associated costs for consumables and/or energy are high and IgG monomer/dimer recovery is always less than 90%.

The present authors have developed a process through which the use of reagents to precipitate high molecular weight aggregates/polymers and/or proteins as described in the prior art has been dispensed with, and surprisingly have achieved virtually total elimination of the polymers generated, with high productivity, lower production costs and easy implementation in comparison with the processes in the prior art. Furthermore, through the use of this process, stability has been achieved for the final product in liquid, preferably formulated in the presence of amino acids or polyalcohols, and it may be kept in liquid for at least 1 year at a temperature of between 2 and 30°C and a pH of 4.6 or above and up to 5.8.

This invention therefore relates to a process for obtaining an IgG composition from a partly purified IgG solution from human plasma which comprises the steps of:

a) diafiltering the partly purified IgG solution;
b) stabilising the solution obtained in step a);
c) heat treating the solution obtained in step b);
d) selectively adsorbing the high molecular weight aggregates and/or polymers from the heat treated solution in step c) through cation chromatography; and
e) diafiltering and formulating the solution obtained in step d).

In one embodiment, there is provided a process for obtaining an IgG composition from an IgG solution partly purified from human plasma having an IgG content of more than 95% in relation to total proteins comprising the steps of:

a) diafiltering the partly purified IgG solution;

b) stabilising the solution obtained in step a);

c) heat treating the solution obtained in step b);

d) selectively adsorbing high molecular weight aggregates and/or polymers from the solution heat treated in step c) through cation chromatography; and

e) diafiltering and formulating the solution obtained in step d).

Through the use of this process a significant reduction in the high molecular weight aggregates/polymer content, that is to say those higher than the dimer of IgG and other unstable proteins, is achieved, giving rise to a solution which essentially contains IgG monomers/dimers that can be formulated in a slightly acid medium, and can be kept in a liquid at ambient temperature without noticeable signs of

CLAIMS

1. A process for obtaining an IgG composition from an IgG solution partly purified from human plasma comprising the steps of:

a) diafiltering the partly purified IgG solution;

b) stabilising the solution obtained in step a);

c) heat treating the solution obtained in step b);

d) selectively adsorbing high molecular weight aggregates and/or polymers from the solution heat treated in step c) through cation chromatography; and

e) diafiltering and formulating the solution obtained in step d).

2. A process according to claim 1, characterised in that the said process is carried out starting with an IgG solution purified from human plasma having an IgG content of more than 95% in relation to total proteins.

3. A process according to claim 1 or 2, characterised in that said process is carried out starting with an IgG solution purified from human plasma having an IgG content of more than 97% in relation to total proteins.

4. A process according to any one of the preceding claims, characterised in that the diafiltration step (a) is carried out until the ethanol concentration is less than 0.5% (weight/volume), preferably less than 0.1% (weight/volume).

5. A process according to any one of the preceding claims, characterised in that the diafiltration step (a) is carried out until the concentration of non-denatured precipitation reagents such as PEG, octanoic acid, compatible non-ionic

detergents or any mixture thereof is less than 2% (weight/volume) and in any case will not give rise to more than 3% of polymer after step c).

6. A process according to any one of the preceding claims, characterised in that the diafiltration step (a) is carried out until the ionic strength of the starting IgG solution is less than 1 mS/cm.

7. A process according to any one of the preceding claims, characterised in that the pH value at the end of step (a) is within the range from 4.2 to 6.0.

8. A process according to any one of the preceding claims, characterised in that the diafiltration step (a) is carried out with water for injection or with a buffer solution of low ionic strength.

9. A process according to claim 8, characterised in that the buffer solution of low ionic strength is a ≤ 5mM solution of acetic acid or sodium acetate, having a pH between 4.0 and 5.0.

10. A process according to any one of the preceding claims, characterised in that the diafiltration step (a) is carried out in tangential flow mode across membranes having a molecular cut-off between 10 kDa and 100 kDa.

11. A process according to any one of the preceding claims, characterised in that in the diafiltration step (a) the proteins are concentrated to a concentration not exceeding some 5% (weight/volume), preferably between 2% and 4% (weight/volume).

12. A process according to any one of the preceding claims, characterised in that sorbitol is used as a stabilising agent in the stabilisation step (b).

