

PATENT SPECIFICATION

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- (21) Application No. 50379/77 (22) Filed 2 Dec. 1977 (19)
(31) Convention Application No. 747063 (32) Filed 3 Dec. 1976 in
(33) United States of America (US)
(44) Complete Specification Published 9 Jan. 1980
(51) INT. CL³ C07G 7/00
(52) Index at Acceptance
C3H 100 120 130 FZ



(54) FRACTIONATION PROCESS

(71) I, MYER LOUIS COVAL, a Canadian citizen of 6241 Chelton Drive, Oakland 94611, California, United States of America, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:-

5 The invention relates to gamma globulins and particularly to a gamma globulin fraction suitable for administration by intravenous injection. 5

The immunoglobulin G fraction of pooled human plasma contains antibodies to many viruses and bacteria. Immunoglobulins are effective in the clinical management of a wide variety of disease states, such as

- 10 1. prophylaxis and therapy of infections, in persons with genetic and nosocomial antibody deficiency states, especially staphylococci, pneumococci, streptococci and H.influenzae; 10
2. prophylaxis in patients with normal immunoglobulin levels, of viral infections (hepatitis, polio, measles, rubeola, rabies, herpes and parotitis), the prophylaxis of tetanus, and of Rh-incompatibility;
- 15 3. therapy of severe bacterial infections: staphylococci, coli, pseudomonas, pyocyanus septicemias, and also for the therapy of some viral infections such as Herpes zoster. 15

The full clinical potential of immunoglobulin G has not been determined because although many doses have been given intramuscularly, the intravenous preparations hitherto prepared are extremely degraded and thus low doses have been used. The major anti-bacterial 20 antibodies in human gamma globulin are against microorganisms which inhabit the upper respiratory tract, the skin and the gastrointestinal tract. The quantity of gamma globulin needed to overcome an experimental *in vivo* infection is proportional to the number of infectious organisms in the inoculum. This has been shown for *Pseudomonas aeruginosa*, *E. coli*, *Proteus*, and *Staphylococcus aureus*. The quantity of gamma globulin needed is also 25 proportional to the specific antibody level in the preparation. With chloramphenicol there is a synergistic action, but with other antibiotics there is only an additive effect. 25

Human immunoglobulins were first isolated on a large scale during the period from 1945 to 1950 at Harvard in F.J. Cohn's laboratory. It was soon observed that intravenous injection of these preparations caused shock reactions in some patients and it was subsequently established that the anticomplementary activity of IgG preparations is responsible for the shock 30 reactions. This anticomplementary activity is due to IgG aggregates formed during the fractionation. 30

In view of the shock reactions associated with the intravenous administration of the immunoglobulins, these therapeutically useful substances have hitherto been administered 35 intramuscularly. 35

However, the intramuscular administration of immunoglobulins has got many limitations:

- a) administration is painful;
 - b) the amount which can be administered is limited;
 - c) proteolysis at the site of injection decreases the available IgG;
 - 40 d) maximum blood levels are attained only after three or four days, which is a serious 40
- handicap in those cases requiring high blood levels of IgG immediately after injection.

Furthermore, intravenous administration of immunoglobulins has wider clinical application because the full dose of IgG enters the blood stream immediately without being degraded at the site of injection and significantly higher blood levels can be attained. These 45 considerations have prompted the search for methods to prepare IgG with low anticom- 45

plementary activity which is suitable for intravenous use. The methods which have been developed hitherto are based on proteolytic or chemical treatment to abolish the anti-complementary properties of the aggregates. Examples of preparations obtained by these methods are:

1. Pepsin-treated Immunoglobulin. In this preparation the protein is extensively degraded to antibody fragments (5S,F(ab')₂). Its usefulness for combatting bacterial infections is however limited because it has a short-life (about 30 hours compared with 20 to 30 days for negative IgG). After combining with antigens, the 5S fragments do not fix complement. It has no application in prophylaxis.

2. Plasmin-treated Immunoglobulin. More than 60% of this preparation is degraded to fragments (Fab and Fc). The remaining 7S globulin has a normal half-life (three to four weeks), but the antibody spectrum is limited.

3. pH 4-treated Immunoglobulin. This preparation has a tendency to become anticomplementary during storage. Its compatibility is therefore restricted and high doses cannot be administered. The half-life is slightly reduced (12 to 14 days) and the antibacterial activity is reduced to an unknown degree.

