理时间具有主要影响,其中降低的进料流导致较低处理通量,这增加了处理泵时间。由于增加的粘度增加了通过保留物通道的压降,所以30 LMH的下限进料流率对于可达到的最终浓度是关键的,因此较低的流率使得能够泵送更粘的溶液。

[0060] 在于约84 g/l海藻糖的存在下运行的实验室规模工艺期间,在最终保留物池中以30 LMH的进料流率和50 psi的进料压力实现了177 g/l的最终浓度。如表8所示,分别测量来自实验室规模运行的洗涤级分的得率。

[0061] 表7. 实验室规模运行条件

步骤	溶液	进料压力 (psig)	保留物压力 (psig)	目标
平衡	10 mM组氨酸, 50 mM NaCl, pH 6.4	20 (± 2)	10 (± 2)	± 0.2 pH单位
浓缩1	VRF产物池	34 (± 6)	16 (± 6)	600-1000 g/m^2
渗滤	35 mM组氨酸 pH 5.3	34 (± 6)	16 (± 6)	> 7 TOV
浓缩2	渗滤池	35 (± 20)	10 (± 10)	超过DS目标20-30%
缓冲液冲洗	20 mM组氨酸, 84 g/L海藻糖, pH 5.5	30 (± 20)	10 (± 10)	浓度依赖性的

[0062] 表8. 实验室规模开发实验的结果

实验	加样挑战 (g/m^2)	浓缩 1 通量(LMH) & 压力($P_{进料}/P_{ret}$)	渗滤通量(LMH) & 压力($P_{进料}/P_{ret}$)	浓缩 2 通量 (LMH) & 压力($P_{进料}/P_{ret}$)	% 得率 Ret /冲洗
无海藻糖	354*	90-20 (35/15)	25-42 (35/15)	35-4 (50/0)	85 /13
有海藻糖	354*	20-90 (35/15)	25-42 (35/15)	45-4 (50/0)	85 /13

*由于材料限制和工艺循环时间,在实验室规模上采用下限加样挑战,并且表示最差情况的得率回收选择。

[0063] 在图4中可以看到实验室规模的工艺通量分布图,其中垂直线表示降低进料流率以保持具有开放保留物(零psi)的低于～50 psi进料压力的起点,其中处理通量中的减少由于减少交叉流率而发生。图5显示了最终浓缩过程中的工艺进料通道压降和进料流率。在实验室规模系统中,当进料压力接近～50 psi时,通过降低进料泵速率手工调节流量。

[0064] *中试规模确认批次*

使用C-滤网膜的UFDF工艺在500 L规模进行以确认可以实现最终浓度目标。该工艺示于表9中,并且在中试工厂中进行的3批的工艺数据示于表10中。

[0065] 结果表明,该工艺实现了高回收,并且浓度满足中间和最终目标。此外,批次13P120J604的赋形剂浓度在21.2 mM组氨酸,85.4 g/l海藻糖和0.051 g/l EDTA测量,其在±15%的目标规格内。

[0066] 表9. 用于中试规模制造的UFDF工艺参数

参数	细节	类型
膜	Millipore PLCTK (30 kDa 纤维素) C-滤网	
膜表面积	500–1350 g/m^2	控制限
操作温度	18-25 °C	控制限
平衡缓冲液	10 mM 组氨酸, 50 mM NaCl, pH 6.4	
预过滤器（Pre-filter）	对于 0.2 um 过滤器≤3000 L/m^2	目标
渗滤缓冲液	35 mM 组氨酸 pH 5.3	
稀释缓冲液	30 mM 组氨酸, 400 g/L 海藻糖, pH 5.4	
最终 UF 冲洗缓冲液	20 mM 组氨酸, 84 g/L 海藻糖, pH 5.5	
过滤器保养/平衡	≥ 10 L/m^2	目标范围
最大入口 & 保留物压力	≤ 80 psig	控制限
浓缩 1 入口压力	设定值 35 psig, 目标范围 22-55 psig	目标范围
浓缩 1 保留物压力	设定值 15 psig, 目标范围 0-40 psig	目标范围
目标交叉流率	0–10 L/分钟/m^2	目标范围
透过物通量	0–50 LMH	预期的
渗滤入口压力	设定值 40 psig, 目标范围 22-55 psig	目标范围
渗滤保留物压力	设定值 15 psig, 目标范围 0-40 psig	目标范围
渗滤浓度	基于 350-450 L 的罐体积 70-90 g/L	目标
	70–110 g/L	目标范围
渗滤体积	最小 8 TOV's	目标范围
透过物 pH 和电导率（渗滤结束）	8 TOVs 后核实 pH 为 5.5 ± 0.20 单位。继续渗滤直到满足目标。	控制限
4X 海藻糖缓冲液添加	以 1:3 比在 DF 后添加 30 mM 组氨酸, 400 g/L 海藻糖 pH 5.4	控制限
浓缩 2 入口压力	设定值 22-55 psig, 目标范围 20-60 psig	目标范围
浓缩 2 保留物压力	设定值 0 psig, 目标范围 0-40 psig	目标范围
保留物浓度	170-190 g/L (依照罐中的材料均衡)	目标
	> 158 g/L (实际保留物浓度)	控制限
缓冲液冲洗	最小体积再循环的漂洗	
UF 池浓缩目标	在 20X EDTA/PS80 掺料前 158 ± 10 g/L	控制限
UF 池密度	密度计算：0.0004 * 浓度 + 1.0126	
后过滤器（Post-filter）	对于 0.2 um 过滤器≤450 L/m^2	目标
处理备忘录	基于在 350 – 450 L 保留物罐体积中 32-42 kg 的渗滤浓度 在渗滤后直接添加 4X 海藻糖缓冲液到罐中。 以总体积（罐中的体积+系统滞留体积）的 1:3 比添加。 循环 10 分钟并浓缩至>158 mg/ml。	

