(2005) 67-74) report the purification of PEGylated rhG-CSF by two consecutive ion-exchange chromatography steps.

A method for purifying PEGylated erythropoietin on a SP Sephacryl S 500 HR column is reported in WO 2012/035037.

WO 1999/057134 reports for protein purification by ion exchange chromatography.

A method for the purification of mono-PEGylated erythropoietin comprising two cation exchange chromatography steps wherein the same type of cation exchange material is used in both cation exchange chromatography steps is reported in WO 2009/010270.

Summary

Herein is reported a method for the purification of a protein conjugate comprising erythropoietin and a single poly (ethylene glycol) residue from reaction by-products or not reacted starting material by a cation exchange chromatography method.

It has been found that by employing a wash step with a washing solution with increased pH value the conjugated protein comprising erythropoietin and a single poly (ethylene glycol) residue can be obtained from a cation exchange chromatography material, such as for example Toyopearl® SP-650, in a single step with high purity and yield in an improved and simplified way.

It has been found that when compared to a purification process for a protein conjugate comprising erythropoietin and a single poly (ethylene glycol) residue that employs two sequential cation exchange chromatography steps, the yield and the quality are at least comparable or better. The one column process allows for obtaining the same quality while increasing the robustness of the process. It also reduces the manufacturing costs and production time. Final yields can be increased without loss of quality of the mono-PEGylated erythropoietin.

Thus, 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 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 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,

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.

In one embodiment the method further comprises the step of re-applying the first solution with a pH of about 2.4 to about 2.7 after step b) and before step c). Thus, in one embodiment 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 Toyopearl® SP-650 chromatography material, to

embodiment the solution with increased or increasing conductivity has a pH value of from pH 2.3 to pH 3.5.

In one embodiment the solution with increased or increasing conductivity has a conductivity that increases step-wise or linearly.

In one embodiment the method is used in large scale protein preparations wherein the chromatography column of step a) has a diameter of at least 30 cm.

In one embodiment the erythropoietin is human erythropoietin. In one embodiment the human erythropoietin has the amino acid sequence of SEQ ID NO: 01 or SEQ ID NO: 02.

In one embodiment the single poly (ethylene glycol) residue has a molecular weight of from 20 kDa to 40 kDa.

Herein is reported a method for obtaining 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 Toyopearl® SP-650M as chromatography material, to which a first solution with a pH of about 2.4 to about 2.7 has been applied,

a1) re-applying the first solution with a pH of about 2.4 to about 2.7,

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.

Description of the Invention

Herein is reported a method for purifying a protein, which comprises one erythropoietin molecule and one poly (ethylene glycol) residue, with a gradient elution method on a cation exchange chromatography column, such as for example