Claims

1. A ~~M~~method for the separation of host cell proteins ~~(HCPs)~~, antibody fragments and low molecular weight substances from solutions containing antibodies, ~~characterized in that~~ said method comprising:
~~an aqueous, clarified cell culture~~ contacting a solution~~, having a pH value in the range of 2 – 11, preferably in the range of 5 – 9, and a conductivity in the range of 1 – 150 mS/cm, and which is~~ containing antibodies~~, is contacted for a period of time~~ with a hydrophobic chromatography material for a period of time
~~in a liquid chromatography column having a diameter ranging from 1 to 100 cm, preferably in the range of 5 to 50 cm, whereby the column is operated at pressures up to 100 bar, preferably at a pressure ranging from 0,2 to 80 bar and~~
whereby the antibodies stay in solution and ~~HCPs~~ host cell proteins, antibody fragments and low molecular weight substances are adsorbed by the hydrophobic chromatography material, ~~which is a rigid, particulate separation material having mean particle diameters in the range of 10 µm to 600 µm, preferably in the range of 20 µm to 150 µm, most preferred in the range of 20 µm to 63 µm, and having pore sizes in the range of 4 – 500 nm, preferably in the range of 10 – 30 nm, most preferred in the range of 13 nm to 25 nm~~
~~and~~
~~which is composed of cross-linked polymers, which in turn are composed of monovinylaromatic monomer and divinylaromatic monomer in~~
~~1.~~ wherein said hydrophobic chromatography material is a particulate material consisting of polystyrene or poly(ethyl)styrene, which is cross-linked with copolymer of divinylbenzene and ethyleneglycol methacrylate in a ratio of 98-:-2 up to 10-:-90–% by weight.

~~2. Method of claim 1, characterized in that the hydrophobic chromatography material is particulate and made of cross-linked vinylbenzene, crosslinked ethylvinylbenzene, polystyrene-divinylbenzene, polystyrene-divinylbenzene, polyethylstyrene-divinylbenzene, or of polyethylstyrene-divinylbenzene ethyleneglycol-dimethylacrylate resin.~~

~~3. Method according to claim 1 or 2, characterized in that a chromatography material is applied which is composed of cross-linked polymer composed of monovinylaromatic monomer and mixture of divinylaromatic and di(meth)acrylester monomer in a ratio 98 : 2 up to 10 : 90 % by weight.~~

~~4. Method according to one or more of the claims 1 – 3, characterized in that particulate chromatography material is applied, consisting of polystyrene or poly(ethyl)styrene, which is cross-linked with copolymer of divinylbenzene and ethylenglycol methacrylate in a ratio of 98 : 2 up to 10 : 90 % by weight.~~

~~5.~~2. The ~~M~~method ~~according to one or more of the claims 1 – 4~~ of claim 1, ~~characterized in that~~ wherein said solution containing antibodies is an aqueous solution ~~is applied~~ having a pH value in the range of ~~5 – 9~~ 2-11 and a conductivity in the range of ~~2 – 50~~ 1-150 mS/cm.

~~6.~~3. The ~~M~~method ~~according to one or more of the claims 1 – 5~~ of claim 1, ~~characterized in that the~~ wherein said solution containing antibodies is an aqueous solution, and said aqueous solution is passed through at a flow rate in the range of 150 ~~–~~ -1000 cm/min~~, preferably in the range of 300 – 900 cm/min~~.

~~7.~~4. The ~~M~~method ~~according to one or more of the claims 1 – 6~~ of claim 1, ~~characterized in that~~ wherein the separation is processed after ~~the~~ a Protein A affinity binding step.

~~8.~~5. The ~~M~~method ~~according to one or more of the claims 1 – 7~~ of claim 1, ~~characterized in that the sequence of purification treatments includes a treatment~~ further comprising contacting the solution with an ion exchange resin.