[0067] 表10．3个中试规模批次的UFDF工艺数据

批次#	单位	13P120J604	13P120J605	13P120J606
UF过滤器类型		30kD Millipore C-滤网	30kD Millipore C-滤网	30kD Millipore C-滤网
UF总面积	m2	1,14	1,14	1,14
加样体积	L	68.9L / 30.56L	81.53L / 26.57L	85.2L / 32.0L
加样浓度	g/L	11.03 g/L / 10.9 g/L	11.04 g/L / 11.22 g/L	8.79 g/L / 11.51 g/L
UF 蛋白质挑战	g/m2	959	950	982
UF 容量挑战	l/m2	87,2	95	103
浓缩1开始时间	AM/PM	08:21	08:21	09:08
浓缩1结束时间	AM/PM	11:38	12:07	15:02

浓缩1透过物体积	L	86,66	97,66	107,16
浓缩1 进料压力	psig	35	35	35
浓缩1 保留物压力	psig	15	15	15
浓缩1透过物压力	psig	0	0	0
浓缩1终点保留物体积	L	12,8	10,4	10,2
浓缩1平均通量	L/m2/h	23,1	22,7	15,9
浓缩1 平均TMP	psig	25	25	25
渗滤开始时间	AM/PM	11:49	12:14	10:05
渗滤结束时间	AM/PM	14:45	16:22	13:39
渗滤透过物体积	L	109	82,76	103
置换体积		8,0	8,0	8,0
渗滤进料压力	psig	40	40	40
渗滤保留物压力	psig	15	15	15
渗滤透过物压力	psig	0	0	0
渗滤平均通量	L/m2/h	32,6	18,8	25,3
渗滤平均 TMP	psig	27,5	27,5	27,5
浓缩2开始时间	AM/PM	15:15	16:45	14:02
浓缩2结束时间	AM/PM	15:55	17:27	14:45
浓缩2进料压力	psig	25 - 55	25 - 55	25 - 55
浓缩2保留物压力	psig	0 - 2	0 - 2	0 - 2
浓缩2透过物压力	psig	0	0	0
浓缩2透过物体积	L	7,1	7,9	6,8
浓缩2平均通量	L/m2/h	9,3	9,9	8,3
浓缩2 平均TMP (psig)		20	20	20
产物池体积	L	6,433	6,337	7,175
产物池浓度	g/L	158,6	157,5	156,6
回收	%	95	92	99
洗涤池体积	L	833	915	790
洗涤池浓度	g/L	62,88	63,20	54,28
洗涤池克数	g	52	58	43
洗涤池比例	g/m2	5,14%	5,79%	3,82%

[0068] *A.3 结论*

上述实验能够在达到药物物质目标的同时显示92-99％的步骤得率。

[0069] 该实施例证实，根据本发明的UFDF方法适合于制备具有在可接受范围内的pH和所有赋形剂浓度的高度浓缩的(150 g/l)博科昔单抗药物物质。可以用其他蛋白质在相同益处的情况下实现上述，尤其是具有特别高粘度的蛋白质。

[0070] B-实施例2

在说明性实施例2中，所考虑的蛋白质是抗体C1GM，特异性结合IL-7R的IL-7R拮抗剂单克隆抗体。该方法已被设计成在药物物质中达到120 g/l的目标产物浓度，其中药物物质在7.0的pH包含以下赋形剂：

- 浓度为20 mM的组氨酸，
- 浓度为100 mM的精氨酸，
- 浓度为50 g/l的蔗糖，
- 浓度为0.02 g/l的PS80(聚山梨醇酯80)，和
- 浓度为0.5 g/l的EDTA。

[0071] 在蛋白质浓度中±10 g/l、赋形剂浓度中±15％和pH值中±0.5的容差的情况下实现上述要求被认为是可以接受的。

[0072] 就得率而论，需要该方法达到超过85％的产物回收。

[0073] 用于下述实验的原材料是已通过MabSelect®和Q膜层析处理的完全纯化的溶液。

[0074] *超滤/渗滤设备*

所有实验均使用装配Pellicon 3®(30KDa，C-滤网，88 cm^2)再生纤维素膜的GE Crossflow系统(300 mL储器)或Quattroflow™泵系统进行。跨膜压力(TransMembrane Pressure)(TMP)保持在大约14-22 psi，$P_{进料}$<55 psi。除非另有说明，否则所有漂洗液都是通过将漂洗缓冲液再循环>15分钟，然后浓缩至系统的最小工作体积产生的。

[0075] *分析测定*

使用Thermo Scientific Nanodrop 2000C™或来自C Technologies Inc.的Solo VPE™进行蛋白质浓度的紫外-可见光分光光度测定法。如通过ARD实验测定的在280 nm的消光系数为1.51 $mL*mg^{-1}*cm^{-1}$。

[0076] *实验*

实验1

原材料用5％的2M NaCl掺料并用2M Tris碱调整至pH 7.0，浓缩至50 g/l，用22 mM组氨酸，110 mM精氨酸pH 7.0渗滤，用5X蔗糖缓冲液(22 mM组氨酸，110 mM精氨酸，275 g/L蔗糖pH 7.0)掺料，并在～34 LMH的进料流率浓缩至146.9 g/L，如表11中详述的。浓缩的溶液的pH为7.00。UF系统用渗滤溶液以单程模式(无再循环)冲洗，从而导致33 g/L的浓度。总得率约为88％。