4. β -propiolactone-treated Immunoglobulin. The molecules are extensively altered, probably forming new antigenic determinants. The half-life is about 10 days. The bacteriolytic activity is reduced.

The four IgG subclasses have different susceptibilities to proteolysis. Thus the pepsin, plasmin and pH 4 (pepsin) preparations mentioned above differ markedly from untreated IgG in their subclass distribution.

As noted above, the undesirable anticomplementary activity which is responsible for the shock reaction produced by the intravenous administration of IgG is due to the aggregates present therein, which are formed during the fractionation procedures used in preparation. The preparations described above are obtained by methods which use procedures to destroy these aggregates after they are formed, in most cases by either chemical or enzymatic degradation. However, such degradation procedures also result in some degradation of the IgG with consequent loss of activity. Such preparations as described above are thus not as active as desired. Little work has been done on developing methods which prevent the formation of aggregates and provide IgG preparations having substantially no anticomplementary activity.

More recently, there has been disclosed in German Offenlegungsschrift 2,357,800, published June 6, 1974, a method for the preparation of a gamma globulin suitable for intravenous administration. This procedure, as well as other published procedures for the preparation of gamma globulin, requires as starting material a relatively purified gamma globulin fraction. However, of greater significance, the gamma globulin obtained by this method still possesses an excessively high anticomplementary activity for intravenous use.

It has also been proposed (U.S. Patent Specification Number 3,763,135) to prepare a material suitable for intravenous injection from Fraction III but the yield is not high and the product still exhibits an appreciable anticomplementary activity.

U.S. Food and Drug Administration standards are available for intramuscularly administered gamma globulin, but not for intravenously administered gamma globulin. Such standards are needed to distinguish between gamma globulin which causes shock-like reactions when given by the intravenous route to sensitive individuals and gamma globulin which does not elicit such reactions.

During the past fifteen years, it has been established that no clinical symptoms are observed, even in highly sensitive recipients, when the level of anticomplementary activity is sufficiently low. With the unit of the standard Mayer two unit assay (EXPERIMENTAL IMMUNOCHEMISTRY. By E. A. Kabat and M. M. Mayer 2nd Ed., p. 133, Thomas, Springfield, Ill., 1961), the safe level is 0.04 to 0.02 units or less of anticomplementary material per milligram of immunoglobulin G, and may be somewhat higher than 0.04, but reactions are routinely observed when the level is 0.4 units per milligram. The designation of gamma globulin preparations as suitable for intravenous use, signifying the absence of clinical reactions, is dependant on a specific low level of anticomplementary activity. It is also necessary to preserve the physiological antibody activity and specificity, in order to provide a clinically safe and effective preparation.

DT-OS 2606118 describes the preparation of a product having less than 0.020 units per mg of anticomplementary activity as measured according to the method of KABAT and MAYER (Experimental Immunochimistry, 2nd ed., pp 224 [1961] Thomas Springfield, Ill.) and suitable for intravenous injection. However, the anticomplementary activity is still appreciable.

We have now found that, using certain specific fractionation steps, a gamma globulin fraction may be isolated which not only retains the properties of native gamma globulin molecules but is also substantially free of aggregates and their anticomplementary activity

and is thus suitable for administration intravenously.

Thus, according to the present invention, there is provided a process for the preparation of a gamma globulin fraction substantially free of anticomplementary activity and suitable for intravenous administration which comprises:

- 5 a) extracting a paste or powder of Fraction II or II + III plasma protein or of a placental extract containing said Fraction with pyrogen-free water at a pH of from 4.9 to 6;
- b) separating the extract thus obtained from solid material and treating said extract with polyethylene glycol in an amount of about 4% weight/volume;
- 10 c) treating the glycol-treated extract optionally after separation from solid material thus precipitated, with ethanol in an amount of from 4 to 12% weight/volume at temperatures of from -6 to 10°C;
- d) separating solid material from the ethanol-treated extract thus obtained to yield a further extract; and
- 15 e) isolating the gamma globulin fraction from said further extract at temperatures of from -6 to 20°C and at a pH of from 7 to 8.2 by means of the addition of either polyethylene glycol in an amount of up to 12% weight/volume or of ethanol in an amount of up to 30% volume/volume, said gamma globulin fraction being substantially free of anticomplementary activity.

