During protein production, product-related impurities include high-order aggregation species, which are complexes comprising product aggregates. These product-related aggregates and impurities may include HCPs and DNA (4). Other non-aggregate product-related impurities include unreacted (unpaired) half antibodies, noncovalently or covalently linked homodimers, and noncovalently linked heterodimers, which are closely related to the product of interest and are challenging to remove by standard processes such as Protein A purification (8).

Bispecific heterodimeric antibodies, comprising heavy and light chains from different monospecific monoclonal antibodies, can be produced in high yields by "knobs-into-holes" strategies (9) which bias the formation of a particular heterodimer. It is based on the principle that "knobs" (replacement of a small amino acid by a large amino acid in a region of one antibody chain) pack preferentially with "holes" (replacement of a large amino acid by a small amino acid in a corresponding region of another antibody chain), to favour selective heterodimerisation between specific chains. However, this method can produce further non-aggregate side-products, for instance resulting from false-paired knob-knob and hole-hole by-products.

There remains a need to provide methods for reducing the amount of non-aggregate product-related impurities.

~~Disclosure of the invention~~Summary

The present ~~invention~~disclosure provides novel methods and uses for reducing the amount of non-aggregate product-related impurities in a buffered solution of monoclonal antibodies (mAbs), by using a depth filter which comprises silica and polyacrylic fiber. The inventors found that using such a depth filter led to a decrease in non-aggregate product related impurities.

As discussed herein, depth-filters are known to reduce certain process-related impurities, such as HCPs and DNAs, as well as aggregate product-related impurities, in recombinant protein manufacturing processes. However, the inventors did not expect the depth filter to reduce non-aggregate product-related impurities. At its broadest, the present ~~invention~~disclosure relates to this unexpected finding.

In another aspect, the present disclosure provides a method of reducing the amount of non-aggregate product-related impurities (NAPRIs) in a buffered solution of monoclonal

antibodies (mAbs) by passing the buffered solution of monoclonal antibodies (mAbs) through a synthetic depth filter comprising silica and polyacrylic fiber, to remove a proportion of the NAPRIs from the buffered solution, wherein the NAPRIs comprise incompletely or incorrectly assembled mAb polypeptides.

Accordingly, one ~~aspect~~example of the ~~invention~~disclosure provides a method of reducing the amount of non-aggregate product-related impurities (NAPRIs) in a buffered solution of monoclonal antibodies (mAbs), wherein the method comprises passing the buffered solution of

monoclonal antibodies (mAbs) through a depth filter comprising silica and polyacrylic fiber, to remove a proportion of the NAPRIs from the buffered solution. The amount of NAPRIs is reduced by passing the buffered solution of mAbs through the depth filter.

In another aspect, the present disclosure provides a method of producing a buffered solution of monoclonal antibodies (mAbs) with a reduced amount of non-aggregate product-related impurities (NAPRIs) by passing a buffered solution of monoclonal antibodies (mAbs) through a synthetic depth filter comprising silica and polyacrylic fiber, to produce the buffered solution of monoclonal antibodies (mAbs) with a reduced amount of non-aggregate product-related impurities (NAPRIs), wherein the NAPRIs comprise incompletely or incorrectly assembled mAb polypeptides.

In a further ~~aspect~~example, this ~~invention~~disclosure provides a method of producing a buffered solution of monoclonal antibodies (mAbs) with a reduced amount of non-aggregate product-related impurities (NAPRIs), wherein the method comprises passing a buffered solution of monoclonal antibodies (mAbs) through a depth filter comprising silica and polyacrylic fiber, to produce the buffered solution of monoclonal antibodies (mAbs) with a reduced amount of non-aggregate product-related impurities (NAPRIs). The amount of NAPRIs is reduced by passing the buffered solution of mAbs through the depth filter.

