Therefore, there is still a need for tailoring effector functions of recombinant IgG antibodies, especially for the provision of means for improving ADCC mediated by therapeutic antibodies.

In WO 2011/012297 a method for producing an immunoglobulin or immunoglobulin fragment with defined glycostructure comprising the steps of providing an affinity chromatography column eluate containing the immunoglobulin or immunoglobulin fragment, incubating the affinity chromatography column eluate with (a1,3)galactosidase of plant origin, e.g. from green coffee beans (EC 3.2.1.22), applying the incubated affinity chromatography column eluate to a protein A chromatography material and recovering the immunoglobulin or immunoglobulin fragment from the protein A chromatography material and thereby producing an immunoglobulin or immunoglobulin fragment with defined glycostructure is reported.

In WO 2015/123754 an enzymatic method is provided for restructuring an affinity ligand bound heterogeneous glycoform antibody sample to a substantially homogenous single desired glycoform antibody sample for therapeutic uses and kits for performing the methods. A method for enzymatically altering the Fc region of an affinity ligand bound antibody from a heterogeneous glycoform to a substantially homogenous single glycoform comprises: contacting the affinity ligand bound heterogeneous glycoform antibody with a reaction buffer designed for a particular glycoform modification for a time sufficient and under conditions to modify the glycoform of the Fc region to a substantially homogeneous single form; optionally adding one or more nucleotide sugars and/or cofactors; and releasing the substantially homogeneous single glycoform antibody sample from said affinity ligand. The invention also encompasses biopharmaceuticals comprising single glycoform mAbs and polyclonal antibodies enzymatically produced for the treatment of cancers and immune disorders as well as compositions comprising the single glycoform antibodies as a biopharmaceutical.

In WO 2016/037947 galactoengineered recombinant antibodies of IgG1 isotype, methods for the production of said antibodies and uses thereof are reported.

Summary

Herein is reported a method for the in vitro glycoengineering of antibodies, in one example of recombinantly produced monoclonal antibodies, wherein the used enzyme(s) is (are) recovered after the modification of the antibody and conditioned

for re-use. The method as reported herein is, amongst other things, an improved method, especially a more economic method, for the modification of antibodies.

With the method as reported herein the enzymes used for the modification of the glycosylation of the antibody can be removed from the antibody preparation resulting in an improved preparation.

The method as reported herein is useful for the modification of any monoclonal antibody without the need of modifications to the preceding up-stream production process steps. The method as reported herein can be integrated into an existing process. Inherently no significant changes to existing antibody producing cell lines are required as the glycostructure modification is provided by the method as reported herein during down-stream processing.

It has been found that it is possible to recover the enzymes use for the modification of the glycosylation of antibodies in a form that allows re-use of the reconditioned enzymes for the same reaction without a detrimental loss of enzymatic activity. It was surprisingly found that the enzymes can be re-used at least once without significant loss of enzymatic activity and conversion efficiency.

As reported herein is a method for the enzymatic preparation/production of an antibody with a modified (substantially homogeneous) glycosylation at an N-glycosylation site comprising the separation of the enzyme(s) employed in the enzymatic modification from the antibody after the modification of the glycosylation and the re-use of the enzymes at least once (in the same enzymatic preparation/production process).

In one example the separation is by a chromatographic step. In one example the chromatographic step is an affinity chromatography step or/and a cation exchange chromatography step. In one example the affinity chromatography step is a protein A affinity chromatography step or an affinity chromatography with an antibody light chain affinity ligand. In one example the cation exchange chromatography step is a strong cation exchange chromatography step. In one example the strong cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups (SP-Sepharose).

In one example the separation is by two chromatographic steps, wherein the first step is an affinity chromatography step and the second step is a cation

exchange chromatography step, wherein the affinity chromatography step is a protein A affinity chromatography step or an affinity chromatography with an antibody light chain affinity ligand, and wherein the cation exchange chromatography step is a cation exchange chromatography step wherein the cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups (SP-Sepharose).

In one example in the enzymatic modification the antibody is either in solution or bound to an antibody (light chain) affinity ligand during the enzymatic modification. In one example the enzymatic modification is in solution.

In one example of the method as reported herein the monoclonal antibody is modified in solution by incubation with one or more glycosylation modifying enzymes to produce a monoclonal antibody preparation with modified glycostructure at an N-glycosylation site, wherein after the incubation the antibody and the one or more enzymes are separated by affinity chromatography or/and cation exchange chromatography, and wherein the enzymes are thereafter re-used at least once in the same reaction.