20 According to a further features of the present invention there is provided a gamma globulin fraction suitable for intravenous administration and having an anticomplementary activity of less than 0.01 units per mg.

The gamma globulin fraction according to the invention is suitable for intravenous injection, shows an antibody spectrum which is substantially unaltered as compared with the starting Fraction and shows substantially no anticomplementary activity *in vitro* even after lyophilization. The anticomplementary activity of the fraction is so low that it cannot be measured by the method of KABAT and MAYER (*loc. cit.*). In a new assay the conditions of this method are altered so that the number of erythrocytes is reduced by a factor of ten; the complement is correspondingly decreased and this assay thus exhibits a ten to eleven fold increase in sensitivity. The anticomplementary activity of the product produced according to the invention is, measured by this method, generally from 0.0005 to 0.0025 units per mg, whereas the product of the above mentioned DT-OS 2606118 has an activity of 0.010 to 0.020 units per mg.

In addition the fraction has a pH within the physiological range and a biological half-life of about 3 to 4 weeks. Further a freeze-dried product may be produced which has a long shelf-life and which is easy to reconstitute.

35 The paste or powder of Fraction II or II + III plasma protein may be obtained as described by Cohn *et al.* in J. Am. Chem. Soc. 68, 459-475 (1946) and contains nearly all of the plasma immunoglobulins in addition to other proteins. Preferably the Fraction will have a protein content of from 20 to 30% weight/weight. Placental extracts containing the Fraction may be obtained according to conventional methods.

40 The extraction with pyrogen-free water is preferably effected at temperatures of from 0 to 5°C. The pyrogen-free water is mixed with the paste or powder, preferably in an amount of from 25 to 45 litres of water per kilogram of paste or powder and optionally in the presence of a salt e.g. sodium chloride. In addition, the pyrogen-free water may contain about 2% weight-volume polyethylene glycol and about 0.2% weight/volume albumin. The suspension thus formed preferably has a conductivity of about $300 \times 10^{-6} \text{ cm}^{-1} \cdot \text{ohm}^{-1}$. Preferably the suspension is left for about 1 hour before separation of the solid material.

45 The treatment with polyethylene glycol is preferably effected at temperatures of from 0 to 5°C, whilst the treatment with ethanol is preferably effected at temperatures of from -6 to 0°C. In addition the ethanol is preferably used in an amount of about 6% weight/volume. Both treatments, i.e. in step (b) and step (c), are preferably effected at a pH of about 5.1.

50 The suspension of the extract in polyethylene glycol is preferably left for about one hour before separation of the solid material. The suspension of the glycol-treated extract in ethanol is preferably left for from 1 to 24 hours, more preferably 2 hours.

55 Separation of the solid material in any of steps (b), (c) and (d) may, for example, be effected by filtration or centrifugation. In the case of a placental extract particular care is required to remove all insoluble material formed at each stage, particularly floating insoluble material.

60 The further extract is adjusted to a pH of from 7 to 8.2, preferably about 8 and optionally sodium chloride is added thereto in a concentration of from 0.0025 to 0.15 M e.g. 0.01 M. The gamma globulin fraction is precipitated by the addition of either polyethylene glycol in an amount of up to 12% e.g. 10 to 12% weight/volume or of ethanol in an amount of up to 30% e.g. 20 to 30% preferably about 25% volume/volume. Separation of the precipitated fraction is preferably effected by centrifugation e.g. by continuous high speed, flow thru centrifugation. Preferred temperatures for the isolation are from -6 to 0°C.

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Throughout the process, the polyethylene glycol used will preferably have a number average molecular weight of from 4000 to 6000. The isolated gamma globulin fraction may, if desired, subsequently be dissolved in a carrier or adjuvant. The carrier or adjuvant may advantageously include human albumin, sodium acetate, potassium acetate, glycine, lactose, and/or mannitol, mannitol being included if it is subsequently desired to lyophilize the solution.

The solution obtained is preferably adjusted to a pH of from 5.0 to 5.5 and may then be stored as a liquid below 10°C or lyophilized. If the solution is to be lyophilized then preferably it will be adjusted to a pH of 6.4 to 6.6. Lyophilization may then be effected according to conventional techniques.

Solutions of the gamma globulin fraction stored below 10° have proved stable for at least one year whilst lyophilized powders have proved stable for at least 2 years.

