

Claims:

1. A process for producing a PEGylated protein mixture the process comprising:
 - a) reacting a non-PEGylated protein with a PEGylation reagent to produce a mixture of reaction products comprising non-PEGylated protein and PEGylated protein, wherein the PEGylated protein comprises mono-PEGylated protein and oligo-PEGylated protein;
 - b) subjecting the mixture of reaction products to an ion exchange chromatography (IEC) step to provide an IEC flow-through solution in which the fraction of PEGylated protein is increased relative to the mixture of reaction products;
the IEC step comprising applying the mixture of reaction products to an IEC material under conditions suitable for binding non-PEGylated protein; and
 - c) collecting the IEC flow-through solution from step b) to provide a PEGylated protein mixture comprising mono-PEGylated protein and oligo-PEGylated protein,
wherein non-PEGylated protein is recovered in step b) by eluting an IEC eluate from the IEC material and the eluted non-PEGylated protein is used in a subsequent PEGylation reaction.
2. The process according to claim 1, wherein the protein is a hormone, a cytokine, an enzyme or an antibody.
3. The process according to claim 1 or claim 2, wherein the protein is erythropoietin.
4. The process according to any one of the preceding claims, wherein the IEC step is an anion exchange (AEC) step.
5. The process according to any one of the preceding claims, wherein two or more cycles of steps a), b) and c) are performed, in which non-PEGylated protein is recovered in step b) by eluting an IEC eluate from the IEC material and the eluted non-PEGylated protein is used in a subsequent PEGylation reaction.
6. The process according to claim 5 wherein the process comprises three, four or five cycles, in which in step b) of each cycle non-PEGylated protein is recovered and used in a subsequent PEGylation reaction.

7. The process according to claims 5 or claim 6, wherein step c) further comprises pooling the flow-through solution collected from each IEC step to provide a PEGylated protein mixture which is a pooled PEGylated protein mixture.
- 5 8. The process according to any one of the preceding claims, wherein eluting non-PEGylated protein from the IEC material uses an elution buffer comprising less than or equal to about 45 mM salt.
9. The process according to any one of the preceding claims, wherein the step b) IEC eluate is added directly to the subsequent PEGylation reaction.
- 10
10. The process according to any one of the preceding claims, in which the non-PEGylated protein is recovered and used in a subsequent PEGylation reaction, and wherein fresh non-PEGylated protein is also added to the subsequent PEGylation reaction in order to maintain substantially constant PEGylation reaction conditions in each PEGylation reaction.
- 15
11. The process according to any one of the preceding claims wherein the IEC material has a binding capacity for the PEGylated protein of less than about 1.5 g/L.
12. The process according to any one of the preceding claims, wherein:
- 20 i. the mixture of reaction products comprises less than 25% oligo-PEGylated protein; and/or
- ii. the IEC flow-through solution comprises at least 90% PEGylated protein.
13. The process according to any one of the preceding claims, wherein the IEC step is an AEC step
- 25 and wherein,
- i. the AEC material is Toyopearl Super Q 650 M; and/or
- ii. the AEC step is performed at pH of about 7.0 to 9.0; and/or
- iii. the AEC step is performed at a conductivity of about 1.0 to 3.0 mS/cm; and/or
- iv. the mixture of reaction products is applied to the AEC material as a AEC load solution
- 30 comprising about 10 – 30 mM bicine and about 1 – 10 mM Na₂SO₄.

14. The process according to any one of the preceding claims, wherein the PEGylation reaction is performed at a pH of about 7.0 to 9.0, and wherein the PEG/protein molar ratio is about 0.6 – 1.0.
15. A process for producing a mono-PEGylated protein composition comprising at least about 90%
5 mono-PEGylated protein, the process comprising:
- subjecting the PEGylated protein mixture comprising mono-PEGylated protein and oligo-PEGylated protein produced by the process of any one of claims 1 to 14 to a purification process separating mono-PEGylated protein and oligo-PEGylated protein; and
- recovering a mono-PEGylated protein composition in which the fraction of mono-
10 PEGylated protein is increased relative to the PEGylated protein mixture, wherein the mono-PEGylated protein composition comprises at least about 90% mono-PEGylated protein.
16. The process of claim 15, wherein the purification process comprises subjecting the PEGylated protein mixture to a hydrophobic interaction chromatography (HIC) step in flow-through mode to provide a
15 HIC flow-through solution in which the fraction of mono-PEGylated protein is increased relative to the PEGylated protein mixture,
- the HIC step comprising applying the PEGylated protein mixture to a HIC material under conditions suitable for binding oligo-PEGylated protein, wherein the HIC flow-through provides the mono-PEGylated protein composition.
- 20
17. The process of claim 15, wherein the purification process comprises subjecting the PEGylated protein mixture to a hydrophobic interaction chromatography (HIC) step in bind and elute mode to provide a HIC eluate in which the fraction of mono-PEGylated protein is increased relative to the PEGylated protein mixture, the HIC step comprising
- 25 applying the second mixture to a HIC material under conditions suitable for binding mono-PEGylated protein and oligo-PEGylated protein,
- eluting the mono-PEGylated protein from the HIC material to provide a HIC eluate, wherein the HIC eluate provides the mono-PEGylated protein.
- 30
18. The process according to any one of claims 15 to 17 wherein the mono-PEGylated protein composition comprises at least about 95%, 98%, 99%, or 99.9% mono-PEGylated protein.
19. The process according to any one of the preceding claims, wherein

- i. the IEC step and HIC step are performed at substantially the same pH; or
- ii. the PEGylation reaction, IEC step and HIC step are performed at substantially the same pH.

20. The process according to any one of the preceding claims, wherein mono-PEGylated protein
5 comprises a PEG residue having a molecular weight of at least about 20kDa.

21. The process according to any one of the preceding claims, further comprising formulating the protein
composition with a pharmaceutically acceptable carrier to provide a pharmaceutical composition.