In another aspect, the present disclosure provides a use of a synthetic depth filter comprising silica and polyacrylic fiber to reduce the amount of non-aggregate product-related impurities (NAPRIs) in a buffered solution of monoclonal antibodies (mAbs), wherein the NAPRIs comprise incompletely or incorrectly assembled mAb polypeptides.

In a further ~~aspect~~example, this ~~invention~~disclosure provides use of a depth filter comprising silica and polyacrylic fiber to reduce the amount of non-aggregate product-related impurities (NAPRIs) in a buffered solution of monoclonal antibodies (mAbs).

In a further aspect, this ~~invention~~disclosure provides a buffered solution of monoclonal antibodies (mAbs) in which the amount of non-aggregate product-related impurities (NAPRIs) has been reduced relative to the amount of the mAbs, produced by performing any of the methods of the ~~invention~~disclosure, or by any of the uses of the ~~invention~~disclosure.

In yet another aspect, the present disclosure provides a method of producing a mAb, the method comprising the steps of:

(a) culturing a host cell comprising a nucleic acid encoding for a mAb so that the mAb is produced along with NAPRIs;

(b) forming a buffered solution of the mAb and NAPRIs;

(c) reducing the amount of NAPRIs by performing the method according to any one of the preceding claims, or by the use according to any one of the preceding claims on the buffered solution of mAb and NAPRIs; and

(d) isolating the mAb from the buffered solution.

In a further ~~aspect~~example, this ~~invention~~disclosure provides a method of ~~a~~ producing a mAb, the method comprising the steps of:

(a) culturing a host cell comprising a nucleic acid encoding for a mAb so that the mAb is produced along with NAPRIs;

(b) forming a buffered solution of the mAb and NAPRIs;

(c) reducing the amount of reduced NAPRIs by performing the methods of the ~~invention~~disclosure or by the uses of the ~~invention~~disclosure; and optionally

(d) isolating the mAb from the buffered solution.

The following ~~embodiment~~examples are ~~embodiment~~examples of the aspects of the ~~invention~~disclosure.

In some ~~embodiment~~examples, the buffered solution of mAbs is a concentrated buffered solution of mAbs. In some ~~embodiment~~examples, the concentration of the concentrated buffered solution of mAbs is between 2 mg/mL and 20 mg/mL, between 5 mg/mL and 15 mg/mL or between 5 mg/mL and 10mg/mL.

In some ~~embodiment~~examples, the mAb is a multispecific antibody. In one ~~embodiment~~example the mAb a bispecific antibody. In some ~~embodiment~~examples, the mAb is an antibody fragment. In some ~~embodiment~~examples, the mAb is an antibody fusion protein comprising an antibody or antibody fragment and another biologically active polypeptide.

In some ~~embodiment~~examples, the buffered solution of monoclonal antibodies is passed through the depth filter at a temperature that is below ambient temperature. For instance, the temperature may be between about 4° C and about 22° C. The temperature may be between about 10° C and about 21° C. The temperature may be between about 15° C and about 20° C.

The present ~~invention~~disclosure allows largescale antibody purification. Large amounts of antibodies can be loaded onto the silica and polyacrylic fiber depth filter. For instance, the load can be over 100 g/m^2, over 200 g/m^2, over 300 g/m^2, over 500 g/m^2, or over 700 g/m^2. The load can be up to 1500 g/m^2, 2000 g/m^2, or 2500 g/m^2. The flow rate can be in the range of between about 1 L/min*m² to about 10 L/min*m², for example 1.5 L/min*m² to about 8 L/min*m². In preferred examples, the flow rate is in the range of between about 3 L/min*m² to about 6 L/min*m², or at about 4.3 L/min*m². The unit 'L/min*m²' denotes the volume (litres) flowing through the filter per minute, per unit area (m²).

Even before passage through the depth filter, the NAPRIs are present at a relatively low concentration in the initial buffered mAb solution, e.g. at least 1.5-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 1000-fold or at least 10,000-fold lower than the concentration of the mAb. This ~~invention~~disclosure allows the NAPRI concentration to be reduced even further relative to the concentration of the mAb.