As indicated above, the gamma globulin fraction exhibits an antibody spectrum which is substantially unaltered as compared with the types and levels of gamma globulin antibodies present in the plasma Fraction whilst still showing substantially no anticomplementary activity. Advantageously the fraction is substantially free of aggregates and decomposition products F(ab)₁, F(ab)₂ and Fc. Preferably the fraction has a sedimentation coefficient of about 7S.

The gamma globulin fractions according to the invention are thus useful in pharmaceutical preparations suitable for intravenous administration. Thus they may be incorporated into pharmaceutical compositions in a form suitable for intravenous administration in association with a carrier or excipient according to conventional methods. Thus, for example they may be dissolved in an aqueous solution buffered to a pH of from 5.4 to 6.7 and containing glycine and albumin. The concentration of the gamma globulin fraction is preferably adjusted to 5° weight/volume. Suitable buffers include, for example, phosphate and sodium acetate-acetic acid systems.

To prevent or reduce any denaturation at a liquid-air or liquid solid interface of the product in solution, it is advantageous to add a surfactant to the pharmaceutical composition. Suitable surfactants are non-ionic surfactants such as the block copolymers of propylene and ethylene oxides such as "Pluronic" (registered Trade Mark) 68 (poloxamer 188) and partial esters of sorbitol and polyoxyethylene oxides of long chain fatty acids such as the Tweens 20, 40, 60, 80 and 85 (the word "Tween" is a registered Trade Mark) (polysorbates 20, 40, 60, 80 and 95), water-soluble substances described in the 1973 edition of the Cosmetic, Toiletry and Fragrance Association, Inc. CTFA Cosmetic Ingredient Dictionary, and fluoro surfactants such as "Zonyl" (registered Trade Mark) FSA, FSB, FSC and FSN. These non-ionic surfactants stabilize proteins against surface denaturation and do not contain as part of their structure any chemical groups which may otherwise interact with or denature proteins.

The gamma globulin fraction according to the present invention when incorporated into pharmaceutical compositions has a longer half-life than other gamma globulin preparations now on the market. The gamma globulin fraction has proved to be useful for intravenous administration in all instances and for all conditions where intravenous administration is desired without any of the usual undesirable effects associated with the intravenous administration of hitherto known gamma globulin fractions. The following non-limiting Examples serve to illustrate the present invention.

Example 1

Cohn fraction II + III paste is suspended in pyrogen-free distilled water at 0-5°C at a concentration of 30 litres per kilo. A range of 20 to 50 may be used. The pH of the suspension is then adjusted to pH 5.1 (range 4.9 to 6.0) with dilute acetic acid (other acids like those mentioned above can be used). The suspension is then filtered or centrifuged at 0-5°C and the precipitate discarded. The filtrate is brought to 4% weight/volume polyethylene glycol 4000 (PEG 4000) (PEG 6000 and 12,000 can be used at other concentrations). The precipitate formed in one hour is removed as in the preceding step. The filtrate is then brought to 6% weight/volume in ethanol (range 4 to 12% weight/volume) with cautious addition at -2°C (range 0 to -6°C). The precipitate is again removed after 1 to 24 hours, preferably 2 hours.

Still at -2°C the solution is then made 0.01 M in NaCl and the pH is adjusted to 8.0 (range 7 to 8.2) with sodium hydroxide (1% weight/volume). The precipitate formed by the addition of ethanol to 25% volume/volume, or of polyethylene glycol 4000 to 10-12% weight/volume preferably 12% weight/volume to the solution still at -2°C is removed by continuous high speed, flow-thru centrifugation. The resulting paste is dissolved in the following solution: heated human albumin, 5 to 25 mg/ml, preferably 5 to 10 mg/ml, sodium acetate 0.025 M, glycine 0.15 M, mannitol 1 to 2% preferably 2% weight/volume, all adjusted with acetic acid to pH 5.1. Lactose may be substituted for mannitol.

The resulting solution, containing 5 to 6% weight/volume IgG can be lyophilized or stored

as a liquid below 10°C. If stored as a liquid, the mannitol is omitted from the dissolving solution. The solution can be lyophilized, freeze-dried, in the following manner: the pH is adjusted to 6.4 to 6.6 with 1% weight/volume sodium hydroxide; after dispensing in vials, in the desired amounts, the solution is then rapidly shell-frozen, and lyophilization is conducted according to current procedures, with care to avoid over-heating after the water has been removed. If lyophilized it is then reconstituted to form a 5 to 6% weight/volume solution of gamma globulin. The solution is stable frozen or at 10°C for at least one year and the freeze-dried powder for at least 2 years. The resulting purity is at least 97%, with the only detectable impurity albumin. The anticomplementary activity is below 0.01 units per mg gamma globulin, range 0.0005 to 0.0025 units. The units are measured by the two-unit assay of Mayer, cited above.

