

A further object of the present invention is to provide a purer liquid biomolecule formulation product with the process, which may be purified easier and faster compared with the prior art processes, whereby the degree of carryover of excipients, particularly of anionic excipient(s), is reduced or even minimized.

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A further object of the present invention is to provide a process, in which the number of diavolume exchanges necessary to clear the excipient(s) is in a reasonable range in order not to impair biomolecule stability and trigger aggregate formation.

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A still further object of the present invention is to provide a process which is also feasible in large scale, provides the desired quality standard and operation efficiency with reasonable costs.

15 SUMMARY

Surprisingly, it was found that the disadvantages known from prior art may be overcome, particularly the carryover problem may be significantly reduced or completely avoided, if an additional diafiltration step, DF1, performed with a liquid medium at a defined high ionic strength, before a second diafiltration (DF2) is employed.

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Therefore, in order to overcome the above mentioned disadvantages, an improved and modified process of double ultra- and diafiltration UF/DF is provided. The process of the present disclosure for the preparation of a highly concentrated liquid formulation containing biomolecules comprising the steps of

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- (a) a first ultrafiltration UF1;
- (b) a first diafiltration DF1;
- (c) a second diafiltration DF2; and

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- (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 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.

- 10 In one aspect, the present disclosure relates to a process for the preparation of a liquid formulation containing biomolecules comprising the steps of

- (a) a first ultrafiltration UF1;
(b) a first diafiltration DF1;
15 (c) a second diafiltration DF2; and
(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 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;

wherein said liquid formulation has a concentration of 70 mg/ml or more.

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The expression "highly concentrated liquid formulation containing biomolecules" in the frame of the present disclosure shall be understood in the sense that the biomolecule(s) is (are) present in the liquid formulation in a concentration of 70 mg/ml or more, or 80 mg/ml or more, or 85 mg/ml or more.

The process of the present disclosure was found to consistently reduce levels of undesired excipient(s) present in the starting liquid biomolecule formulation, preferably below the limit of detection, and resulted in stable liquid formulations of the biomolecules contained. In case the biomolecules are selected to be proteins, the process yielded solutions with only enough exchange-medium counterions to balance proteins' inherent charge and permit the protein to self-buffer. Biomolecule integrity and biomolecule quality was generally found to be acceptable or completely unchanged.

Furthermore, it was completely unexpected that any kind of unwanted excipient(s) present in the starting liquid biomolecule formulation can be removed with the process of the present disclosure performed under solution conditions such as positively charged excipient(s), negatively charged excipient(s) and neutral excipient(s).

A further advantage of the process according to the disclosure resides in the fact that it is very mild with regard to physical stress exerted onto the proteins. This can be concluded from a high degree of monomer content throughout the steps of the process.

In another aspect, the present disclosure relates to a liquid formulation containing biomolecules prepared by a process as defined herein; wherein said liquid formulation has a concentration of 70 mg/ml or more.

DETAILED DESCRIPTION OF THE INVENTION

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context.

For example, the expressions "liquid formulation", "solution" "soluble" and "dissolved" or "solved" according to the present invention should be understood in their broadest meaning and include all kind of mixtures of a solid or liquid in a liquid medium such as true solutions, dispersions, suspensions and the like, unless otherwise stated.

The expression "highly concentrated" should be understood in the sense that the liquid biomolecule formulation is provided in a concentration which is higher than the starting concentration, preferably significantly higher than before. The exact increase of the concentration provided depends from each single case, the biomolecule and medium chosen as well as conditions and parameters of the ultrafiltration and diafiltration equipments used.

As used herein, the expression "ultrafiltration" or "UF" and similar terms refer to any technique in which a liquid formulation is subjected to a semi-permeable membrane that retains biomolecules, for example proteins, while allowing solvent and solute molecules smaller than the biomolecule to pass through. In the present

invention ultrafiltration is used to increase the concentration of biomolecules, for example proteins, in a liquid formulation.

CLAIMS

1. A process for the preparation of a liquid formulation containing biomolecules
5 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;
wherein said liquid formulation has a concentration of 70 mg/ml or more.

- 20 2. The process according to claim 1,
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 20 mM up to the limit of solubility
of the salt, preferably from 100 mM to 1000 mM, more preferably from 150
25 mM to 750 mM, most preferably from 200 mM to 500 mM
and
the liquid medium C has a low ionic strength indicated in form of a
concentration which is in the range of from 0 mM to 150 mM, preferably
from 0 mM to 100, more preferably from 0 mM to 75 mM, most preferably
30 from 0 mM to 50 mM.

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
5 strength of the liquid medium C so that the difference between the ionic
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.