In one example of the method as reported herein the monoclonal antibody is bound to an affinity ligand, especially an antibody light chain affinity ligand, for enzymatic on-column modification to produce a monoclonal antibody preparation with modified glycostructure at an N-glycosylation site, wherein after the reaction the enzymes are separated from the antibody and re-used at least once in the same reaction.

Thus, in one example the method as reported herein for the enzymatic preparation/production of an antibody with a modified (substantially homogeneous) glycosylation at an N-glycosylation site comprises the following steps

a) incubating an antibody that has a (non-modified) glycosylation at the N-glycosylation site with one or more enzymes for a time sufficient and under conditions suitable to modify the at the N-glycosylation site to a defined (substantially homogeneous) form (homogeneous glycosylation),

b) separating the antibody with a modified glycosylation at the N-glycosylation site from the one or more enzymes and thereby producing

the antibody with a modified glycosylation at the N-glycosylation site and one or more recycled enzymes, and

c) repeating step a) with the one or more recycled enzymes of step b) at least once.

One aspect of the disclosure relates to a method for the enzymatic production of an antibody with a modified glycosylation at an N-glycosylation site, comprising the following steps

a) incubating an antibody that has a glycosylation at an N-glycosylation site with one or more enzymes for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site,

b) separating the antibody with a modified glycosylation at the N-glycosylation site from the one or more enzymes in a cation exchange chromatography, wherein the cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups, and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and one or more recycled enzymes, and

c) repeating step a) with the one or more recycled enzymes of step b) at least once.

In one example in the incubating is either in solution or with the antibody bound to an antibody (light chain) affinity ligand. In one example the incubating is in solution.

It was surprisingly found that an antibody light chain affinity ligand bound antibody can be effectively enzymatically modified as if the antibody would be in solution.

Thus, in one example the method as reported herein for the enzymatic preparation/production of an antibody with a modified (substantially homogeneous) glycosylation at an N-glycosylation site comprises the following steps

a) incubating in solution an antibody that has a (non-modified) glycosylation at the N-glycosylation site with one or more enzymes for a time sufficient and under conditions suitable to modify the

glycosylation at the N-glycosylation site to a defined (substantially homogeneous) form (homogeneous glycosylation),

b) separating in one or more chromatography steps the antibody with a modified glycosylation at the N-glycosylation site from the one or more enzymes and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and one or more recycled enzymes, and

c) repeating step a) with the one or more recycled enzymes of step b) at least once.

Thus, in one example the method as reported herein for the enzymatic preparation/production of an antibody with a modified (substantially homogeneous) glycosylation at an N-glycosylation site comprises the following steps

a) incubating in solution an antibody that has a (non-modified) glycosylation at the N-glycosylation site with one or more enzymes for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site to a defined (substantially homogeneous) form (homogeneous glycosylation),

b) separating the antibody with a modified glycosylation at the N-glycosylation site from the one or more enzymes in a cation exchange chromatography, and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and one or more recycled enzymes, and

c) repeating step a) with the one or more recycled enzymes of step b) at least once.

Thus, in one example the method as reported herein for the enzymatic preparation/production of an antibody with a modified (substantially homogeneous) glycosylation at an N-glycosylation site comprises the following steps

a) incubating in solution an antibody that has a (non-modified) glycosylation at the N-glycosylation site with one or more enzymes for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site to a defined (substantially homogeneous) form (homogeneous glycosylation),

b) separating the antibody with a modified glycosylation at the N-glycosylation site from the one or more enzymes in an affinity chromatography and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and one or more recycled enzymes, and

c) repeating step a) with the one or more recycled enzymes of step b) at least once.

Thus, in one example the method as reported herein for the enzymatic preparation/production of an antibody with a modified (substantially homogeneous) glycosylation at an N-glycosylation site comprises the following steps

a) incubating in solution an antibody that has a (non-modified) glycosylation at the N-glycosylation site with two or more enzymes for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site to a defined (substantially homogeneous) form (homogeneous glycosylation),

b) separating the antibody with a modified glycosylation at the N-glycosylation site from the two or more enzymes in an affinity chromatography and thereby producing the antibody with a modified glycosylation at the N-glycosylation site,

c) separating the two or more enzymes separated from the antibody in step b) in a cation exchange chromatography, and

d) repeating step a) with the two or more separated enzymes of step c) at least once.

