

CLAIMS

1. A process for the preparation of a highly concentrated liquid formulation
5 containing biomolecules comprising the steps of
- (a) a first ultrafiltration UF1;
 - (b) a first diafiltration DF1;
 - (c) a second diafiltration DF2; and
 - 10 (d) a second ultrafiltration UF2;
- wherein an aqueous solution of one or more salts, as liquid medium B, is
used for step (b) and water or an aqueous solution of one or more salts, as
liquid medium C, is used for step (c); wherein the one or more salts used for
15 step (b) are the same or different from the one or more salts used for step (c)
and wherein the liquid medium B has an ionic strength which is higher than
the ionic strength of the liquid medium C.
2. The process according to claim 1,
20 characterized in that
- the liquid medium B has a high ionic strength indicated in form of a
concentration which is in the range of from about 20 mM up to the limit of
solubility of the salt, or from about 100 mM to 1000 mM, or from about 150
mM to 750 mM, or from about 200 mM to 500 mM
- 25 and
- the liquid medium C has a low ionic strength indicated in form of a
concentration which is in the range of from about 0 mM to 150 mM, or from
about 0 mM to 100, or from about 0 mM to 75 mM, or from about 0 mM to
50 mM.
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3. The process according to claim 1 or 2,
characterized in that
the liquid medium B has an ionic strength which is higher than the ionic
strength of the liquid medium C so that the difference between the ionic
5 strength of the liquid medium B and the ionic strength of the liquid medium
C indicated in form of a concentration is at least about 100 mM, or at least
about 200 mM, or at least about 500 mM.
4. The process according to any of the preceding claims 1 to 3,
10 characterized in that
the liquid biomolecule formulation used in step (a) contains a liquid medium
A which is an aqueous solution and contains one or more excipients, the
liquid medium A is exchanged with liquid medium C by means of liquid
medium B in steps (b) and (c), whereby the liquid biomolecule formulation
15 obtained in step (c) and (d) has a reduced content of said excipient(s).
5. The process according to claim 4,
characterized in that
the excipients are selected from a group consisting of
20 excipients charged or neutral in aqueous solution;
- the excipients being selected from the group consisting of additives used in
the preparation or processing of biomolecules; unwanted substances or ions
such as impurities contained in the starting liquid biomolecule formulation;
25 undesired side-products formed during the manufacturing process of the
biomolecule; decomposition or degradation products of starting,
intermediate or end products formed during the production of the
biomolecule.
- 30 6. The process according to claim 5,

characterized in that

the additives, unwanted substances or ions originate from cell components or debris, degradation products of bacteria such as endotoxines, DNA, RNA, undesired lipids, HCP (Host cell proteins), lipopolysaccharides (LPS) or parts thereof; sugars; detergents such as positively charged, negatively charged and also non-ionic species; any kind of negatively or positively charged ions resulting from salts.

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7. The process according to any of the preceding claims 1 to 6,
10 characterized in that
the salts are selected from organic salts and/or inorganic salts.
8. The process according to claim 7,
characterized in that
15 the inorganic salt is selected from the group consisting of alkali salts or alkaline earth salts of sulfates, nitrates, phosphates, carbonates, halogenides, borates, silicates.
9. The process according to any of the preceding claims 7 or 8,
20 characterized in that
the inorganic salt is selected from the group of pharmaceutically acceptable inorganic salts comprising sodium salts; calcium salts; magnesium salts or combinations thereof.
- 25 10. The process according to claim 9,
characterized in that
the sodium salts comprise sodium halides, sodium sulfate, or sodium borate;
the calcium salts comprise calcium halides, calcium sulfate, or calcium
30 borate;

the magnesium salts comprise magnesium halides, magnesium sulfate or magnesium borate.

11. The process according to claim 10,
5 characterized in that
the halides comprise chlorides.
12. The process according to any of the preceding claims 1 to 11,
characterized in that
10 liquid medium B comprises sodium chloride in a concentration from about
150 to about 900 mM, or from about 200 to about 700 mM, or from about
400 to about 600 mM, or from about 450 to about 550 mM.
13. The process according to any of the preceding claims 1 to 12,
15 characterized in that
the salt is an organic and/or inorganic buffer salt.
14. The process according to claim 13,
characterized in that
20 the buffer salt is selected from the group consisting of N-(2-acetamido)-
aminoethanesulfonic acid (ACES) and salts thereof, acetic acid and salts
thereof, aconitic acid and salts thereof, adipic acid and salts thereof,
ascorbic acid and salts thereof, N-(2-Acetamido)-iminodiacetic acid (ADA)
and salts thereof, ammonia and salts thereof, ammonium chloride, 2-amino-
25 2-methyl-1-propanol (AMP), 2-amino-2-methyl-1,3-propanediol, ammediol
(AMPD), N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfo-
nic acid (AMPSO) and salts thereof, N,N-bis-(2-hydroxyethyl)-2-
aminoethanesulfonic acid (BES) and salts thereof, benzoic acid and salts
thereof, bicarbonates such as sodium hydrogen carbonate, N,N'-bis(2-
30 hydroxyethyl)-glycine (bicine), Tris buffers such as tris(hydroxymethyl)-