Similarly, the absolute amount of NAPRI (e.g. measured in moles) relative to the absolute amount of mAb is reduced (and the absolute amount of NAPRI in solution is reduced following passage through the depth filter). Moreover, the NAPRI concentration may be reduced after the solution has passed through the depth filter, relative to the NAPRI concentration before the solution passes through the depth filter (although this is not necessarily always the case, e.g. if wash fractions are included in the eluted solution, thus increasing its volume).

The reduction in NAPRI concentration relative to the mAb concentration can be expressed as a reduction in the following ratio; [mAb]:[NAPRI]. This ratio is increased after the solution has passed through the depth filter.

Typically, the non-aggregate product-related impurities (NAPRI) is a polypeptide comprised of incompletely or incorrectly assembled polypeptide chains of the mAb. In some ~~embodiment~~examples, the NAPRI is a polypeptide lacking one or more polypeptide chains of the mAb. In some ~~embodiment~~examples, the NAPRI is a polypeptide comprising a different polypeptide chain arrangement than the mAb. In some ~~embodiment~~examples, the non-aggregate product-related impurities (NAPRIs) comprise a polypeptide that have a different amino acid sequence and/or different antibody chain configuration from the mAb.

In one ~~embodiment~~example the mAb is a multispecific antibody consisting of four different polypeptide chains and the NAPRI is a polypeptide (a) lacking one or more of said four polypeptide chains, or (b) comprising two or more of the same of said four different polypeptide chains. In one ~~embodiment~~example the mAb is a multispecific antibody comprising two heavy chains that are associated with each other, wherein one heavy chain comprises a knob mutation and the other heavy chain comprises a hole mutation, and the NAPRI is a polypeptide comprising two heavy chains comprising a knob mutation that are associated with each other, or two heavy chains comprising a hole mutation that are associated with each other. In one ~~embodiment~~example the mAb is a multispecific antibody comprising two Fab fragments, wherein a first Fab fragment comprises a first light chain comprising in N- to C-terminal direction a VL domain and a CL domain and a first heavy chain comprising in N- to C-terminal direction a VH domain and a CH1 domain; and wherein a second Fab fragment comprises either (a) a second light chain comprising in N- to C-terminal direction a VL domain and a CH1 domain and a second heavy chain comprising in N- to C-terminal direction a VH domain and a CL domain, (b) a second light chain comprising in N- to C-terminal direction a VH domain and a CH1 domain and a second heavy chain comprising in N- to C-terminal direction a VL domain and a CL domain, or (c) a second light chain comprising in N- to C-terminal direction a VH domain and a L domain and a second heavy chain comprising in N- to C-terminal direction a VL domain and a CH1 domain; and wherein the NAPRI is a polypeptide, wherein (a) the first light chain and the second heavy chain are associated, (b) the second light chain and the first heavy chain are associated, (c) two first heavy chains are associated, (d) two second heavy chains are associated, or (e) the polypeptide lacks at least one of the first light chain, first heavy chain, second light chain and second heavy chain.

In some ~~embodiment~~examples, the NAPRI comprises two heavy chains which are the same as each other. In some ~~embodiment~~examples, the NAPRI comprises two heavy chains having the same amino acid sequence. In some ~~embodiment~~examples, the NAPRIs comprise two heavy chains that are the same as each other and/or two light chains that are the same as each other. For

instance, where the product is a bispecific monoclonal antibody that has been engineered to pair via knob-hole interactions, the NAPRI may comprise knob-knob and/or hole-hole false-paired chains. In some ~~embodiment~~examples the non-aggregate product-related impurities (a) have false chain configurations; (b) have a loss of parts, optionally missing a light chain; (c) have an addition of parts, optionally wherein the impurity is a monomer with a light chain; (d) is a ¾ antibody; (e) is a light chain mispairing; (f) is a knob/knob antibody; or (g) is a hole/hole antibody. In some ~~embodiment~~examples the NAPRI may be a light chain dimer, free light chain, a heavy chain dimer wherein one of the heavy chains is truncated, a heavy chain monomer, or a 1 + 1 clipping dimer.