In one example the cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups (SP-Sepharose). It has been found that the method is not working with a matrix of crosslinked poly(styrene divinylbenzene).

In one example the one or more enzymes is one enzyme. In one example the one enzyme is a galactosyltransferase or a sialyltransferase.

In one example the two or more enzymes are two enzymes. In one example the two or more enzymes are a galactosyltransferase and a sialyltransferase.

In one example the galactosyltransferase is **β**4GalT1.

In one example the sialyltransferase is ST6.

In one example the sialyltransferase is ST6Gal1 or ST6Gal2.

In one example the N-glycosylation site is in the Fab or in the Fc-region.

In one example the cation exchange chromatography comprises the following steps

i) applying the solution comprising a galactosyltransferase and/or a sialyltransferase and the antibody with the modified N-glycosylation site to a (strong) cation exchange chromatography material,

ii) optionally washing the (strong) cation exchange chromatography material (to remove unbound compounds from the (strong) cation exchange chromatography material),

iii) applying a first solution to the (strong) cation exchange chromatography material and thereby recovering the galactosyltransferase (if present) from the (strong) cation exchange chromatography material,

iv) applying a second solution to the (strong) cation exchange chromatography material and thereby recovering the antibody with the modified N-glycosylation site from the (strong) cation exchange chromatography material, and

v) applying a linear gradient to the (strong) cation exchange chromatography material and thereby recovering the sialyltransferase (if present) from the (strong) cation exchange chromatography material.

In one example the solution of step i) is a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5. In one example the solution of step i) comprises about 50 mM MES and has a pH value of about pH 6.4.

In one example the solution of step ii) is a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5. In one example the solution of step ii) comprises about 50 mM MES and has a pH value of about pH 6.4.

In one example the solution of step iii) is a tris(hydroxymethyl)aminomethane (TRIS) buffered solution with a pH value from pH 6.6 to pH 8.0. In one example the solution of step iii) comprises about 40 mM TRIS and has a pH value of about pH 7.4.

In one example the solution of step iv) is a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5 comprising about 75 mM to about 125 mM sodium chloride (NaCl). In one example the solution of step iv) comprises about 30 mM MES, about 90 mM NaCl and has a pH value of about pH 5.6.

It has been found that a reduction of the pH value from 7.4 to below pH 6, e.g. pH 5.6, results in the elution of aggregated galactosyltransferase.

In one example the linear gradient is from the solution of step iv) to a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5 comprising about 750 mM to about 1250 mM sodium chloride (NaCl). In one example the linear gradient is from the solution of step iv) to a solution comprising about 50 mM MES, about 1000 mM NaCl with a pH value of about pH 6.4.

In one example the repeating is for 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 times. In one example the repeating is for 1 to 5 times. In one example the repeating is for 1 to 3 times.

In one example is the method as reported herein for the enzymatic modification of the glycosylation at an N-glycosylation site of an antibody (to a substantially homogeneous glycosylation) wherein the antibody is bound to an antibody light chain affinity ligand during the enzymatic modification.

In one example the method comprises the following steps:

- incubating the antibody light chain affinity ligand-bound monoclonal antibody with a glycosylation at the N-glycosylation site with one or more enzymes for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site to a defined (substantially homogeneous) form (homogeneous glycosylation).

In one example the method comprises prior to the incubation step the step of

- binding the monoclonal antibody with glycosylation at the N-glycosylation site to an antibody light chain affinity ligand,

and after the incubation step the step of

- releasing the antibody with a defined (substantially homogeneous) glycosylation at the N-glycosylation site from the antibody light chain affinity ligand.

In one example the method comprises the following steps in the following order

- applying a (buffered) solution comprising the antibody with glycosylation at the N-glycosylation site to an antibody light chain affinity ligand bound to a solid phase (antibody light chain affinity ligand chromatography material) whereby the antibody is bound to the ligand (resulting in a ligand-bound antibody),
- optionally washing the solid phase with a buffered solution,
- enzymatically modifying the glycosylation at the N-glycosylation site of the antibody by either
    - applying a first (buffered) solution comprising a first glycosylation modifying enzyme (and a first activated sugar residue) for a time sufficient and under conditions suitable for the enzymatic modification to the ligand-bound antibody, optionally washing the modified ligand-bound antibody, applying a second (buffered) solution comprising a second glycosylation modifying enzyme (and a second activated sugar) for a time sufficient and under conditions suitable for the enzymatic modification to the modified ligand-bound antibody, optionally washing the two-times modified ligand-bound antibody,

