

antibody comprising one or more disulfide bonds has the capacity, under appropriate conditions, to reduce the disulfide bond, for example to a form –SH. In one embodiment the reducing agent itself comprises a single thiol group, two thiol groups or three or more thiol groups. Alternatively, the reducing agent does not comprise a thiol group itself.

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Thiol as employed herein refers to a group comprising the entity –SH.

In one embodiment the reducing agent is selected from the group comprising: glutathione (GSH), ethylene sulfite, 2-mercaptoethanol (BME), 2-mercaptoethylamine including salts thereof such as hydrochloride (also referred to as BMEA, bMEA or B-mea, β -mea or β mea), cysteine, such as cysteine-HCl, phosphorous acid and dithiothreitol (DTT), TCEP (tris(2-carboxyethyl)phosphine), THP (tris(hydroxypropyl)phosphine).

In one embodiment the reducing agent comprises a single thiol group. Advantageously if a reducing agent comprising a single thiol is employed the desired disulfide bond between the variable regions of monomers forms naturally i.e. without the need to perform a specific oxidation step. Examples of reducing agents comprising a single thiol group include, but are not limited to, glutathione, mercaptoethanol (such as 2-mercaptoethanol), mercaptoethylamine (such as 2-mercaptoethylamine) and cysteine (such as cysteine-HCl).

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In one embodiment the thiol reducing agent is mercaptoethanol (such as 2-mercaptoethanol), mercaptoethylamine (such as 2-mercaptoethylamine), and in particular 2-mercaptoethylamine (also referred to as BMEA, bMEA or B-mea, β -mea or β mea).

In one embodiment the reducing agent is added before the antibody composition is in the temperature range of the present disclosure. In one embodiment the step a) is performed after treatment with a reducing agent, in particular wherein the reducing agent is removed prior to heating. The stronger the reducing agent the more likely it is to be suited for use in a pre-treatment step and removed prior to step a). Accordingly, in one embodiment wherein the reducing agent is selected from phosphorous acid, DDT, TCEP and THP, the reducing agent is removed prior to step a). If required the reducing agent may be removed prior to heating by routine techniques including diafiltration and the like. Use of TCEP may require an oxidation step to reintroduce the desired disulfide bonds in the final antibody molecules.

In one embodiment the reducing agent is not removed before the temperature is brought into the range, 30 to 60°C. The embodiment wherein the reducing agent remains in the composition during heating in step a) is particularly of benefit for reducing agents which contain a single thiol group such as the reducing agents selected from glutathione, mercaptoethanol (such as 2-mercaptoethanol), mercaptoethylamine (such as 2-mercaptoethylamine) and cysteine (such as cysteine-HCl).

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Claims:

1. A method for increasing the percentage of monomer in a composition of recombinantly expressed antibody molecules characterised in that the antibody molecule comprises at least one Fv with specificity for an antigen of interest comprising one VH and one VL wherein the antibody molecule is selected from the list consisting of a Fab-dsFv, a Fab-dsscFv, and a Fab-2xdsscFv, said method comprises:
 - a) a thermal conversion step of holding the composition comprising the antibody molecule at a temperature in the range 45 to 55°C, for example 50 to 55°C for a period of at least 1 hour,
 - b) wherein step a) is performed in the presence of a reducing agent or after treatment with a reducing agent, wherein the reducing agent is a thiol reducing agent which comprises a single thiol group and the reducing agent is present at a concentration of 1 mM to 100 mM.
2. A method according to claim 1, wherein the temperature is 50°C
3. A method according to claim 1 or claim 2, wherein the reducing agent is selected from the group comprising: glutathione (GSH), 2-mercaptoethanol (BME), 2-mercaptoethylamine (BMEA), cysteine-HCl and dithiothreitol (DTT).
4. A method according to claim 3, wherein the reducing agent is selected from 2-mercaptoethanol (BME) and 2-mercaptoethylamine (BMEA), in particular 2-mercaptoethylamine.
5. A method according to any one of claims 1 to 4, wherein the concentration of reducing agent is in the range 30 to 90mM, 60 to 90 mM or 70 to 80 mM.
6. A method according to any one of claims 1 to 5, wherein the period is in the range 1 to 70 hours, for example 3 to 10 hours, such as 4 to 6 hours, in particular 5 hours.
7. A method according to claim 6, wherein step a) is performed at a temperature in the range 45 to 55°C, for a period of 4 to 6 hours and in step b) the reducing agent is BMEA or BME and is at a concentration in the range 60 to 90mM.
8. A method according to claim 7, wherein step a) is performed at a temperature of 50°C, for a period of 5 hours and in step b) the reducing agent is BMEA or BME and is at a concentration 70 to 80mM, for example 75 mM.
9. A method according to any one of claims 1 to 8, wherein the reducing agent is employed in the presence of an amino acid.