In some ~~embodiment~~examples, the buffered solution has a pH of about 4.0 to about 7.5. In some ~~embodiment~~examples, the buffered solution has a pH of about 4.0 to about 7.2. In some ~~embodiment~~examples, the buffered solution has a pH of about 4.0 to about pH 5.5. The buffered solution may comprise sodium acetate or sodium citrate. The buffered solution may comprise sodium acetate. In some ~~embodiment~~examples, the buffered solution may comprise 150 mM sodium acetate. In some ~~embodiment~~examples, the buffered solution may comprise 10mM or 50mM sodium citrate. In some ~~embodiment~~examples, the buffered solution may comprise histidine. In some ~~embodiment~~examples, the buffered solution may comprise acetic acid. In some ~~embodiment~~examples, the pH of the solution is buffered with Tris.

In some ~~embodiment~~examples, the concentration of a non-aggregate product-related impurity (NAPRI) has been measured after the amount of NAPRIs has been reduced according to the methods of the ~~invention~~disclosure.

In some ~~embodiment~~examples, the buffered solution of mAbs (or buffered solution of mAb) may have been subjected to chromatography (before the depth filter is used). In some ~~embodiment~~examples, the buffered solution of mAbs may have been subjected to affinity chromatography, anion exchange chromatography, cation exchange chromatography or multimodal (mixed mode) chromatography. In some ~~embodiment~~examples, the buffered solution of mAbs may have been subjected to affinity chromatography, for instance using a Protein A resin, a Protein L resin, an Fc-selective resin, a Kappa light chain-selective resin, or a Lambda light chain-selective resin. Additionally or alternatively to a previous chromatography, e.g. affinity chromatography, in some ~~embodiment~~examples, the buffered solution of mAbs may have been subjected to ion exchange chromatography, for instance on an anion exchange or cation exchange column, or a multimodal (mixed mode) chromatography. The buffered solution mAbs may been concentrated by subjection to affinity chromatography.

The depth filter may be a multi-layer depth filter comprising multiple levels of depth filter media. Preferably, the depth filter is a double layer depth filter. Preferably, the depth filter does not contain diatomaceous earth. The depth filter may be a Millistak+® HC Pro Synthetic Depth Filter X0SP in some ~~embodiment~~examples.

The methods and uses of this ~~invention~~disclosure may further include a step of identifying the presence or absence of NAPRI, or measuring the NAPRI concentration, in the buffered solution of mAbs after the solution has passed through the depth filter. The amount of NAPRI remaining can be measured. Similarly, the amount of mAb can be measured after the solution has passed through the depth filter. In some ~~embodiment~~examples, the amount of NAPRI remaining can be measured by Capillary Electrophoresis SDS Page or Size Exclusion Chromatography. In some ~~embodiment~~examples, the NAPRI is heavy chain dimer where one heavy chain is truncated; a heavy chain; or a product light chain, and its amount can be measured with Capillary Electrophoresis SDS Page in a reducing environment. In some ~~embodiment~~examples, the NAPRI is a LMW; a 1+1 dimer (a heavy chain that has been truncated) a 1+1 clipping dimer (a heavy chain truncated and clipped in the hinge region) a hole-hole mispairing; a hole-hole mispairing; a half-hole; a light chain or a light chain dimer, and its amount can be measured with Size Exclusion Chromatography and/or with Capillary Electrophoresis SDS Page in a non-reducing environment. In some ~~embodiment~~examples, the NAPRI is a HMW, and its amount can be measured with hydrophobic interaction chromatography and/or with Capillary Electrophoresis SDS Page in a non-reducing environment. In some ~~embodiment~~examples, the HMW NAPRI is a knob-knob mispairing. In some ~~embodiment~~examples, the NAPRI is a heavy chain knob, or a heavy chain hole.

