more poly (ethylene glycol) residues per erythropoietin molecule to a column, comprising Toyopearl® SP-650 as chromatography material, to which a first solution with a pH of about 2.4 to about 2.7 has been applied,

b) applying a second solution with an increased pH value with respect to the first solution (with a pH of about 2.4 to about 2.7),

c) applying a solution with increased or increasing conductivity to the column and thereby recovering the protein, which comprises erythropoietin and a single poly (ethylene glycol) residue.

Herein is reported a method for obtaining/purifying/producing a protein, which comprises erythropoietin and a single poly (ethylene glycol) residue, comprising the following steps:

a) applying a solution comprising a mixture of erythropoietin and conjugates of erythropoietin and poly (ethylene glycol) with one or more poly (ethylene glycol) residues per erythropoietin molecule to a column, comprising a chromatography material that has a matrix of methacrylate with a sulfopropyl as functional group, to which a first solution with a pH of about 2.4 to about 2.7 has been applied,

b) applying a second solution with an increased pH value with respect to the first solution (with a pH of about 2.4 to about 2.7),

c) applying a solution with increased or increasing conductivity to the column and thereby recovering the protein, which comprises erythropoietin and a single poly (ethylene glycol) residue.

Herein is reported as one aspect a method for purifying a protein, which comprises erythropoietin and a single poly (ethylene glycol) residue, comprising the following steps:

a) applying a solution comprising a mixture of erythropoietin and conjugates of erythropoietin and poly (ethylene glycol) with one or more poly (ethylene glycol) residues per erythropoietin molecule to a column, comprising a chromatography material that has a matrix of methacrylate with a sulfopropyl as functional group, to which a first solution with a pH of 2.4 to 2.7 has been applied,

Patent Claims

1. A method for purifying a protein, which comprises erythropoietin and a single poly (ethylene glycol) residue, comprising the following steps:

   a) applying a solution comprising a mixture of erythropoietin and conjugates of erythropoietin and poly (ethylene glycol) with one or more poly (ethylene glycol) residues per erythropoietin molecule to a column, comprising a chromatography material that has a matrix of methacrylate with a sulfopropyl as functional group, to which a first solution with a pH of 2.4 to 2.7 has been applied,

   b) applying a second solution with an increased pH value with respect to the first solution,

   c) applying a solution with increased or increasing conductivity to the column and thereby recovering the protein, which comprises erythropoietin and a single poly (ethylene glycol) residue.

2. The method according to claim 1, characterized in that the method further comprises the step of re-applying the first solution with a pH of 2.4 to 2.7 after step b) and before step c).

3. The method according to any one of claims 1 or 2, characterized in that the second solution with an increased pH value is a solution with a pH of 2.7 to 3.0.

4. The method according to any one of claims 1 to 3, characterized in that the second solution with an increased pH value is a solution with a constant conductivity value.

5. The method according to any one of claims 1 to 4, characterized in that the second solution with an increased pH value and the first solution with a pH of 2.4 to 2.7 have about the same constant conductivity value.

6. The method according to any one of claims 1 to 5, characterized in that the second solution with an increased pH value and/or the first solution with a pH of 2.4 to 2.7 have a constant conductivity value of 17 mS/cm to 19 mS/cm.

7. The method according to any one of claims 1 to 6, characterized in that the second solution with an increased pH value has a pH of 2.7 to 3.0 and has a conductivity value of 17 mS/cm to 19 mS/cm.

8. The method according to any one of claims 1 to 7, characterized in that the solution comprising a mixture of erythropoietin and conjugates of erythropoietin and poly (ethylene glycol) with one or more poly (ethylene glycol) residues per erythropoietin molecule is not adjusted to a conductivity value of about 19 mS/cm.

9. The method according to any one of claims 1 to 8, characterized in that the solution with increasing conductivity is a solution with increasing sodium chloride concentration.

10. The method according to any one of claims 1 to 9, characterized in that the solution with increasing conductivity has a conductivity that increases linearly or step-wise.

11. The method according to any one of claims 1 to 10, characterized in that the method is used in large scale protein preparations wherein the chromatography column of step a) has a diameter of at least 30 cm.

12. The method according to any one of claims 1 to 11, characterized in that the erythropoietin is human erythropoietin.

13. The method according to claim 12, characterized in that the human erythropoietin has the amino acid sequence of SEQ ID NO: 01 or SEQ ID NO: 02.

14. The method according to any one of claims 1 to 13, characterized in that the single poly (ethylene glycol) residue has a molecular weight of from 20 kDa to 40 kDa.