10 4. The process according to any of the preceding claims 1 to 3,
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
15 medium B in steps (b) and (c), whereby the liquid biomolecule formulation
obtained in step (c) and (d) has a reduced content of said excipient(s).

5. The process according to claim 4,
characterized in that
20 the excipients are selected from a group consisting of
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
25 contained in the starting liquid biomolecule formulation; 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.

6. The process according to claim 5, wherein said unwanted substances or ions are impurities.
- 5 7. 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; sugars;
detergents; any kind of negatively or positively charged ions resulting from
salts.
- 10 8. The process according to claim 7, wherein said degradation products of
bacteria are selected from endotoxines, DNA, RNA, undesired lipids, HCP
(Host cell proteins), lipopolysaccharides (LPS) or parts thereof.
- 15 9. The process according to claim 7, wherein said detergents are selected
from positively charged, negatively charged and non-ionic species.
10. The process according to any of the preceding claims 1 to 9,
characterized in that
20 the salts are selected from organic salts and/or inorganic salts.
11. The process according to claim 10,
characterized in that
the inorganic salt is selected from the group consisting of alkali salts or
25 alkaline earth salts of sulfates, nitrates, phosphates, carbonates,
halogenides, borates, silicates.
12. The process according to any of the preceding claims 10 or 11,
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.

- 5 13. The process according to claim 12,
characterized in that
the sodium salts comprise sodium halides, sodium sulfate, or sodium
borate;
the calcium salts comprise calcium halides, calcium sulfate, or calcium
10 borate;
the magnesium salts comprise magnesium halides, magnesium sulfate or
magnesium borate.
14. The process according to claim 13,
15 characterized in that
the halides comprise chlorides.
15. The process according to any of the preceding claims 1 to 14,
characterized in that
20 liquid medium B comprises sodium chloride in a concentration from 150 to
900 mM, preferably from 200 to 700 mM, more preferably from 400 to 600
mM, most preferably from 450 to 550 mM.
16. The process according to any of the preceding claims 1 to 15,
25 characterized in that
the salt is an organic and/or inorganic buffer salt.
17. The process according to claim 16,
characterized in that

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-2-methyl-1-propanol (AMP), 2-amino-2-methyl-1,3-propanediol, ammediol (AMPD), *N*-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid (AMPSO) and salts thereof, *N,N*-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) and salts thereof, benzoic acid and salts thereof, bicarbonates, sodium hydrogen carbonate, *N,N'*-bis(2-hydroxyethyl)-glycine (bicine), Tris buffers, 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, 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, glycyglycine, *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-
5 {{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).

10 18. The process according to claim 16 or 17,
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,
15 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
20 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,
25 alanine, arginine, glutamine, glycine, histidine, lysine, and proline.

19. The process according to any of the preceding claims 1 to 18,
characterized in that
the liquid medium C consists of water.

20. The process according to any of the preceding claims 1 to 19,
characterized in that
the biomolecule and the excipient(s) to be removed from the liquid
biomolecule formulation have opposite charges.

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21. The process according to any of the preceding claims 1 to 20,
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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22. The process according to any of the preceding claims 1 to 21,
characterized in that
the biomolecule comprises a positively charged protein and the excipient(s)
to be removed by the process comprise(s) anions.

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23. The process according to any of the preceding claims 1 to 22,
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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24. The process according to any of the preceding claims 1 to 23,
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
medium cycles, whereby $y = 2$ to 10, or $y = 2$ to 8, or $y = 2$ to 6.

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25. The process according to any of the preceding claims 1 to 24,
characterized in that

the ultrafiltration UF1 of step (a) is used to concentrate the liquid biomolecule formulation, up to 10%-70%, preferably 15%-60%, more preferably 25%-50% of the volume compared with the initial concentration of the liquid biomolecule formulation.

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26. The process according to any of the preceding claims 1 to 25, characterized in that the ultrafiltration UF2 of step (d) is used to concentrate the liquid biomolecule formulation to the desired value.

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27. The process according to any of the preceding claims 1 to 26, 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 step (c) and step (d) directly follow one after the other whereby no intermediate process step is performed in between.

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- 20 28. The process according to any of the preceding claims 1 to 27, characterized in that 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, 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.

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29. The process according to any of the preceding claims 1 to 28,
characterized in that
the process steps (a) to (d) are performed at room temperature of 20°C to
25°C.

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30. The process according to any of the preceding claims 1 to 29,
characterized in that
the process steps (a) to (d) are performed using a tangential flow filtration
(TFF) system or a centrifugal filtration system.

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31. Liquid formulation containing biomolecules prepared by a process according
to any of claims 1 to 30;
wherein said liquid formulation has a concentration of 70 mg/ml or more.

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