In some ~~embodiment~~examples, the total concentration or amount of non-aggregate product-related impurities (NAPRIs), or a non-aggregate product-related impurity (NAPRI) is less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 8%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2% or less than 1%.

In some ~~embodiment~~examples, the concentration of a non-aggregate product-related impurity (NAPRI) is measured by Size Exclusion Chromatography (SEC), optionally wherein the measurement is performed using high performance liquid chromatography.

In some ~~embodiment~~examples, the concentration of a non-aggregate product-related impurity (NAPRI) is measured by Capillary Electrophoresis SDS Page (CE-SDS), optionally wherein the measurement is performed using a LabChip device or a LabChip GXII device.

Claims

1. A method of reducing the amount of non-aggregate product-related impurities (NAPRIs) in a buffered solution of monoclonal antibodies (mAbs) by passing the buffered solution of monoclonal antibodies (mAbs) through a synthetic depth filter comprising silica and polyacrylic fiber, to remove a proportion of the NAPRIs from the buffered solution,

wherein the NAPRIs comprise incompletely or incorrectly assembled mAb polypeptides.

2. A method of producing a buffered solution of monoclonal antibodies (mAbs) with a reduced amount of non-aggregate product-related impurities (NAPRIs) by passing a buffered solution of monoclonal antibodies (mAbs) through a synthetic depth filter comprising silica and polyacrylic fiber, to produce the buffered solution of monoclonal antibodies (mAbs) with a reduced amount of non-aggregate product-related impurities (NAPRIs),

wherein the NAPRIs comprise incompletely or incorrectly assembled mAb polypeptides.

3. Use of a synthetic depth filter comprising silica and polyacrylic fiber to reduce the amount of non-aggregate product-related impurities (NAPRIs) in a buffered solution of monoclonal antibodies (mAbs),

wherein the NAPRIs comprise incompletely or incorrectly assembled mAb polypeptides.

4. The method according to any one of the preceding claims, or the use according to claim 3, wherein the mAb is a multispecific antibody.

5. The method according to any one of the preceding claims, or the use according to any one of the preceding claims, wherein the mAb is an antibody fusion protein comprising an antibody or antibody fragment and another biologically active polypeptide.

6. The method according to any one of the preceding claims, or the use according to any one of the preceding claims, wherein the buffered solution of monoclonal antibodies is passed through the depth filter at a temperature that is below ambient temperature.

7. The method according to any of the preceding claims, or the use according to any one of the preceding claims, wherein the NAPRI is a polypeptide lacking one or more polypeptide chains of the mAb.

~~8. The method according to any of the preceding claims, or the use according to any one of the preceding claims~~, and/or wherein the NAPRI is a polypeptide comprising a different polypeptide chain arrangement than the mAb.

~~9~~8. The method according to any one of claims 1-6, or the use according to any one of claims 3-6, wherein the NAPRI comprises two heavy chains having the same amino acid sequence.

~~10. The method according to any one of the preceding claims, or the use according to any one of the preceding claims, wherein the buffered solution of mAbs has been subjected to affinity chromatography.~~

~~11~~9. The method according to any one of the preceding claims, or the use according to any one of the preceding claims, wherein the depth filter is a multi-layer depth filter comprising multiple levels of depth filter media.

~~12~~10. The method according to any one of the preceding claims, or the use according to any one of the preceding claims, wherein the depth filter does not contain diatomaceous earth.

~~13~~11. The method according to any one of the preceding claims, or the use according to any one of the preceding claims, further comprising measuring the NAPRI concentration in the buffered solution of mAbs after it has passed through the depth filter.

~~14~~12. The method according to any one of the preceding claims, or the use according to any one of the preceding claims, wherein the buffered solution of mAbs has been subjected to chromatography before passing through the synthetic depth filter.