aminomethane, [bis-(2-hydroxyethyl)-imino]-tris-(hydroxymethylmethane) (Bis-Tris), 1,3-bis[tris(hydroxymethyl)-methylamino]propane (Bis-Tris-Propane), boric acid and salts thereof, dimethylarsinic acid (Cacodylate) and salts thereof, 3-(cyclohexylamino)-propanesulfonic acid (CAPS) and salts thereof, 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO) and salts thereof, carbonic acid and salts thereof, carbonates such as sodium carbonate, cyclohexylaminoethanesulfonic acid (CHES) and salts thereof, citric acid and salts thereof, 3-[N-bis(hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (DIPSO) and salts thereof, formic acid and salts thereof, gluconic acid and salts thereof, glyceric acid and salts thereof, glutamic acid and salts thereof, glycines such as glycylglycine, N-(2-hydroxyethyl)-piperazine-N'-ethanesulfonic acid (HEPES) and salts thereof, N-(2-hydroxyethyl)-piperazine-N'-3-propanesulfonic acid (HEPPS, EPPS) and salts thereof, N-(2-hydroxyethyl)-piperazine-N'-2-hydroxypropanesulfonic acid (HEPPSO) and salts thereof, imidazoles, lactic acid and salts thereof, malic acid and salt thereof, maleic acid and salts thereof, 2-(N-morpholino)-ethanesulfonic acid (MES) and salts thereof, 3-(N-morpholino)-propanesulfonic acid (MOPS) and salts thereof, 3-(N-morpholino)-2-hydroxypropanesulfonic acid (MOPSO) and salts thereof, phosphoric acid and salts thereof, piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) and salts thereof, piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) (POPSO) and salts thereof, pyridines, succinic acid and salts thereof, 3-[[tris(hydroxymethyl)-methyl]-amino]-propanesulfonic acid (TAPS) and salts thereof, 3-[N-tris(hydroxymethyl)-methylamino]-2-hydroxypropanesulfonic acid (TAPSO) and salts thereof, tartaric acid and salts thereof, taurine (2-aminoethanesulfonic acid, AES and salts thereof), triethanolamine (TEA), 2-[tris(hydroxymethyl)-methylamino]-ethanesulfonic acid (TES) and salts thereof, and N-[tris(hydroxymethyl)-methyl]-glycine (tricine).

30 15. The process according to claim 13 or 14,

characterized in that

the buffer salt or the buffer being a biological buffer which is an amino acid, in an aqueous solution, selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine;

or the buffer salt or the buffer being a biological buffer which is selected from the group consisting of phosphoric acid and salts thereof, citric acid and salts thereof, tris, succinic acid and salts thereof, malic acid and salts thereof, tartaric and salts thereof, acetic acid and salts thereof, lactic acid and salts thereof, aconitic acid and salts thereof, ascorbic acid and salts thereof, glutamic acid and salts thereof, ammoniumchloride, triethanolamine, alanine, arginine, glutamine, glycine, histidine, lysine, and proline.

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16. The process according to any of the preceding claims 1 to 15, characterized in that the liquid medium C consists or essentially consists of water.

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17. The process according to any of the preceding claims 1 to 16, characterized in that the biomolecule and the excipient(s) to be removed from the liquid biomolecule formulation have opposite charges.

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18. The process according to any of the preceding claims 1 to 17, characterized in that the biomolecule is positively charged and the excipient(s) to be removed by the process is(are) negatively charged excipient(s).

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19. The process according to any of the preceding claims 1 to 18,

characterized in that
the biomolecule comprises a positively charged protein and the excipient(s)
to be removed by the process comprise(s) anions.

- 5 20. The process according to any of the preceding claims 1 to 19,
characterized in that
the process step (b) is repeated several times prior to perform the
subsequent step (c), or the exchange of liquid medium B is performed with x
medium cycles, whereby x = 2 to 10, or x = 2 to 8, or x = 2 to 6.
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21. The process according to any of the preceding claims 1 to 20,
characterized in that
the process step (c) is repeated several times prior to perform the
subsequent step (d), or the exchange of liquid medium C is performed with y
15 medium cycles, whereby y = 2 to 10, or y = 2 to 8, or y = 2 to 6.
22. The process according to any of the preceding claims 1 to 21,
characterized in that
the ultrafiltration UF1 of step (a) is used to concentrate the liquid
20 biomolecule formulation, up to about 10%-70%, or about 15%-60%, or about
25%-50% compared with the initial concentration of the liquid biomolecule
formulation.
23. The process according to any of the preceding claims 1 to 22,
25 characterized in that
the ultrafiltration UF2 of step (d) is used to concentrate the liquid
biomolecule formulation to the desired value.
24. The process according to any of the preceding claims 1 to 23,
30 characterized in that

step (b) and step (c) follow directly one after the other whereby no intermediate process step is performed in between,
and/or step (a) and step (b) follow directly one after the other whereby no intermediate process step is performed in between, and/or

5 step (c) and step (d) directly follow one after the other whereby no intermediate process step is performed in between.

25. The process according to any of the preceding claims 1 to 24,
characterized in that
- 10 the biomolecules are selected from the group consisting of small molecules, monomers or biopolymers;
or the biomolecules are selected from the group consisting of lipids, vitamins; hormones, neurotransmitter; amino acids, nucleotides, monosaccharides; proteins, peptides; nucleic acids; oligosaccharides, polysaccharides,
- 15 or the biomolecules are selected from the group consisting of proteins, peptides, nucleic acids, oligosaccharides, and polysaccharides;
or the biomolecules are selected from the group consisting of proteins and peptides.
- 20 26. The process according to any of the preceding claims 1 to 25,
characterized in that
the process steps (a) to (d) are performed at room temperature of about 20°C to about 25°C.
- 25 27. The process according to any of the preceding claims 1 to 26,
characterized in that
the process steps (a) to (d) are performed using a tangential flow filtration (TFF) system or a centrifugal filtration system.

28. Highly concentrated liquid formulation containing biomolecules prepared by a process according to any of claims 1 to 27.