immobilized on a solid phase comprising silica or glass; (b) removing contaminants bound to the solid phase by washing the solid phase with a hydrophobic electrolyte solvent; and (c) recovering the protein from the solid phase.

In WO2011/038894 a protein A chromatography method with a pronounced depletion of host cell protein and DNA by specific wash steps prior to the recovery of the immunoglobulin from the protein A chromatographic material is reported.

In WO2013/177118 compositions and methods for the isolation and purification of antibodies from a sample matrix are reported.

In WO2013/033517 methods for separating a polypeptide of interest (such as an antibody) from a virus are reported.

A method for purifying a protein, including one or more chromatographic processes, in which an amino acid; or a dipeptide, an oligopeptide, or a polyamino acid thereof is included in a buffer solution used in at least one chromatographic process (equilibration buffer, wash buffer, and elution buffer), thereby purifying a high-purity protein with a very small quantity of the impurity (e.g., polymers or host cell proteins) is reported in EP2583973.

Summary

Herein is reported a method for the production of an antibody with reduced content of host cell proteins by purifying the antibody with an affinity chromatography step.

In more detail it has been found that by the method of the current disclosure which uses a low conductivity aqueous solution in a wash step of an affinity chromatography prior to the recovery of an antibody from the chromatographic material, that the content of certain host cell proteins in a solution comprising the antibody can be reduced. Accordingly, it has been found that the content of phospholipases (in particular phospholipase B-like 2 (PLBL2)) can be reduced. It has been found that the PLBL2 content can be reduced 100-fold or more if the antibody is of the IgG4 isotype.

As reported herein is the use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of a host cell protein wherein the protein A chromatography is used to purify a human IgG1 or a human IgG4 isotype antibody.

One aspect as reported herein is the use of of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of a host cell protein wherein the protein A chromatography is used to purify a human IgG4 or IgG1 isotype antibody, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

In one embodiment the human IgG4 isotype antibody is an antibody against P-selectin, or an bispecific antibody against factor IXa and factor X, or an antibody against IL-13, or an antibody against amyloid beta. In one embodiment the human IgG1 isotype antibody is an antibody against Influenza B, or an antibody against VEGF-A, or an antibody against CD22, or a bispecific antibody against HER3 and EGFR, or an antibody against amyloid beta, or an antibody against Her2, or a bispecific antibody against Ang2 and VEGF-A, or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

In one embodiment the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

In one embodiment the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

In one embodiment the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.

In one embodiment the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.

In one embodiment the low conductivity aqueous solution has a pH of about 7 or higher.

In one embodiment the low conductivity aqueous solution wash step is preceded or succeeded by a high conductivity aqueous solution wash step.

In one embodiment the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.

In one embodiment an intermediate wash step is performed with a medium conductivity aqueous solution between the low conductivity aqueous solution wash step and the high conductivity aqueous solution wash step.

In one embodiment the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.

In one embodiment the high (or medium) conductivity aqueous solution comprises Histidine.

As reported herein is a method for producing a human IgG4 or IgG1 isotype antibody comprising the steps of

a) cultivating a cell comprising a nucleic acid encoding a human IgG4 or IgG1 isotype antibody,

b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 or IgG1 isotype antibody with a protein A chromatography material,

d) washing the protein A chromatography material with a low conductivity aqueous solution,

e) recovering the human IgG4 or IgG1 isotype antibody from the protein A chromatography material

and thereby producing the human IgG4 or IgG1 isotype antibody.

One aspect as reported herein is a method for producing a human IgG4 or IgG1 isotype antibody comprising the steps of

a) cultivating a cell comprising a nucleic acid encoding a human IgG4 or IgG1 isotype antibody,

b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 or IgG1 isotype antibody with a protein A chromatography material,

d) washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less,

e) recovering the human IgG4 or IgG1 isotype antibody from the protein A chromatography material

and thereby producing the human IgG4 or IgG1 isotype antibody.

As reported herein is a method for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 or IgG1 isotype antibody,

b) purifying the human IgG4 or IgG1 isotype antibody with a protein A chromatography method/step, comprising washing the protein A chromatography material with a low conductivity aqueous solution.

One aspect as reported herein is a for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 or IgG1 isotype antibody,

b) purifying the human IgG4 or IgG1 isotype antibody with a protein A chromatography method/step, comprising washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

In one embodiment the human IgG4 isotype antibody is an antibody against P-selectin or a bispecific antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta. In one embodiment the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or a bispecific antibody against HER3 and EGFR or an antibody against amyloid beta or an antibody against Her2 or a bispecific antibody against Ang2 and VEGF-A, or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

In one embodiment the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

In one embodiment the content of a host cell protein is reduced and the (specific) host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

In one embodiment the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.

In one embodiment the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.

In one embodiment the low conductivity aqueous solution has a pH of about 7 or higher.

In one embodiment the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution and/or with a medium conductivity aqueous solution before or after washing the protein A chromatography material with low conductivity aqueous solution.

In one embodiment the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.

In one embodiment the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.

In one embodiment the high or medium conductivity aqueous solution comprises Histidine.

Detailed Description of the Invention

Herein is reported an improved affinity chromatography method and use comprising the washing of the affinity chromatography material with a low conductivity aqueous solution.

It has been found that specific host cell proteins can be reduced with a wash step with a low conductivity aqueous solution, when this wash step is used in an affinity chromatography step, e.g. a protein A chromatrography step. The affinity chromatography step is used in a purification or production method for antibodies. The low conductivity aqueous solution wash step is particularly effective to reduce the content of phospholipase B-like 2 (PLBL2).