[0077] 表11-用精氨酸缓冲液pH 7.0进行的渗滤和浓缩。

笔记簿	706263-77
泵	Quattro Flow
UF 蛋白质挑战(g/m^2)	348
浓缩 1	
加样体积(L)	1.36
加样浓度(g/L)	2.25
进料流率(LMH)	变化的
进料压力(psi)	<50
浓缩 1 时间(分钟)	320
浓缩 1 浓度(g/L)	49.8
渗滤	
渗滤缓冲液	22 mM 组氨酸, 110 mM 精氨酸, pH 7.0
置换体积 (TOV)	8
进料流率@ DF (LMH)	300
进料压力@ DF (psi)	40
TMP @ DF (psi)	20
透过物流率@ DF (LMH)	11.5-16.5
渗滤时间(分钟)	220
浓缩 2	
掺料溶液	22 mM 组氨酸, 110 mM 精氨酸, 275 g/L 蔗糖 pH 7.0
TMP @ 浓缩 2 (psi)	<25
最大进料压力(psi)	50
结束时的流率(LMH)	34
浓缩 2 时间(分钟)	未记录
最终浓度(g/L)	146.9

[0078] 表12-14显示赋形剂、CGE和SEC测定结果。最终浓缩的材料中的组氨酸、精氨酸和蔗糖浓度都在所需值的±10％以内。在UF工艺过程中没有形成新的聚集，也没有任何碎片水平的变化。

[0079] 表12-精氨酸缓冲液pH 7.0的工艺赋形剂浓度。

样品名称	蛋白质浓度(g/L)	精氨酸(mM)	组氨酸(mM)	蔗糖(g/L)
渗滤液	50.1	113.7	22.6	N/A
掺料后	38.2	110.0	21.9	55.0
最终浓缩物	146.9	108.5	19.8	50.0

系统漂洗液	33	111.1	22.1	49.9
渗滤缓冲液	N/A	116.1	23.6	N/A
5X蔗糖缓冲液	N/A	87.6	15.8	277.6

[0080] 表13-精氨酸缓冲液pH 7.0实验的nrCGE和rCGE结果

样品	nrCGE			rCGE		
	% IgG	%碎片	%其他	% HC + LC	%碎片	%其他
抗-IL-7R 参考	97.5	2.5	0	98.7	0.5	0.8
渗滤液	96.2	3.8	0	98.8	0.5	0.7
掺料后	96.2	3.8	0	99.1	0.3	0.5
最终浓缩物	96.3	3.7	0	98.9	0.5	0.6
系统漂洗液	96.8	3.2	0	99.1	0.3	0.6

[0081] 表14-精氨酸缓冲液pH 7.0实验的SEC结果

样品名称	% HMMS	% LMMS	% 单体
渗滤液	0.6	0.2	99.2
掺料后	0.6	0.2	99.2
最终浓缩物	0.6	0.2	99.2
系统漂洗液	0.6	0.2	99.2

[0082] *实验2*

重复实验，如表15中所详述的，唯一的区别是计算掺料体积的方式。掺料之前UF系统的总体积是根据依在储器中添加体积加系统滞留体积为转移的总材料均衡(总加样除以渗滤液浓度)计算的。在5X蔗糖掺料后，蛋白质在～34 LMH进料流率和$P_{进料}$<50 psi浓缩至183.6 g/L。

[0083] 表15-用pH 7.0的精氨酸缓冲液进行的渗滤和浓缩。

笔记簿	706263-78
泵	Quattro Flow
UF 蛋白质挑战(g/m^2)	387
浓缩 1	
加样体积(L)	1.5
加样浓度(g/L)	2.27
进料流率(LMH)	变化的
进料压力(psi)	<50
浓缩 1 时间(分钟)	400
浓缩 1 浓度(g/L)	43
渗滤	
渗滤缓冲液	22 mM 组氨酸, 110 mM 精氨酸, pH 7.0
置换体积 (TOV)	10
进料流率@ DF (LMH)	300
进料压力@ DF (psi)	28
TMP @ DF (psi)	20
透过物流率@ DF (LMH)	13.4-19.1
渗滤时间(分钟)	300
浓缩 2	
掺料溶液	22 mM 组氨酸, 110 mM 精氨酸, 275 g/L 蔗糖 pH 7.0
TMP @ 浓缩 2 (psi)	<25
最大进料压力(psi)	50
结束时的流率(LMH)	34
浓缩 2 时间(分钟)	未记录
最终浓度(g/L)	183.6

[0084] 表16显示最终材料中赋形剂浓度、组氨酸、精氨酸和蔗糖浓度在所需目标值的±10％内。在如何计算掺料体积中的区别看来对最终赋形剂浓度没有任何显著影响。

[0085] 表16-精氨酸pH 7.0缓冲液的赋形剂浓度

样品名称	蛋白质浓度(g/L)	精氨酸(mM)	组氨酸(mM)	蔗糖(g/L)
渗滤液	43.71	112.9	21.8	ND
掺料后	36.2	113.1	22.1	55.6
最终浓缩物	182	106.9	19.4	48.5
5X掺料缓冲液	N/A	102.1	22.2	266.7