13. A process according to claim 12, characterised in that the concentration of sorbitol used in the stabilisation step (b) is less than 50% (weight/weight), and preferably is between 30% and 35% by weight.

14. A process according to any one of the preceding claims, characterised in that the pH is adjusted to between 4.6 and 5.2 in the stabilisation step (b).

15. A process according to any one of the preceding claims, characterised in that the heat treatment (step c) is carried out at a temperature between 55°C and 63°C, for a time of between 1 and 24 hours.

16. A process according to any one of the preceding claims, characterised in that the heat treatment (step c) is carried out at a temperature of 60 ± 1°C, for 10-11 hours.

17. A process according to any one of the preceding claims, characterised in that the selective adsorption step is carried out in a strong cation exchange chromatography column.

18. A process according to claim 17, characterised in that the strong cation exchange resin comprises at least one of the cationic sulfone groups such as sulfonyl, sulfonic, or sulfopropyl groups covalently bonded to an insoluble synthetic perfusion matrix comprising polymethacrylate or polystyrene whose particle size varies between 20 and 100 µm.

19. A process according to any one of the preceding claims, characterised in that in the selected adsorption step the injection flow is 5-30 column volumes/hour.

20. A process according to any one of the preceding claims, characterised in that sodium chloride is added to the solution heat treated in step c) up to a final concentration of between 0.2 and 0.5 M (mol/litre).

21. A process according to claim 20, characterised in that after the addition of sodium chloride the pH of the solution in step c) is adjusted to between 4.2 and 5.5.

22. A process according to any one of the preceding claims, characterised in that in the selective adsorption step between 1 and 10 litres of resin are used for each kg of IgG (dry) requiring purification, which is equivalent to a loading of between 100 and 1000 mg of IgG/ml of resin.

23. A process according to any one of the preceding claims, characterised in that in the selective adsorption step elution is carried out using a decreasing saline gradient.

24. A process according to any one of the preceding claims, characterised in that the process comprises at least one additional viral inactivation/elimination treatment step.

25. A process according to claim 22, characterised in that the additional viral inactivation/elimination treatment steps are carried out before or after the heat treatment step.

26. A process according to claim 22, characterised in that the additional viral inactivation/elimination treatment step is carried out by incubation at acid pH in the presence or absence of pepsin, or by treatment with non-ionic detergents, ionic solvents or by nanofiltration.

27. A process according to any one of the preceding claims, characterised in that the diafiltration step (e) is carried out with water for injection or with a buffer solution of low ionic strength.

28. A process according to any one of the preceding claims, characterised in that in the diafiltration step (e) stabilisers for the final formulation are added.

29. A process according to claim 28, characterised in that the pH of the final formulation is between 4.6 and 5.8.

30. A process according to any one of the preceding claims, characterised in that the diafiltration step (e) is carried out in the tangential flow mode through membranes having a molecular cut-off between 10 kDa and 100 kDa.

31. A process according to any one of the preceding claims, characterised in that in the diafiltration step (e) the proteins are concentrated to a value of between 5% and 22% (w/v).

Claims

1. A process for obtaining an IgG composition from an IgG solution partly purified from human plasma having an IgG content of more than 95% in relation to total proteins comprising the steps of:

a) diafiltering the partly purified IgG solution;
b) stabilising the solution obtained in step a);
c) heat treating the solution obtained in step b);
d) selectively adsorbing high molecular weight aggregates and/or polymers from the solution heat treated in step c) through cation chromatography; and
e) diafiltering and formulating the solution obtained in step d).

2. A process according to claim 1, **characterised in that** said process is carried out starting with an IgG solution purified from human plasma having an IgG content of more than 97% in relation to total proteins.

3. A process according to claim 1 or 2, **characterised in that** the diafiltration step (a) is carried out until the ethanol concentration is less than 0.1% (weight/volume).

4. A process according to any one of the preceding claims, **characterised in that** the diafiltration step (a) is carried out until the concentration of non-denatured precipitation reagents such as PEG, octanoic acid, compatible nonionic detergents or any mixture thereof is less than 2% (weight/volume) and in any case will not give rise to more than 3% of polymer after step c).