Example 2

In pyrogen-free, distilled water containing 1 to 4%, preferably 2% weight/volume polyethyleneglycol 4000 and 0.1 to 1%, preferably 0.2% weight/volume of human albumin Cohn fraction II paste or powder is dissolved at 0 to 5°C to yield a protein solution of 1 to 5%, preferably 2% weight/weight. The pH is adjusted to 5.1 (range 4.9 to 6.0, preferably 5.0 to 5.8), and the resuting precipitate formed after one hour is removed by filtration or by centrifugation.

The PEG concentration of the filtrate is raised to 4 per cent with a 50% weight/volume PEG-4000 solution or with dry PEG 4000 powder. Ethanol is then added to 6% weight/volume with slow addition so the temperature does not rise above 2°C. The precipitate formed is removed after one to 12 hours, preferably 12 hours. Still keeping the temperature below 2°C, the pH is then raised to 8.0 (range 6.8 to 8.1) and the resulting precipitate is collected by centrifugation after raising the ethanol concentration to 25% volume/volume and dissolved under the same conditions as in the first example.

The product contains over 99% gamma globulin, when measured before the addition of albumin in the dissolving solution. There is no detectable inhibition of hemolysis when 50 mg of the gamma globulin per ml, (a 5 per cent solution) is assayed in the standard Mayer assay, on below 0.01 units per mg IgG. The final solution contains only the added albumin, which has been previously heated by conventional procedures to remove any hepatitis B virus. No other proteins can be detected by conventional techniques such as cellulose acetate electrophoresis, immuno-electrophoresis, or immunodiffusion.

Example 3

Placental gamma globulin isolated by conventional procedures can be treated as in Example 2. Because placental gamma globulin as isolated conventionally contains a few per cent of impurities, which are plasma proteins, additional care is taken to remove all insoluble material at each step, particularly floating insoluble material before the pH is adjusted in the first step, and at all other steps. The 6% weight/volume ethanol filtrate is made 0.01 M in NaCl as in first example. The other steps are all as in Example 2. The purity of the product is at least 98 per cent gamma globulin, when measured (before the addition of the dissolving solution, which contains albumin, 5 to 10 mg/ml), by the same techniques as in Example 2.

The same dissolving solutions are used as in the first example, with mannitol omitted if the product is desired as a solution, and not to be lyophilized.

Example 4

Immunoglobulin G paste or fraction II, or dried fraction II powder of placental origin can be highly purified and also used to produce a product suitable for intravenous use by:

1. Suspending the paste or powder at 1% weight/volume concentration (range 0.3 to 5 per cent weight/volume) in water containing two per cent weight/volume polyethylene glycol 4000 (PEG 2000, 6000, 8000 and 12,000 average molecular weight may also be used), and 0.2 per cent weight/volume heated human albumin at 1°C (range 0 to 5 degrees centigrade). The insoluble floating material is removed by skimming.
2. The pH is then adjusted to 5.1 (range 4.9 to 6.0) with acetic or hydrochloric or citric or other acids. The precipitate is removed at one degree centigrade after one hour, by filtration or centrifugation.
3. The polyethylene glycol 4000 concentration is then raised to four per cent weight/volume. The floating material is again removed. Then the precipitate is removed by filtration or centrifugation.
4. Ethanol is added to six per cent weight/volume (range 2-12% weight/volume) by slow addition at -6°C (range -2° to -15°C).
5. The precipitate and floating material are removed as in the preceding steps.
6. Sodium chloride is added to 0.01 normality (range 0.025 to 0.15).
7. The pH is then raised to 8.0 (range 7 to 8).
8. Ethanol is added to 25% volume/volume final concentration slowly at -6°C.
9. The precipitate is collected by centrifugation.
10. The precipitate is dissolved to a final concentration of 5 to 6 per cent weight/volume on

the basis of determinations with known weights of paste and known volumes of the following solution: 0.5 per cent weight/volume human heated albumin (range 0.3 to 5 per cent weight/volume), sodium acetate (0.0125 or 0.025 N), glycine 0.15 N, pH 5.1 with acetic acid. The pH of the dissolved paste is adjusted to 6.6 with alkali such as 1 per cent weight/volume sodium hydroxide or potassium hydroxide or tris (hydroxymethyl)aminomethane.