[0086] *实验3*

指定最终制剂后第三次重复实验，且结果在表中详述。渗滤后，如实验2中概述的那样计算5X掺料溶液的添加体积。在～34 LMH进料流率和$P_{进料}$<50 psi将蛋白质浓缩至190 g/L。UF系统以单程模式用20 mM组氨酸，100 mM精氨酸，50 g/L蔗糖，pH 7.0漂洗。合并的保留物和漂洗液池中的蛋白质浓度为151 g/L，从而导致约84％的总得率。

[0087] 表17-用pH 7.0的精氨酸缓冲液进行的渗滤和浓缩。

笔记簿	706263-79
泵	Quattro Flow
UF 蛋白质挑战(g/m^2)	500
浓缩 1	
加样体积(L)	1.96
加样浓度(g/L)	2.25
进料压力(psi)	<50
浓缩 1 时间(分钟)	未记录
浓缩 1 浓度(g/L)	45.4
渗滤	
渗滤缓冲液	22 mM 组氨酸, 110 mM 精氨酸, pH 7.0
置换体积 (TOV)	8
进料流率@ DF (LMH)	450
进料压力@ DF (psi)	30
TMP @ DF (psi)	25
透过物流率@ DF (LMH)	18
渗滤时间(分钟)	300
浓缩 2A	
掺料溶液	22 mM 组氨酸, 110 mM 精氨酸, 275 g/L 蔗糖 pH 7.0
TMP @ 浓缩 2 (psi)	<25
最大进料压力(psi)	50
结束时的流率(LMH)	34
浓缩 2 时间(分钟)	未记录
最终浓度(g/L)	190

[0088] 表18总结了赋形剂浓度，表明组氨酸、精氨酸和蔗糖浓度均在所需值的±10％内。表19和表20表明在UFDF工艺过程中没有形成额外的聚集或碎片。

[0089] 表18-精氨酸pH 7.0缓冲液的赋形剂浓度

样品	蛋白质浓度(g/L)	组氨酸(mM)	精氨酸(mM)	蔗糖(g/L)

渗滤液	45.4	22	110.4	N/A
掺料后	37.3	22	110.9	55.5
浓缩2	190	19.5	102	49.7
系统漂洗液	37.6	20.5	102.2	52.2
最终抗-IL-7R 材料	151.6	19.9	102.7	48.9
渗滤缓冲液	N/A	22.2	110.3	N/A
5X掺料缓冲液	N/A	22.2	110.4	274.4
漂洗缓冲液	N/A	20	99.1	51.8

[0090] 表19-精氨酸pH 7.0缓冲液的nrCGE和rCGE结果

样品	nrCGE			rCGE		
	% IgG	% 碎片	%其他	% HC + LC	% 碎片	%其他
抗-IL-7R 参考	97.1	2.9	<0.3	98.7	0.5	0.8
抗-IL-7R 渗滤液	96.2	3.8	<0.3	98.9	0.4	0.7
抗-IL-7R 掺料	96.1	3.9	<0.3	99	0.4	0.7
抗-IL-7R 浓缩物	95.2	4.5	0.3	99	0.3	0.8
抗-IL-7R 漂洗液	96.1	3.9	<0.3	99	0.3	0.7
抗-IL-7R 最终	95.3	4.4	0.3	99	0.3	0.7

[0091] 表20-精氨酸pH 7.0缓冲液的SEC结果

样品名称	% 总HMMS	% 总LMMS	% 单体
抗-IL-7R 标准	0.6	0.2	99.1
抗-IL-7R 渗滤液	0.5	0.3	99.3
抗-IL-7R 掺料的	0.5	0.3	99.3
抗-IL-7R 浓缩物	0.8	0.3	98.9
抗-IL-7R 漂洗液	0.5	0.3	99.3
抗-IL-7R 最终	0.7	0.3	99

[0092] 如上面实验3中所进行的工艺导致所有赋形剂和所考虑的蛋白质的可接受浓度值,并且看来对聚集体或碎片形成没有影响。这个工艺将按比例扩大到中试规模,以确保其如所预期的进行。

[0093] *中试规模UFDF工艺*

使用Millipore® C-滤网再生纤维素膜并使用从500 L规模生物反应器纯化的材料在中试工厂中测试上述开发的UF工艺(实验3)。

[0094] 在表21中详述的单元运行过程中,起始产物浓度为2.92 g/L的86 L原材料用5%的2 M NaCl掺料,然后浓缩至44.6 g/L。然后将材料用22 mM组氨酸,110 mM精氨酸,pH 7.0以大约150 LMH的进料流率和$P_{进料}$<40 psi进行渗滤。8 TOV渗滤后,将保留物用22 mM组氨酸,110 mM精氨酸,275 g/l蔗糖pH 7.0掺料,并再循环10分钟,然后浓缩至191.4 g/L。浓缩过程在30 LMH透过物流率和$P_{进料}$<50 psi停止。然后以单程模式用20 mM组氨酸,100 mm组氨酸,50 g/L蔗糖,pH 7.0冲洗导轨。总得率约为87%。整个UF工艺大约需要5个小时才能完成。

[0095] 通过混合保留物池、漂洗液池和另外的漂洗缓冲液，产生了浓度为135.4 g/L的UF池。通过Millipore® 05/0.2 um Opticap Express SHC以59 L/m^2通过量过滤池。将20X EDTA和PS80赋形剂缓冲液掺料到UF池中以生产最终浓度为129.4 g/L的药物物质。