5. A process according to any one of the preceding claims, **characterised in that** the diafiltration step (a) is carried out until the ionic strength of the starting IgG solution is less than 1 mS/cm.

6. A process according to any one of the preceding claims, **characterised in that** the pH value at the end of step (a) is within the range from 4.2 to 6.0.

7. A process according to any one of the preceding claims, **characterised in that** the diafiltration step (a) is carried out with water for injection or with a buffer solution of low ionic strength.

8. A process according to claim 7, **characterised in that** the buffer solution of low ionic strength is a ≤ 5mM solution of acetic acid or sodium acetate, having a pH between 4.0 and 5.0.

9. A process according to any one of the preceding claims, **characterised in that** the diafiltration step (a) is carried out in tangential flow mode across membranes having a molecular cut-off between 10 kDa and 100 kDa.

10. A process according to any one of the preceding claims, **characterised in that** in the diafiltration step (a) the proteins are concentrated to a concentration not exceeding some 5% (weight/volume), preferably between 2% and 4% (weight/volume).

11. A process according to any one of the preceding claims, **characterised in that** sorbitol is used as a stabilising agent in the stabilisation step (b).

12. A process according to claim 11, **characterised in that** the concentration of sorbitol used in the stabilisation step (b) is less than 50% (weight/weight), and preferably is between 30% and 35% by weight.

13. A process according to any one of the preceding claims, **characterised in that** the pH is adjusted to between 4.6 and 5.2 in the stabilisation step (b).

14. A process according to any one of the preceding claims, **characterised in that** the heat treatment (step c) is carried out at a temperature between 55°C and 63°C, for a time of between 1 and 24 hours.

15. A process according to any one of the preceding claims, **characterised in that** the heat treatment (step c) is carried out at a temperature of 60 ± 1°C, for 10-11 hours.

16. A process according to any one of the preceding claims, **characterised in that** the selective adsorption step is carried out in a strong cation exchange chromatography column.

17. A process according to claim 16, **characterised in that** the strong cation exchange resin comprises at least one of the cationic sulfone groups such as sulfonyl, sulfonic, or sulfopropyl groups covalently bonded to an insoluble synthetic perfusion matrix comprising polymethacrylate or

polystyrene whose particle size varies between 20 and 100 µm.

18. A process according to any one of the preceding claims, **characterised in that** in the selected adsorption step the injection flow is 5-30 column volumes/hour.

19. A process according to any one of the preceding claims, **characterised in that** sodium chloride is added to the solution heat treated in step c) up to a final concentration of between 0.2 and 0.5 M (mol/litre).

20. A process according to claim 19, **characterised in that** after the addition of sodium chloride the pH of the solution in step c) is adjusted to between 4.2 and 5.5.

21. A process according to any one of the preceding claims, **characterised in that** in the selective adsorption step between 1 and 10 litres of resin are used for each kg of IgG (dry) requiring purification, which is equivalent to a loading of between 100 and 1000 mg of IgG/ml of resin.

22. A process according to any one of the preceding claims, **characterised in that** in the selective adsorption step elution is carried out using a decreasing saline gradient.

23. A process according to any one of the preceding claims, **characterised in that** the process comprises at least one additional viral inactivation/elimination treatment step.

24. A process according to claim 23, **characterised in that** the additional viral inactivation/elimination treatment

steps are carried out before or after the heat treatment step.

25. A process according to claim 23, **characterised in that** the additional viral inactivation/elimination treatment step is carried out by incubation at acid pH in the presence or absence of pepsin, or by treatment with non-ionic detergents, ionic solvents or by nanofiltration.

26. A process according to any one of the preceding claims, **characterised in that** the diafiltration step (e) is carried out with water for injection or with a buffer solution of low ionic strength.

27. A process according to any one of the preceding claims, **characterised in that** in the diafiltration step (e) stabilisers for the final formulation are added.

28. A process according to claim 27, **characterised in that** the pH of the final formulation is between 4.6 and 5.8.

29. A process according to any one of the preceding claims, **characterised in that** the diafiltration step (e) is carried out in the tangential flow mode through membranes having a molecular cut-off between 10 kDa and 100 kDa.

30. A process according to any one of the preceding claims, **characterised in that** in the diafiltration step (e) the proteins are concentrated to a value of between 5% and 22% (w/v).