

Claims:

1. A process for producing a mono-PEGylated protein composition comprising at least about 90% mono-PEGylated protein, the process comprising:
 - a) providing a first mixture comprising non-PEGylated protein and PEGylated protein, wherein the PEGylated protein comprises mono-PEGylated protein and oligo-PEGylated protein
 - b) subjecting the first mixture 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 first mixture; the IEC step comprising applying the first mixture to an IEC material under conditions suitable for binding non-PEGylated protein;
 - c) collecting the IEC flow-through solution from step b) to provide a second mixture comprising mono-PEGylated protein and oligo-PEGylated protein; and
 - d) subjecting the second mixture to a hydrophobic interaction chromatography (HIC) step to provide a mono-PEGylated protein composition in which the fraction of mono-PEGylated protein is increased relative to the second mixture, wherein the mono-PEGylated protein composition comprises at least about 90% mono-PEGylated protein.
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 ion exchange chromatography (IEC) step is an anion exchange chromatography (AEC) step.
5. 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.
6. The process according to any one of the preceding claims wherein:
 - i. the first mixture comprises less than 25% oligo-PEGylated protein; and/or
 - ii. the IEC flow-through solution comprises at least 90% PEGylated protein; and/or
 - iii. the mono-PEGylated protein composition comprises at least about 95%, 98%, 99%, or 99.9% mono-PEGylated protein.
7. The process according to any one of the preceding claims, wherein step a) further comprises performing a PEGylation reaction comprising reacting the non-PEGylated protein with a PEGylation reagent.

8. The process according to claim 7, 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.
9. The process according to claim 7 or claim 8, comprising
 - performing a first cycle comprising steps a), b) and c), wherein step b) further comprises eluting non-PEGylated protein from the IEC material to provide an IEC eluate, and
 - performing a second cycle of steps a), b) and c), in which the non-PEGylated protein eluted in step b) of the first cycle is added to the PEGylation reaction of step a).
10. The process according to claim 9, wherein eluting non-PEGylated protein from the IEC material uses an elution buffer comprising less than or equal to about 45 mM salt.
11. The process according to claim 9 or claim 10, wherein the process comprises three, four or five cycles, and wherein step b) of each cycle comprises eluting non-PEGylated protein from the IEC material to provide an IEC eluate, and wherein the non-PEGylated protein eluted in step b) is added to the PEGylation reaction of step a) in the next cycle.
12. The process according to any one of claims 9 to 11, wherein the IEC eluate from step b) of a cycle is added directly to the PEGylation reaction of step a) of the next cycle.
13. The process according to any one of claims 9 to 12, in which the non-PEGylated protein eluted in step b) of a cycle is added to the PEGylation reaction of step a) of the next cycle, and wherein fresh non-PEGylated protein is also added to step a) in order to maintain substantially constant PEGylation reaction conditions in step a) of each cycle.
14. The process according to any one of the preceding claims, comprising performing two or more cycles of steps a), b) and c), wherein
 - step c) further comprises pooling the flow-through solution collected from each IEC step to provide a second mixture which is a pooled second mixture, and wherein
 - step d) comprises subjecting the second mixture to an HIC step.
15. The process according to any one of the preceding claims, wherein the IEC step is an AEC step 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 first mixture is applied to the AEC material as a AEC load solution comprising about 10 – 30 mM bicine and about 1 – 10 mM Na₂SO₄.

16. The process according to any one of the preceding claims, wherein step d) comprises subjecting the second mixture to a HIC step in flow through mode to provide a HIC flow-through solution in which the fraction of mono-PEGylated protein is increased relative to the second mixture,
the HIC step comprising applying the second mixture to a HIC material under conditions suitable for binding oligo-PEGylated protein, wherein the HIC flow-through provides the mono-PEGylated protein composition.
17. The process according to any one of claims 1 to 15, wherein step d) comprises subjecting the second mixture to a 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 second mixture, the HIC step comprising
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.
18. The process according to claim 16, wherein
- the HIC material is Toyopearl Phenyl 650M; and/or
 - the HIC step is performed at a pH of about 7.0 to 9.0; and/or
 - the HIC step is performed at a conductivity of about 30-40 mS/cm; and/or
 - the second mixture is applied to the HIC material as a HIC load solution comprising about 25 mM bicine and about 390 mM Na₂SO₄.
19. The process according to any one of the preceding claims, wherein
- the IEC step and HIC step are performed at substantially the same pH; or
 - 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 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.