[0096] 表21-中试规模UF工艺数据

UFDF 系统和加样负荷	
膜类型	Millipore 30K C 滤网 RC
膜面积, m2	2.28
UF 蛋白质挑战(g/m^2)	123
浓缩 1	
加样体积(L)	96
加样浓度(g/L)	2.923
浓缩 1 时间(分钟)	~120
浓缩 1 浓度(g/L)	44.6
渗滤	
渗滤缓冲液	22 mM 组氨酸, 110 mM 精氨酸 pH 7.0
置换体积 (TOV)	8
保留物流率@ DF (LMH)	131.6
进料压力@ DF (psi)	29
TMP @ DF (psi)	24
透过物流率@ DF (LMH)	18.4
渗滤时间(分钟)	80
浓缩 2	
掺料溶液	22 mM 组氨酸, 110 mM 精氨酸, 275 g/L 蔗糖 pH 7.0
TMP @浓缩 2 (psi)	25
保留物流率@结束 (LMH)	30
浓缩 2 最终体积(L)	0.738
浓缩 2 时间(分钟)	~30
浓缩 2 浓度(g/L)	191.4
浓缩 2 回收(%)	50.35
漂洗	
漂洗缓冲液	20 mM 组氨酸, 100 mM 精氨酸, 50 g/L 蔗糖 pH 7.0
漂洗最终体积(mL)	1057
漂洗浓度(g/L)	97.3
漂洗回收(%)	36.7

[0097] 表22中总结了赋形剂浓度，其显示最终池中的组氨酸、精氨酸和蔗糖浓度在目标值的±15％内。在实验室规模工艺开发期间的目标范围设定为所有赋形剂浓度目标值的±10％，但大规模的验收范围设定为±15％以允许在按比例放大过程中的宽容度。

[0098] 表23和表24总结了来自运行的产物质量结果，其显示没有检测到聚集或碎片中的增加。

[0099] 表22-中试规模运行赋形剂浓度结果

样品名称	浓度(g/L)	精氨酸(mM)	组氨酸(mM)	蔗糖(g/L)
渗滤池	44.6	111.4	22.2	N/A
5X掺料的池	32.9	111.2	22.1	46.3
浓缩2保留物	191.4	105.8	20.1	43.9

最终漂洗池	97.3	101.8	19.9	46.4
药物物质	135.4	103.9	20.2	43.8
UF缓冲液	N/A	110.6	22.2	N/A
5X掺料	N/A	112.7	22.5	269.5
漂洗缓冲液	N/A	101.0	20.4	46.9

[0100] 表23-中试规模运行SEC结果

样品名称	% 总HMMS	% 总LMMS	% 单体
抗-IL-7R参考	0.6	0.4	99.0
UF加样	0.5	0.4	99.2
浓缩2 UF保留物	0.7	0.4	98.9
最终漂洗池	0.6	0.3	99.1
药物物质	0.6	0.3	99.0

[0101] 表24-中试规模运行nrCGE结果

样品	% IgG	% 碎片	% 其他
抗-IL-7R参考	96.5	3.1	0.3
UF加样	96.7	3.3	0.0
渗滤池	97.3	2.7	0.0
浓缩2 UF保留物	96.2	3.4	0.3
最终漂洗池	97.1	2.9	0.0
药物物质	96.5	3.5	0.0

[0102] 结论

总之，上述实验证实了用于所考虑的抗-IL-7R抗体的>120 mg/ml药物物质的UFDF方法的成功工艺开发。UFDF工艺包括初始浓度，渗滤，最终浓缩前的蔗糖掺料，然后用剩余的赋形剂掺料。开发的工艺中的pH和所有赋形剂浓度都在可接受的范围内。

序列表

<110> PFIZER INC.
Glynn, Judy K
Chen, Brian X
LaCasse, Daniel P
<120> 制备治疗用蛋白质制剂的方法和由这种方法生产的抗体制剂
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Arg Leu Gln Ala Gln Ala Ala Arg Arg Gly Tyr Leu Thr Lys Ile Leu

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115 120 125

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Ile Gly Ala Ser Ser Asp Cys Ser Thr Cys Phe Val Ser Gln Ser Gly
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Ser Gly Pro Thr Arg Met Ala Thr Ala Val Ala Arg Cys Ala Pro Asp
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Glu Glu Leu Leu Ser Cys Ser Ser Phe Ser Arg Ser Gly Lys Arg Arg
485 490 495
Gly Glu Arg Met Glu Ala Gln Gly Gly Lys Leu Val Cys Arg Ala His
500 505 510
Asn Ala Phe Gly Gly Glu Gly Val Tyr Ala Ile Ala Arg Cys Cys Leu
515 520 525
Leu Pro Gln Ala Asn Cys Ser Val His Thr Ala Pro Pro Ala Glu Ala
530 535 540
Ser Met Gly Thr Arg Val His Cys His Gln Gln Gly His Val Leu Thr
545 550 555 560
Gly Cys Ser Ser His Trp Glu Val Glu Asp Leu Gly Thr His Lys Pro
565 570 575
Pro Val Leu Arg Pro Arg Gly Gln Pro Asn Gln Cys Val Gly His Arg
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595 600 605
Lys Val Lys Glu His Gly Ile Pro Ala Pro Gln Glu Gln Val Thr Val
610 615 620
Ala Cys Glu Glu Gly Trp Thr Leu Thr Gly Cys Ser Ala Leu Pro Gly
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645 650 655
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
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35 40 45
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Ala Arg Gln Gly Asp Tyr Met Gly Asn Asn Trp Gly Gln Gly Thr Leu
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Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
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Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
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Thr Lys Val Asp Lys Lys Val Ala Pro Glu Leu Leu Gly Gly Pro Ser
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Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
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Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
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Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
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Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15
Thr Val Thr Ile Ser Cys Thr Arg Ser Ser Gly Ser Ile Asp Ser Ser
20 25 30
Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ser Pro Thr Thr Val
35 40 45
Ile Tyr Glu Asp Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80
Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Phe
85 90 95
His His Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gln Pro
100 105 110
Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu
115 120 125
Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro
130 135 140
Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala
145 150 155 160
Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala
165 170 175
Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg
180 185 190
Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr
195 200 205
Val Ala Pro Thr Glu Cys Ser
210 215