11. If the solution is to be lyophilized, 2 per cent weight/volume mannitol is added (range 1 to 3 per cent weight/volume).

12. The resulting solution or freeze-dried powder contains no known impurities, and has less than 0.01 units of anticomplementary activity in the two unit complement assay of Mayer.

13. If the precipitate from step 9 is analyzed it contains more than 98% gamma globulin.

WHAT I CLAIM IS:

1. A process for the preparation of a gamma globulin fraction substantially free of anticomplementary activity and suitable for intravenous administration which comprises:

a) extracting a paste or powder of Fraction II or II + III plasma protein or of a placental extract containing said Fraction with pyrogen-free water at a pH of from 4.9 to 6;

b) separating the extract thus obtained from solid material and treating said extract with polyethylene glycol in an amount of about 4% weight/volume;

c) treating with glycol-treated extract, optionally after separation from solid material thus precipitated, with ethanol in an amount of from 4 to 12% weight/volume at temperatures of from -6 to 10°C;

d) separating solid material from the ethanol-treated extract thus obtained to yield a further extract; and

e) isolating the gamma globulin fraction from said further extract at temperatures of from -6 to 20°C and at a pH of from 7 to 8.2 by means of the addition of either polyethylene glycol in an amount of up to 12% weight/volume or of ethanol in an amount of up to 30% volume/volume, said gamma globulin fraction being substantially free of anticomplementary activity.

2. A process as claimed in claim 1 wherein the Fraction has a protein content of from 20 to 30% weight/weight.

3. A process as claimed in claim 1 or claim 2 wherein the pyrogen-free water and the paste or powder are used in amounts of from 25 to 45 litres of water per kilogram of paste or powder.

4. A process as claimed in any of the preceding claims wherein the extraction with pyrogen-free water is effected at temperatures of from 0 to 5°C.

5. A process as claimed in any of the preceding claims wherein the conductivity of the suspension of the paste or powder in the pyrogen-free water is about $300 \times 10^{-6} \text{ cm}^{-1} \text{ ohm}^{-1}$.

6. A process as claimed in any of the preceding claims wherein the treatment with polyethylene glycol in step (b) is effected at temperatures of from 0 to 5°C.

7. A process as claimed in any of the preceding claims wherein the treatment with ethanol in step (c) is effected at temperatures of from -6 to 0°C.

8. A process as claimed in claim 7 wherein the treatment with ethanol in step (c) is effected at a temperature of about -2°C.

9. A process as claimed in any of the preceding claims wherein, in the treatment with ethanol in step (c), the ethanol is present in an amount of about 6% weight/volume.

10. A process as claimed in any of the preceding claims wherein the treatment with polyethylene glycol in step (b) and/or the treatment with ethanol in step (c) is effected at a pH of about 5.1.

11. A process as claimed in any of the preceding claims wherein the further extract is adjusted to a pH of about 8 before isolation of the gamma globulin fraction.

12. A process as claimed in any of the preceding claims wherein sodium chloride is added to the further extract in a concentration of about 0.01 M before isolation of the gamma globulin fraction.

13. A process as claimed in any of the preceding claims wherein the isolation of the gamma globulin fraction is effected at temperatures of from -6 to 0°C.

14. A process as claimed in any of the preceding claims wherein isolation of the gamma globulin fraction is effected by means of the addition of polyethylene glycol in an amount of from 10 to 12% weight/volume.

15. A process as claimed in any of claims 1 to 13 wherein isolation of gamma globulin fraction is effected by means of the addition of ethanol in an amount of from 20 to 30% volume/volume.

16. A process as claimed in claim 15 wherein isolation of the gamma globulin fraction is effected by means of the addition of ethanol in an amount of about 25% volume/volume.

17. A process as claimed in any of the preceding claims wherein the polyethylene glycol used has a number average molecular weight of from 4000 to 6000.