    or

    - applying a first (buffered) solution comprising a first glycosylation modifying enzyme (and a first activated sugar) for a time sufficient and under conditions suitable for at least a partial enzymatic modification to the ligand-bound antibody, applying after a defined period of time a second (buffered) solution comprising a second glycosylation

modifying enzyme (and a second activated sugar) for a time sufficient and under conditions suitable for the enzymatic modification to the modified ligand-bound antibody, optionally washing the two-times modified ligand-bound antibody,

or

- applying a (buffered) solution comprising a first and a second glycosylation modifying enzyme (and a first and second activated sugar) for a time sufficient and under conditions suitable for the enzymatic modification of the ligand-bound antibody, optionally washing the modified ligand-bound antibody,

- releasing the antibody with a defined glycosylation at the N-glycosylation site from the antibody light chain affinity ligand.

The antibodies as used in the methods as reported herein can be any antibody or antibody fragment, including Fab fragments, single chain antibodies, multispecific antibodies and antibody fusions.

Thus, in one example the antibody is selected from the group of antibodies consisting of an antibody Fab fragment, a full length antibody, a bivalent monospecific antibody, a bispecific antibody, a bivalent bispecific antibody, a trivalent bispecific antibody, a tetravalent bispecific antibody, a trivalent trispecific antibody, and a tetravalent tetraspecific antibody.

In one example the antibody is a bivalent monospecific antibody.

In one example the antibody is a bivalent or trivalent or tetravalent bispecific antibody.

In one example the antibody is a chimeric or humanized or human antibody.

In one example the antibody is a polyclonal antibody preparation.

In one example the antibody is a monoclonal antibody.

In one example the antibody (preparation) is an antibody (preparation) of the human IgG class. In one example the antibody is an antibody of the human IgG1 or IgG4 subclass.

In one example the defined glycosylation is a glycosylation selected from the group consisting of G2 glycoform, G0 glycoform, M3 glycoform, S2 glycoform, A2B glycoform, A2BG2 glycoform and S1 glycoform.

In one example the defined glycosylation is a glycosylation selected from the group consisting of galactose as the terminal sugar, GlcNAc as the terminal sugar, mannose as the terminal sugar and sialic acid as the terminal sugar.

In one example the antibody is a recombinantly produced antibody.

As reported herein is an antibody produced with a method as reported herein.

As reported herein is a pharmaceutical formulation comprising an antibody with defined glycosylation produced by a method as reported herein.

There is also provided a method for the recombinant production of an antibody or fragment thereof with defined glycosylation at an N-glycosylation site, comprising the steps of

a) recombinantly producing an antibody (of IgG1 isotype) or a fragment thereof in a (mammalian or CHO) cell, which comprises nucleic acids encoding the antibody or fragment thereof, to obtain an antibody or fragment thereof with glycosylation at the N-glycosylation site,

b) isolating (recovering and optionally purifying) the antibody or fragment thereof with heterogeneous glycosylation at the N-glycosylation site,

c) enzymatically modifying the antibody or fragment thereof with glycosylation at the N-glycosylation site with galactosyltransferase and/or a sialyl transferase to obtain an antibody or fragment thereof with defined at the N-glycosylation site, which comprises a relative amount of at least 70 % bi-galactosylated antibodies (G2F glycoform) (wherein 100 % corresponds to the amount of G0F, G1F and G2F

glycoforms) at the N-glycosylation site, and subsequent separation of the of the modified antibody from the enzyme(s) with a method as reported herein,

d) optionally purifying the modified antibody or fragment thereof by one or more chromatography steps,

and thereby producing an antibody or fragment thereof with defined glycosylation at the N-glycosylation site.

In one example the first glycosylation modifying enzyme is a galactosyltransferase.

In one example the first glycosylation modifying enzyme is a galactosyltransferase and the second glycosylation modifying enzyme is a sialyltransferase.

In one example the galactosyltransferase is **β**4GalT1.

In one example the sialyltransferase is ST6.

In one example the sialyltransferase is ST6Gal1 or ST6Gal2.

In one example the (first) buffered solution comprises UDP-Gal.

In one example the (second) buffered solution comprises CMP-NANA.