<210> 17
<211> 5
<212> PRT
<213> 人工的
<220>
<223> 合成的构建体
<400> 17
Asp Ser Val Met His
1 5

<210> 18
<211> 17
<212> PRT
<213> 人工的
<220>
<223> 合成的构建体
<400> 18
Leu Val Gly Trp Asp Gly Phe Phe Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15
Gly

<210> 19
<211> 8
<212> PRT
<213> 人工的
<220>
<223> 合成的构建体
<400> 19
Gln Gly Asp Tyr Met Gly Asn Asn
1 5
<210> 20
<211> 13
<212> PRT
<213> 人工的
<220>
<223> 合成的构建体
<400> 20
Thr Arg Ser Ser Gly Ser Ile Asp Ser Ser Tyr Val Gln
1 5 10
<210> 21
<211> 7
<212> PRT
<213> 人工的
<220>
<223> 合成的构建体
<400> 21
Glu Asp Asp Gln Arg Pro Ser
1 5
<210> 22
<211> 9
<212> PRT
<213> 人工的
<220>
<223> 合成的构建体
<400> 22
Gln Ser Tyr Asp Phe His His Leu Val
1 5

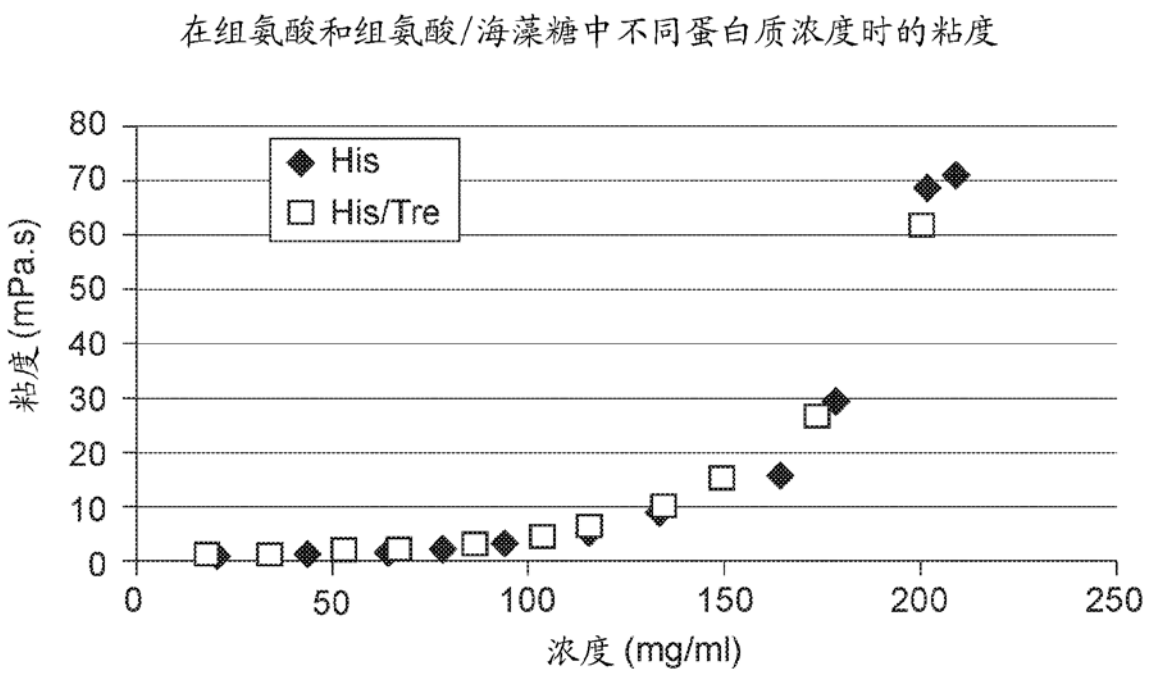
在组氨酸和组氨酸/海藻糖中不同蛋白质浓度时的粘度
His
His/Tre
粘度 (mPa.s)
浓度 (mg/ml)
0
10
20
30
40
50
60
70
80
0
50
100
150
200
250