(13)



- the basis of determinations with known weights of paste and known volumes of the following solution: 0.5 per cent weight/volume human heated albumin (range 0.3 to 5 per cent weight/volume), sodium acetate (0.0125 or 0.025 N), glycine 0.15 N, pH 5.1 with acetic acid. The pH of the dissolved paste is adjusted to 6.6 with alkali such as 1 per cent weight/volume sodium hydroxide or potassium hydroxide or tris (hydroxymethyl)aminomethane.
11. If the solution is to be lyophilized, 2 per cent weight/volume mannitol is added (range 1 to 3 per cent weight/volume).
12. The resulting solution or freeze-dried powder contains no known impurities, and has less than 0.01 units of anticomplementary activity in the two unit complement assay of Mayer.
13. If the precipitate from step 9 is analyzed it contains more than 98% gamma globulin.
- WHAT I CLAIM IS:
1. A process for the preparation of a gamma globulin fraction substantially free of anticomplementary activity and suitable for intravenous administration which comprises:
- 15 a) extracting a paste or powder of Fraction II or II + III plasma protein or of a placental extract containing said Fraction with pyrogen-free water at a pH of from 4.9 to 6;
- 15 b) separating the extract thus obtained from solid material and treating said extract with polyethylene glycol in an amount of about 4% weight/volume;
- 20 c) treating with glycol-treated extract, optionally after separation from solid material thus precipitated, with ethanol in an amount of from 4 to 12% weight/volume at temperatures of from -6 to 10°C;
- 20 d) separating solid material from the ethanol-treated extract thus obtained to yield a further extract; and
- 25 e) isolating the gamma globulin fraction from said further extract at temperatures of from -6 to 20°C and at a pH of from 7 to 8.2 by means of the addition of either polyethylene glycol in an amount of up to 12% weight/volume or of ethanol in an amount of up to 30% volume/volume, said gamma globulin fraction being substantially free of anticomplementary activity.
- 30 2. A process as claimed in claim 1 wherein the Fraction has a protein content of from 20 to 30% weight/weight.
- 30 3. A process as claimed in claim 1 or claim 2 wherein the pyrogen-free water and the paste or powder are used in amounts of from 25 to 45 litres of water per kilogram of paste or powder.
- 35 4. A process as claimed in any of the preceding claims wherein the extraction with pyrogen-free water is effected at temperatures of from 0 to 5°C.
- 35 5. A process as claimed in any of the preceding claims wherein the conductivity of the suspension of the paste or powder in the pyrogen-free water is about $300 \times 10^{-6} \text{ cm}^{-1} \text{ ohm}^{-1}$.
6. A process as claimed in any of the preceding claims wherein the treatment with polyethylene glycol in step (b) is effected at temperatures of from 0 to 5°C.
- 40 7. A process as claimed in any of the preceding claims wherein the treatment with ethanol in step (c) is effected at temperatures of from -6 to 0°C.
- 40 8. A process as claimed in claim 7 wherein the treatment with ethanol in step (c) is effected at a temperature of about -2°C.
9. A process as claimed in any of the preceding claims wherein, in the treatment with ethanol in step (c), the ethanol is present in an amount of about 6% weight/volume.
- 45 10. A process as claimed in any of the preceding claims wherein the treatment with polyethylene glycol in step (b) and/or the treatment with ethanol in step (c) is effected at a pH of about 5.1.
- 50 11. A process as claimed in any of the preceding claims wherein the further extract is adjusted to a pH of about 8 before isolation of the gamma globulin fraction.
- 50 12. A process as claimed in any of the preceding claims wherein sodium chloride is added to the further extract in a concentration of about 0.01 M before isolation of the gamma globulin fraction.
- 55 13. A process as claimed in any of the preceding claims wherein the isolation of the gamma globulin fraction is effected at temperatures of from -6 to 0°C.
- 55 14. A process as claimed in any of the preceding claims wherein isolation of the gamma globulin fraction is effected by means of the addition of polyethylene glycol in an amount of from 10 to 12% weight/volume.
- 60 15. A process as claimed in any of claims 1 to 13 wherein isolation of gamma globulin fraction is effected by means of the addition of ethanol in an amount of from 20 to 30% volume/volume.
- 60 16. A process as claimed in claim 15 wherein isolation of the gamma globulin fraction is effected by means of the addition of ethanol in an amount of about 25% volume/volume.
- 65 17. A process as claimed in any of the preceding claims wherein the polyethylene glycol used has a number average molecular weight of from 4000 to 6000.