In one example the incubation is at room temperature (20 - 25 °C, preferably about 22 °C).

In one example the incubation is at 25 °C.

In one example the incubation is at 37 °C.

In one example the incubation is for 7 to 48 hours.

In one example the solution comprises a chromatographically purified antibody, the (first) glycosylation modifying enzyme is GalT1, and the incubation with the (first) glycosylation modifying enzyme is for 24 hours at 20-27 °C or 37 °C. In one example the incubation is at room temperature (about 22 °C).

In one example the solution comprises a chromatographically purified antibody, the (second) glycosylation modifying enzyme is ST6, and the incubation with the (second) glycosylation modifying enzyme is for 24 hours at 20-27°C or 37 °C. In one example the incubation is at room temperature (about 22 °C).

In one example the solution is a buffered, cell-free cultivation supernatant comprising the antibody, the first glycosylation modifying enzyme is GalT1, the second glycosylation modifying enzyme is ST6, which is added 6 to 24 hours, preferably 24 hours, after the first glycosylation modifying enzyme, the total incubation time is 24 hours to 48 hours, preferably 30 hours, at 20-27°C or 37 °C. In one example the incubation is at room temperature (about 22 °C).

As reported herein is a method for producing an antibody comprising the following steps in the following order:

- providing a cell comprising a nucleic acid encoding the antibody or a fragment thereof comprising at least an antibody light chain,
- cultivating the cell under conditions suitable for the expression of the antibody or fragment thereof with glycosylation at an N-glycosylation site (the fragment comprises at least on light chain that can specifically be bound by the antibody light chain affinity chromatography material used in one of the next steps),
- recovering the antibody or fragment thereof from the cell or the cultivation medium,
- optionally applying a solution comprising antibody or fragment thereof to an antibody light chain affinity chromatography column under conditions suitable for binding of the antibody or fragment thereof to the affinity chromatography material,
- modifying the glycosylation of the antibody or fragment thereof at the N-glycosylation site with a method as reported herein, and
- recovering the modified antibody or fragment thereof with a defined glycosylation at the N-glycosylation site,

and thereby producing an antibody.

In one example comprises the method the following step as final step:

- purifying the modified antibody or fragment thereof with one to three chromatography steps.

As reported herein is a chromatographic method for the separation of a mixture comprising a galactosyltransferase or/and a sialyltransferase and an antibody using a cation exchange chromatography material comprising the following steps

i) applying the solution comprising the galactosyltransferase and/or the sialyltransferase and the antibody to a (strong) cation exchange chromatography material,

ii) optionally washing the (strong) cation exchange chromatography material (to remove unbound compounds from the (strong) cation exchange chromatography material),

iii) applying a first solution to the (strong) cation exchange chromatography material and thereby recovering the galactosyltransferase (if present) from the (strong) cation exchange chromatography material,

iv) applying a second solution to the (strong) cation exchange chromatography material and thereby recovering the antibody from the (strong) cation exchange chromatography material, and

v) applying a linear gradient to the (strong) cation exchange chromatography material and thereby recovering the sialyltransferase (if present) from the (strong) cation exchange chromatography material.

It has been found that with this method the separated galactosyltransferase as well as the separated sialyltransferase can be re-used in an enzymatic conversion without significant loss of enzymatic activity or/and selectivity for multiple times, i.e. at least three times.

In one example the solution of step i) is a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5. In one

example the solution of step i) comprises about 50 mM MES and has a pH value of about pH 6.4.

In one example the solution of step ii) is a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5. In one example the solution of step ii) comprises about 50 mM MES and has a pH value of about pH 6.4.

In one example the solution of step iii) is a tris(hydroxymethyl)aminomethane (TRIS) buffered solution with a pH value from pH 6.6 to pH 8.0. In one example the solution of step iii) comprises about 40 mM TRIS and has a pH value of about pH 7.4.

In one example the solution of step iv) is a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5 comprising about 75 mM to about 125 mM sodium chloride (NaCl). In one example the solution of step iv) comprises about 30 mM MES, about 90 mM NaCl and has a pH value of about pH 5.6.

It has been found that a reduction of the pH value from 7.4 to 5.6 results in the elution of aggregated galactosyltransferase.

In one example the linear gradient is from the solution of step iv) to a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5 comprising about 750 mM to about 1250 mM sodium chloride (NaCl). In one example the linear gradient is from the solution of step iv) to a solution comprising about 50 mM MES, about 1000 mM NaCl with a pH value of about pH 6.4.

