

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.

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

- (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 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.

15 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
20 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)
25 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.

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In another aspect, the present disclosure relates to a highly concentrated liquid formulation containing biomolecules prepared by a process as defined herein.

DETAILED DESCRIPTION OF THE INVENTION

10 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
15 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
20 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.

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