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18. A process as claimed in any of the preceding claims wherein the pyrogen-free water additionally contains about 2% weight/volume polyethylene glycol and about 0.2% weight/volume albumin.
19. A process as claimed in any of the preceding claims wherein the isolated gamma globulin fraction is subsequently dissolved in a carrier or adjuvant.
20. A process as claimed in claim 19 wherein the carrier or adjuvant includes human albumin, sodium acetate, potassium acetate, glycine, lactose and/or mannitol.
21. A process as claimed in claim 19 or claim 20 wherein the solution of the isolated gamma globulin fraction in the carrier or adjuvant is adjusted to a pH of from 5.0 to 5.5.
22. A process as claimed in claim 21 wherein the solution is subsequently adjusted to a pH of 6.4 to 6.6.
23. A process as claimed in claim 22 wherein the solution is lyophilized.
24. A process as claimed in any of the preceding claims wherein the Fraction has a protein content of from 20 to 30% weight/weight, the conductivity of the suspension of the paste or powder in the pyrogen-free water is about $300 \times 10^{-6} \text{ cm}^{-1} \text{ ohm}^{-1}$ and isolation of the gamma globulin fraction is effected by means of the addition of either polyethylene glycol in an amount of from 10 to 12% weight/volume or ethanol in an amount of from 20 to 30% volume/volume.
25. A process as claimed in any of claims 1 to 23 wherein the extraction with pyrogen-free water is effected in the presence of a salt.
26. A process as claimed in claim 25 wherein the salt is sodium chloride.
27. A process for the preparation of a gamma globulin fraction substantially free of anticomplementary activity substantially as herein described.
28. A process for the preparation of a gamma globulin fraction substantially as herein described in any of Examples 1 to 4.
29. Gamma globulin fractions substantially free of anticomplementary activity whenever prepared by a process as claimed in any of claims 1 to 28.
30. Gamma globulin fractions as claimed in claim 29 being substantially free of aggregates and decomposition products $F(ab)_1$, $F(ab)_2$ and Fc .
31. Gamma globulin fractions as claimed in claim 29 or claim 30 having a sedimentation coefficient of about 7S.
32. A gamma globulin fraction suitable for intravenous administration and having an anticomplementary activity of less than 0.01 units per mg.
33. A fraction as claimed in claim 32 having an anticomplementary activity of from 0.0005 to 0.0025 units per mg.
34. A fraction as claimed in claim 32 or claim 33 substantially free of aggregates and decomposition products $F(ab)_1$, $F(ab)_2$ and Fc .
35. A fraction as claimed in any of claims 32 to 34 having a sedimentation coefficient of about 7S.
36. A fraction as claimed in any of claims 32 to 35 being soluble in water.
37. A fraction as claimed in any of claims 32 to 36 having an antibody spectrum and subclass distribution substantially the same as the plasma from which said fraction is obtained.
38. A fraction as claimed in any of claims 32 to 37 in lyophilized form.
39. A fraction as claimed in claim 38 which is stable for at least 2 years.
40. A gamma globulin fraction as claimed in claim 32 substantially as herein described.
41. A gamma globulin fraction substantially as herein described in any of Examples 1 to 4.
42. Pharmaceutical compositions in a form suitable for intravenous administration comprising, as active ingredient, a gamma globulin fraction as claimed in claim 32 in association with a carrier or excipient.
43. Compositions as claimed in claim 42 wherein the gamma globulin fraction is as defined in any of claims 33 to 41.
44. Compositions as claimed in claim 42 or claim 43 wherein the gamma globulin fraction is dissolved in an aqueous solution buffered to a pH of from 5.4 to 6.7 and containing glycine and albumin.
45. Compositions as claimed in claim 44 wherein the gamma globulin concentration is 5% weight/volume.
46. Compositions as claimed in any of claims 42 to 45 containing a surfactant.
47. Compositions as claimed in claim 46 wherein the surfactant is non-ionic.
48. Compositions as claimed in claim 47 wherein the surfactant is a block copolymer of propylene and ethylene oxides, a partial ester of sorbitol and a polyoxyethylene oxide of a long chain fatty acid or a fluoro surfactant.
49. Compositions as claimed in claim 42 substantially as herein described.



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For the Applicants,
FRANK B. DEHN & CO.,
Chartered Patent Agents,
Imperial House,
15-19 Kingsway,
LONDON, W.C.2.

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Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1979.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from
which copies may be obtained.