图 1

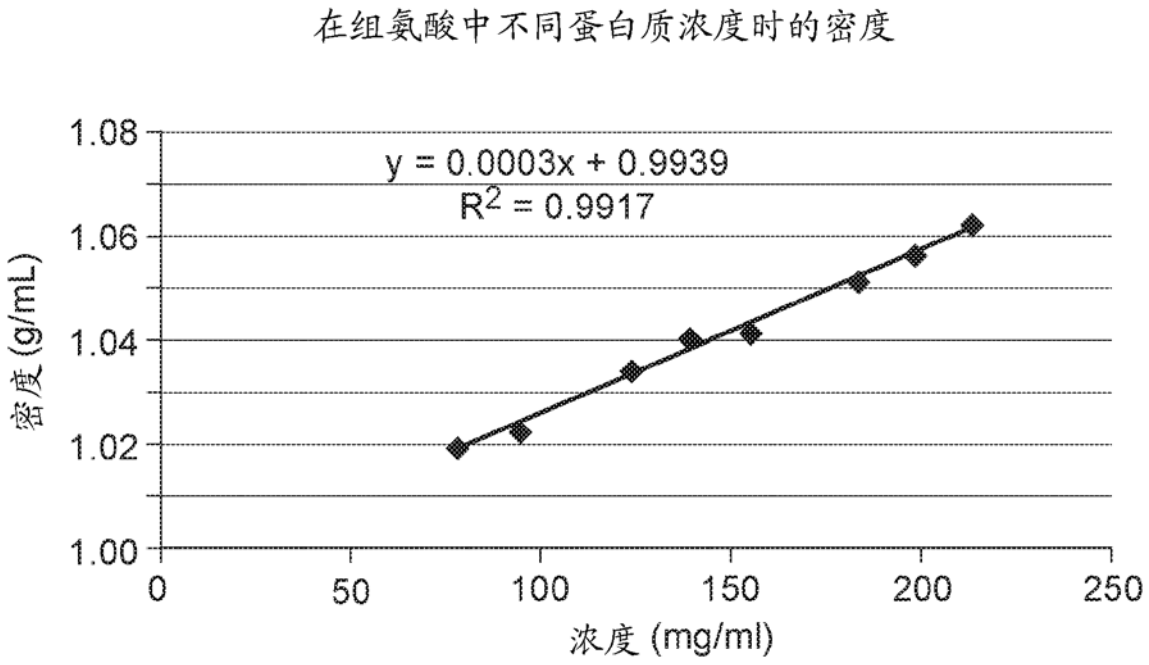
在组氨酸中不同蛋白质浓度时的密度
y = 0.0003x + 0.9939
R2 = 0.9917
密度 (g/mL)
浓度 (mg/ml)
1.00
1.02
1.04
1.06
1.08
0
50
100
150
200
250

图 2

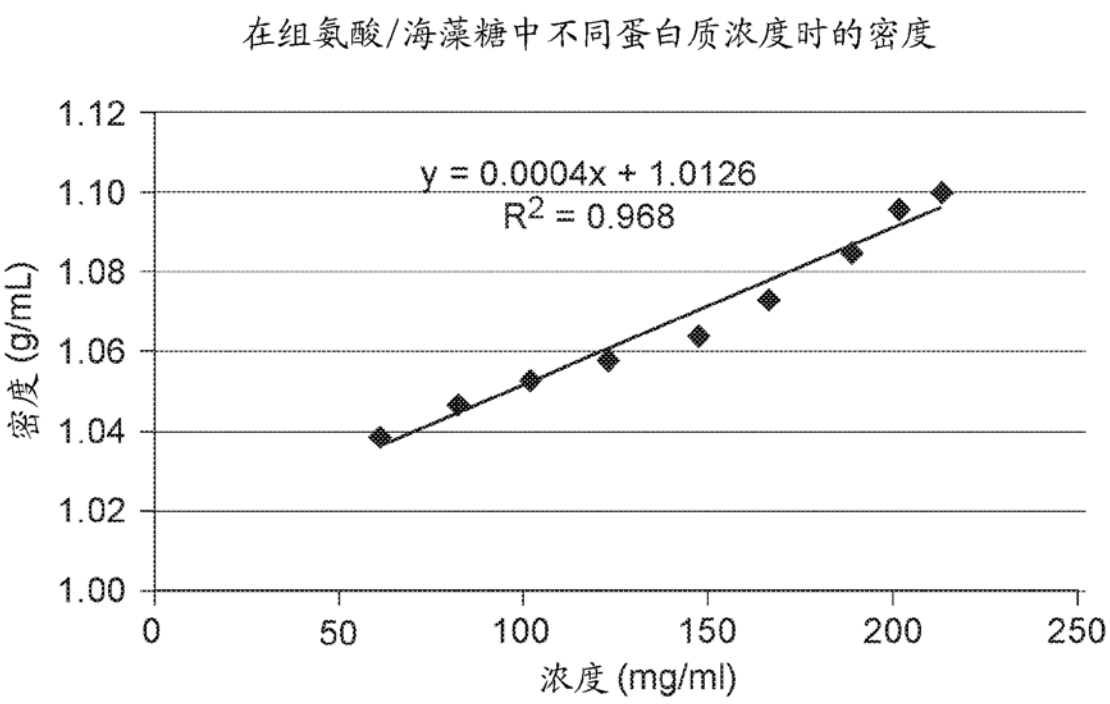
在组氨酸/海藻糖中不同蛋白质浓度时的密度
y = 0.0004x + 1.0126
R2 = 0.968
密度 (g/mL)
浓度 (mg/ml)
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0
50
100
150
200
250