~~15. The method or the use according to claim 14, wherein the chromatography is affinity chromatography, anion exchange chromatography, cation exchange chromatography or multimodal (mixed mode) chromatography.~~

~~16. The method or the use according to claim 15, wherein the chromatography is affinity chromatography using a Protein A resin, a Protein L resin, an Fc-selective resin, a Kappa light chain-selective resin, or a Lambda light chain-selective resin.~~

~~17~~13. The method or the use according to any one of the preceding claims ~~14-16~~, wherein the chromatography ~~is~~ comprises one or more of ion exchange chromatography, anion

exchange chromatography, cation exchange chromatography, multimodal (mixed mode) chromatography, or affinity chromatography.

~~18. The method or the use according to claim 17, wherein the ion exchange chromatography used an anion exchange column, a cation exchange column, or multimodal (mixed mode) chromatography.~~

~~19~~14. A buffered solution of monoclonal antibodies (mAbs) in which the amount of non-aggregate product-related impurities (NAPRIs) has been reduced relative to the amount of the mAbs, produced by performing the method according to any one of the preceding claims, or by the use according to any one of the preceding claims.

~~20~~15. A method of producing a mAb, the method comprising the steps of:

(a) culturing a host cell comprising a nucleic acid encoding for a mAb so that the mAb is produced along with NAPRIs;

(b) forming a buffered solution of the mAb and NAPRIs;

(c) reducing the amount of NAPRIs by performing the method according to any one of the preceding claims, or by the use according to any one of the preceding claims on the buffered solution of mAb and NAPRIs; and

(d) isolating the mAb from the buffered solution.

~~21~~16. The method according to claim ~~20~~15, wherein the buffered solution of mAbs has been subjected to chromatography before passing through the synthetic depth filter.

~~22. The method according to claim 20, wherein the chromatography is affinity chromatography, anion exchange chromatography, cation exchange chromatography or multimodal (mixed mode) chromatography.~~

~~23. The method according to claim 22, wherein the chromatography is affinity chromatography using a Protein A resin, a Protein L resin, an Fc-selective resin, a Kappa light chain-selective resin, or a Lambda light chain-selective resin.~~

~~24~~17. The method according to ~~any one of~~ claim~~s 21-23~~15 or 16, wherein the chromatography ~~is~~ comprises one or more of ion exchange chromatography~~.~~

~~25. The method according to claim 24, wherein the ion exchange chromatography used an~~ , an anion exchange column, a cation exchange column, ~~or~~ multimodal (mixed mode) chromatography, or affinity chromatography.

~~26~~18. The method or the use according to any one of the preceding claims, wherein the buffered solution of monoclonal antibodies is passed through the depth filter at a temperature of between ~~about~~ 10° C and ~~about~~ 21° C, or between ~~about~~ 15° C and ~~about~~ 20° C.

~~27~~19. The method or the use according to any one of the preceding claims, wherein the buffered solution of monoclonal antibodies is passed through the depth filter at mass load of in the range of ~~about~~ 100 g/m^2 to ~~about~~ 2500 g/m^2, ~~about~~ 300 g/m^2 to ~~about~~ 2000 g/m^2, or ~~about~~ 500 g/m^2 to ~~about~~ 1500 g/m^2.

~~28~~20. The method or the use according to any one of the preceding claims, wherein the buffered solution of monoclonal antibodies has a pH in the range of ~~about~~ 4.0 to ~~about~~ 7.5, ~~about~~ 4.0 to ~~about~~ 7.2, or ~~about~~ 4.0 to ~~about~~ 5.5 when it passes through the depth filter.

~~29~~21. The method or the use according to any one of the preceding claims, wherein the buffered solution of monoclonal antibodies is passed through the depth filter at a flow rate in the range of ~~about~~ 1 L/min*m² to ~~about~~ 10 L/min*m², ~~about~~ 1.5 L/min*m² to ~~about~~ 8 L/min*m², or at a flow rate that is about 4.3 L/min*m².