In one example the N-glycosylation site is in the Fab or in the Fc-region.

Detailed Description of the Invention

DEFINITIONS

As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and is

Patent Claims

1. A method for the enzymatic production of an antibody with a modified glycosylation at an N-glycosylation site, comprising the following steps

   a) incubating an antibody that has a glycosylation at an N-glycosylation site with one or more enzymes for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site,

   b) separating the antibody with a modified glycosylation at the N-glycosylation site from the one or more enzymes in a cation exchange chromatography, wherein the cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups, and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and one or more recycled enzymes, and

   c) repeating step a) with the one or more recycled enzymes of step b) at least once.

2. The method according to claim 1, wherein step a) comprises incubating the antibody that has the glycosylation at the N-glycosylation site with one or more enzymes and one or more activated sugar residues for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site to a defined form.

3. The method according to claim 1 or 2, wherein the incubating is either in solution or with the antibody bound to an antibody affinity ligand.

4. The method according to claim 3, wherein the incubating is either in solution or with the antibody bound to an antibody light chain affinity ligand or an antibody Fc-region affinity ligand.

5. The method according to any one of claims 1 to 4, wherein the one or more enzymes are a galactosyltransferase and a sialyltransferase.

6. The method according to claim 5, wherein the galactosyltransferase is β4GalT1.

7. The method according to any one of claims 5 to 6, wherein the sialyltransferase is ST6.

8. The method according to any one of claims 1 to 7, wherein the cation exchange chromatography comprises the following steps

i) applying the solution comprising a galactosyltransferase and/or a sialyltransferase and the antibody with the modified glycosylation at the N-glycosylation site to the cation exchange chromatography material,

ii) optionally washing the cation exchange chromatography material,

iii) applying a first solution to the cation exchange chromatography material and thereby eluting the galactosyltransferase from the cation exchange chromatography material,

iv) applying a second solution to the cation exchange chromatography material and thereby eluting the antibody with the modified glycosylation at the N-glycosylation site from the cation exchange chromatography material, and

v) applying a linear gradient to the cation exchange chromatography material and thereby eluting the sialyltransferase from the cation exchange chromatography material.

9. The method according to claim 8, wherein the cation exchange chromatography comprises the following steps

i) applying the solution comprising a galactosyltransferase and/or a sialyltransferase and the antibody with the modified glycosylation at the N-glycosylation site to the cation exchange chromatography material,

ii) optionally washing the cation exchange chromatography material to remove unbound compounds from the cation exchange chromatography material but without eluting the antibody with the modified glycosylation at the N-glycosylation site,

iii) applying a first solution to the cation exchange chromatography material and thereby eluting the galactosyltransferase, if present, from the cation exchange chromatography material,

iv) applying a second solution to the cation exchange chromatography material and thereby eluting the antibody with the modified glycosylation at the N-glycosylation site from the cation exchange chromatography material, and

v) applying a linear salt gradient to the cation exchange chromatography material and thereby eluting the sialyltransferase, if present, from the cation exchange chromatography material

10. The method according to claim 8 or 9, wherein

- the solution of step i) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5,
- the solution of step ii) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5,
- the solution of step iii) is a tris(hydroxymethyl)aminomethane buffered solution with a pH value from pH 6.6 to pH 8.0,
- the solution of step iv) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5 comprising about 75 mM to about 125 mM sodium chloride, and
- the linear gradient is from the solution of step iv) to a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5 comprising about 750 mM to about 1250 mM sodium chloride.

11. The method according to any one of claims 1 to 10, wherein the repeating is for 1 to 3 times.

12. The method according to any one of claims 1 to 11, wherein the antibody is a bivalent monospecific antibody or a bivalent bispecific antibody or an antibody Fab fragment.

13. The method according to any one of claims 1 to 12, wherein the antibody is a chimeric or humanized or human antibody.

14. The method according to any one of claims 1 to 13, wherein the antibody is a monoclonal antibody.

15. The method according to any one of claims 1 to 14, wherein the antibody is an antibody of the human IgG1 or IgG4 subclass.

16. The method according to any one of claims 1 to 15, wherein the N-glycosylation site is a Fab region N-glycosylation site or the Fc-region N-glycosylation site at asparagine residue 297, where the numbering is according to Kabat).

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