图 3

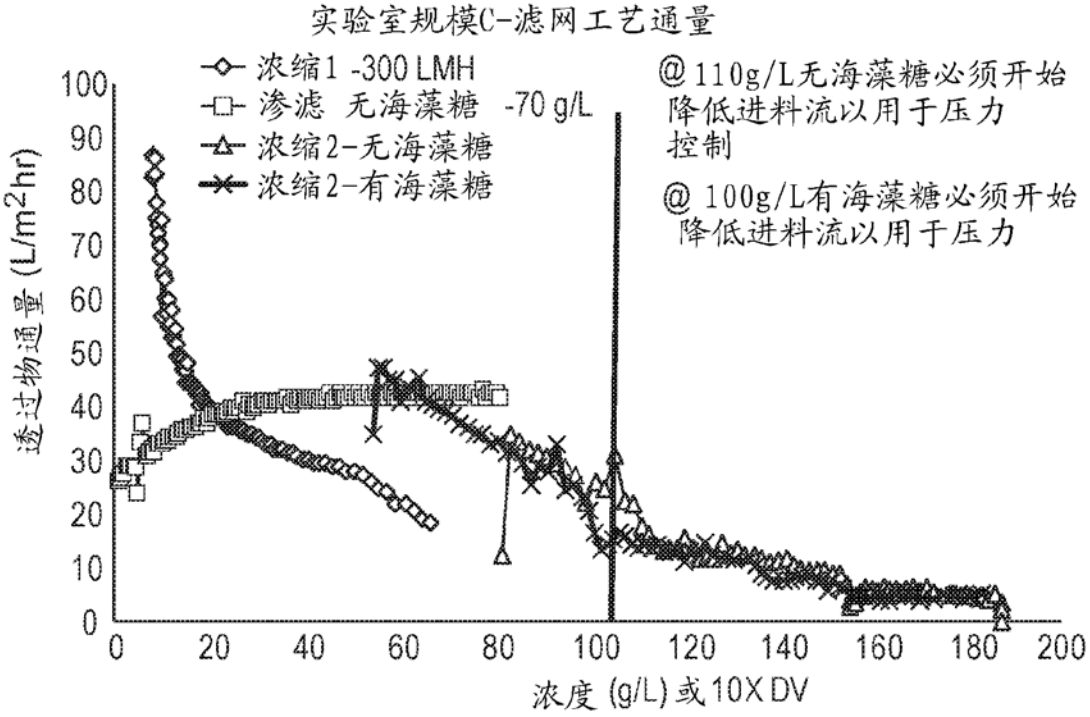
实验室规模C-滤网工艺通量
浓缩1 -300 LMH
渗滤 无海藻糖 -70 g/L
浓缩2-无海藻糖
浓缩2-有海藻糖
@ 110g/L无海藻糖必须开始降低进料流以用于压力控制
@ 100g/L有海藻糖必须开始降低进料流以用于压力
透过物通量 (L/m2hr)
浓度 (g/L) 或10X DV
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
200

图 4

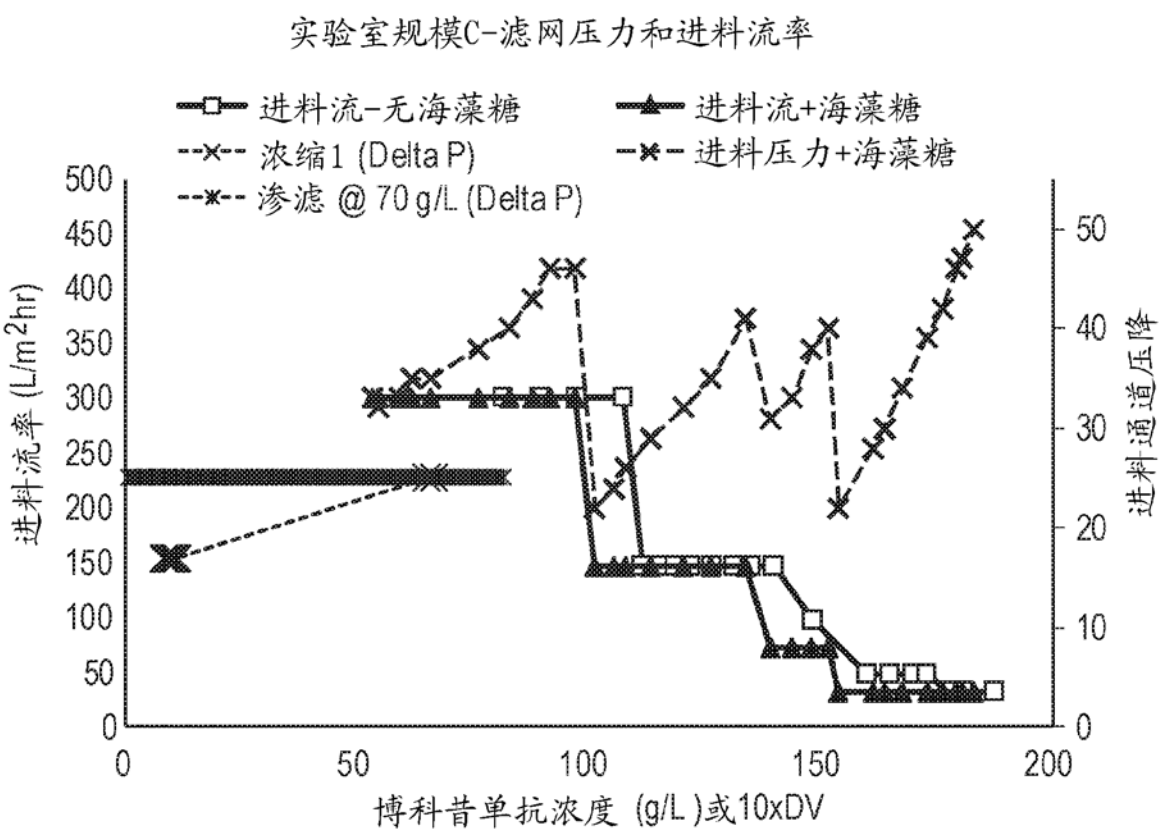
实验室规模C-滤网压力和进料流率
进料流-无海藻糖
进料流+海藻糖
浓缩1 (Delta P)
进料压力+海藻糖
渗滤 @ 70 g/L (Delta P)
进料流率 (L/m2hr)
进料通道压降
博科昔单抗浓度 (g/L)或10xDV
0
50
100
150
200
250
300
350
400
450
500
10
20
30
40

图 5