6. The method of claim 1, wherein said particulate material has mean particle diameters in the range of 10 µm to 600 µm.

7. The method according to claim 6, wherein said hydrophobic chromatography material has pore sizes in the range of 4-500 nm.

8. The method of claim 1, wherein said hydrophobic chromatography material has pore sizes in the range of 4-500 nm.

9. The ~~M~~method ~~according to one or more of the claims 1 – 8~~ of claim 1, ~~characterized in that at least one separation treatment~~ further comprising contacting said solution with an ion exchange resin, specific for the separation of Protein A, ~~is combined with a treatment with porous hydrophobic polymer beads as characterized in claim 1~~ resulting in a depletion of up to <10 ng ~~HCP~~ host cell proteins and removal of leached Protein A.

10. The method according to claim 9, wherein said hydrophobic chromatography material is hydrophobic polymer beads.

11. The method of claim 1, wherein said solution containing antibodies is an aqueous solution having a pH value in the range of 5-9 and a conductivity in the range of 2-50 mS/cm.

12. The method of claim 1, wherein said solution containing antibodies is an aqueous solution, and said aqueous solution is passed through at a flow rate in the range of 300-900 cm/min.

13. The method of claim 1, wherein said particulate material has mean particle diameters in the range of 20 µm to 150 µm.

14. The method of claim 1, wherein said particulate material has mean particle diameters in the range of 20 µm to 63 µm.

15. The method of claim 1, wherein said hydrophobic chromatography material has pore sizes in the range of 10-30 nm.

16. The method of claim 1, wherein said hydrophobic chromatography material has pore sizes in the range of 13 nm to 25 nm.

17. The method of claim 1, wherein said hydrophobic chromatography material is rigid hydrophobic porous polymer beads having mean particle

diameters in the range of 10 µm to 600 µm with pore sizes in the range of 4-500 nm.

18. A method for the separation of host cell proteins, antibody fragments and low molecular weight substances from solutions containing antibodies, said method comprising:
contacting a solution containing antibodies with a hydrophobic chromatography material for a period of time whereby the antibodies stay in solution and host cell proteins, antibody fragments and low molecular weight substances are adsorbed by the hydrophobic chromatography material,
wherein said hydrophobic chromatography material is poly(ethyl)styrene crosslinked with divinylbenzene or poly(ethyl)styrene crosslinked with divinylbenzene and ethylene glycol dimethylacrylate.

19. ~~Method according to claim 9, characterized that treatment(s) with ion exchange resin(s), specific for the separation of Protein A, are (is) combined with a treatment with~~ A method for the separation of host cell proteins, antibody fragments and low molecular weight substances from solutions containing antibodies, said method comprising:
contacting a solution containing antibodies with a hydrophobic chromatography material for a period of time whereby the antibodies stay in solution and host cell proteins, antibody fragments and low molecular weight substances are adsorbed by the hydrophobic chromatography material,
~~10.~~ wherein said hydrophobic chromatography material is hydrophobic polymer beads consisting of cross-linked vinylbenzene, poly(ethyl)styrene-divinylbenzene, or ~~of~~ polystyrene-divinylbenzene ethyleneglycol-dimethylacrylate resin.

~~11. Use of hydrophobic chromatographic separation materials having pore sizes in the range of 4 nm to 500 nm, preferably in the range of 10 nm – 30 nm, most preferred in the range of 13 nm to 25 nm for the separation of host cell proteins (HCPs), antibody fragments and low molecular weight substances from solutions containing antibodies in a method according to claims 1 - 10.~~

~~12. Use of hydrophobic, rigid polymer beads, having mean particle diameters in the range of 10µm to 600 µm, preferably in the range of 20 µm to 150 µm, most preferred in the range of 20 µm to 63 µm, and pore sizes in the range of 4 nm to 500 nm, preferably in the range of 10 nm – 30 nm, most preferred in the range of 13 nm to 25 nm, for the separation of host cell proteins (HCPs), antibody fragments and low molecular weight substances from solutions containing antibodies in a method according to claims 1 - 10.~~