10. A method according to claim 9, wherein the amino acid is selected from arginine, lysine and proline.
11. A method according claim 9 or claim 10, wherein the concentration of amino acid is in the range 0.01 to 1.0 M, for example 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 M.
12. A method according to any one of claims 1 to 11, wherein the antibody is at a concentration in the range 0.5g/L to 5g/L in the composition, for example 2 to 3 g/L.
13. A method according to any one of claims 1 to 12, wherein the thermal step is performed in the presence of concomitant stirring.
14. A method according to claim 13, wherein the stirring in the range 100 to 1200 rpm.
15. A method according to any one of claims 1 to 14, wherein reducing agent is added to the recombinant antibody composition before the temperature is raised to be in the range 45 to 55°C.
16. A method according to any one of claims 1 to 14, wherein reducing agent is added to the recombinant antibody composition after the temperature of the recombinant antibody composition has been raised to the range 45 to 55°C.
17. A method according to any one of claims 1 to 16, comprising the further step of downstream purification.
18. A method according to claim 17, wherein downstream processing comprises the step chromatography.
19. A method according to claim 18, wherein the chromatography is hydrophobic interaction chromatography.
20. A method according to claim 18, wherein the chromatography is ion exchange chromatography.
21. A method according to any one of claims 1 to 20, wherein the antibody is a Fab-dsFv.
22. A method according to claim 21, wherein the antibody is a bispecific antibody fusion protein which binds human OX40 and human serum albumin comprising:

a heavy chain comprising, in sequence from the N-terminal, a first heavy chain variable domain (V_{H1}), a C_{H1} domain and a second heavy chain variable domain (V_{H2}),

a light chain comprising, in sequence from the N-terminal, a first light chain variable domain (V_{L1}), a C_L domain and a second light chain variable domain (V_{L2}),

wherein said heavy and light chains are aligned such that V_{H1} and V_{L1} form a first antigen binding site and V_{H2} and V_{L2} form a second antigen binding site,

wherein the antigen bound by the first antigen binding site is human OX40 and the antigen bound by the second antigen binding site is human serum albumin.

23. A method according to claim 22, wherein the first variable domain of the heavy chain (V_{H1}) comprises the sequence given in SEQ ID NO:1 for CDR-H1, the sequence given in SEQ ID NO:2 for CDR-H2 and the sequence given in SEQ ID NO:3 for CDR-H3 and the first variable domain of the light chain (V_{L1}) comprises the sequence given in SEQ ID NO:4 for CDR-L1, the sequence given in SEQ ID NO:5 for CDR-L2 and the sequence given in SEQ ID NO:6 for CDR-L3,

wherein the second heavy chain variable domain (V_{H2}) has the sequence given in SEQ ID NO:11 and the second light chain variable domain (V_{L2}) has the sequence given in SEQ ID NO: 12 and

the second heavy chain variable domain (V_{H2}) and second light chain variable domain (V_{L2}) are linked by a disulfide bond.