FRACTIONATION OF PLASMA USING GLYCINE AND POLYETHYLENE GLYCOL

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FROZEN OR FRESH CITRATED PLASMA (1.8 M GLYCINE)

PLASMA SUPERNATANT

1.1 DILUTION WITH 0.9% NaCl
PEG TO 30%

SALTS
PLASMA PROTEIN
PPT

DILUTION WITH 0.9% NaCl
TO ORIGINAL VOLUME

0.5% TRICALCIUM PHOSPHATE

UNABSORBED PROTEIN
PROTHROMBIN COMPLEX PPT

0.1 M NaCITRATE
ELUATE
5% PEG

20% PEG
TRICALCIUM PHOSPHATE

SALTS
PROTHROMBIN COMPLEX

AHF PRECIPITATE

3.5% PEG

10% PEG

FIBRINOGEN PPT

SUPT.
AHF PRECIPITATE

1.8 M GLYCINE

SUPT.
AHF CONCENTRATE

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FRACTIONATION OF PLASMA USING GLYCINE AND POLYETHYLENE GLYCOL
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ABSTRACT OF THE DISCLOSURE
Concentrates of antihemophilic factor A and prothrombin complex are prepared from citrated blood plasma by an initial fractionation with glycine followed by multiple fractionations of the AHF-containing precipitate and the prothrombin complex-containing supernate with polyethylene glycol, the AHF-containing fraction being given an additional fractionation with glycine and the prothrombin complex-containing fraction being given an intermediate adsorption with tribasic calcium phosphate.

CROSS-REFERENCES TO RELATED APPLICATIONS
This is a continuation-in-part of co-pending application Ser. No. 679,240, filed Oct. 30, 1967 now abandoned and co-pending application Ser. No. 834,883, filed June 19, 1969 now U.S. Pat. 3,560,475. Application Ser. No. 679,240 is a continuation-in-part of application Ser. No. 634,839, filed May 1, 1967 now abandoned, and has been referred as a continuation application May 1, 1970.

This invention relates to a method for the purification of blood coagulation components and, more particularly, to the preparation of concentrates of antihemophilic factor A and prothrombin complex from citrated blood plasma.

The process of blood coagulation is a complicated physiological activity and involves the interaction of numerous substances found in normal whole blood. It is known that certain factors associated with the blood coagulation mechanism are absent or seriously deficient in certain individuals. In those patients suffering from classical hemophilia, antihemophilic factor A (AHF, Factor VIII) is deficient. In those patients afflicted with hemophilia B, plasma thromboplastin component (PTC, Factor IX) is missing from the blood.

In the development of modern blood banking programs involving the collection and storage of large quantities of blood and blood components, the establishment of adequate preservation systems is critical. Since World War II it has been common practice to collect blood in a solution of citric acid, sodium citrate and dextrose, known as ACD blood. The problem of preserving blood is much simplified, however, when it is reduced to preservation of various blood components since it is easier to meet the environmental requirements of the separate components than of whole blood.

Moreover, it is wasteful and even detrimental to the patient to administer more blood components than required. Thus, the hemophiliac needing certain blood coagulation factors ideally is given only those factors required or at least a purified concentrate of those factors.

For the treatment of classical hemophilia, antihemophilic factor A fractions, 20 to 35 times purified in terms of activity per milligram of protein and 10 times concentrated, have been prepared by glycine-precipitation of either fresh or frozen plasma by Webster et al., Amer.


A major disadvantage in the use of plasma cryoprecipitate fractions as starting material for the preparation of antihemophilic factor A is that in practice only about 30% yield of AHF is obtained whereas yields of AHF on the order of about 70% are obtained by the glycine-precipitation of whole plasma. However, when citrated plasma is used for the AHF fractionation, the AHF-depleted plasma generally is unsuitable for preparation of plasma thromboplastin component (PTC, Factor IX) and other coagulation factors of the prothrombin complex. That is, citrated plasma is known to inhibit the adsorption of anticoagulation factors on calcium phosphate, as shown by Steinbuch and Solier, Propl. Geriatol Perelv. Krovi. II, pp. 15–21 (1966) (see Chem. Abstracts 66, p. 9226 (1967)).

Consequently, a method by which citrated plasma could be used directly for the preparation of separate concentrates of antihemophilic factor A and prothrombin complex would find much use in the field of blood coagulation therapy.

Accordingly, it is an object of this invention to provide a method for the purification of blood coagulation components.

It is another object of this invention to provide a method for the preparation of concentrates of AHF and prothrombin complex from citrated blood plasma.

Other objects and advantages will be apparent to those skilled in the art after reading the disclosure herein.

In accordance with the present invention, fresh or frozen citrated plasma is mixed with glycine, using a final glycine concentration of from about 1.3 to about 1.8 molar, to produce an AHF-containing precipitate and a prothrombin complex-containing plasma supernant. Each of the respective precipitate and supernate fractions are subjected to multiple fractionations with polyethylene glycol, the AHF-containing fraction being given an additional precipitation with glycine and the prothrombin complex-containing fraction being given an intermediate adsorption with tribasic calcium phosphate.

While the specification concludes with claims particularly pointing out and distinctly claiming the subject matter regarded as forming the present invention, it is believed that the invention will be better understood from the following detailed description taken in connection with the accompanying drawing which illustrates a preferred embodiment of the method of preparing the separate concentrates of antihemophilic factor A and prothrombin complex from citrated blood plasma.

As used herein, the term "prothrombin complex" refers to a concentrate of blood proteins which are active in the coagulation process comprising principally prothrombin (Factor II), proconvertin (Factor VII), antihemophilic factor B (Factor IX) and Stuart-Prower factor (Factor X).

After separation of the AHF-containing fraction from the prothrombin complex-containing fraction by the glycine precipitation step, each of the respective fractions is separately subjected to several polyethylene glycol precipitation steps. The polyethylene glycol used as a precipitating agent in this invention is a high molecular weight polymer which is generally produced by reacting ethyl-
ene oxide with ethylene glycol or water and has the following structure:

\[ \text{HO(CHO)CHOH} \]

in which \( n \) represents the average number of oxyethylene groups. According to the present invention the polyethylene glycol should be non-toxic and can range in molecular weight from about 200 to about 20,000. It preferably has a molecular weight of from about 440 to about 6,000, PEG 4,000, which is a polyethylene glycol product having an average molecular weight of about 4,000, is the preferred product of this group. The precipitation with these polyethylene glycol polymers is preferably conducted at normal room temperature (about 25°C).

In the preferred method of the invention for obtaining the prothrombin complex, the plasma supernatant from the glycine precipitation step is suspended in normal physiological saline (about 0.9% NaCl), preferably to a dilution of about 1:1, the pH is adjusted to from about 6.8 to about 7.2, and tribasic calcium carbonate is added. The resulting suspension is precipitated with polyethylene glycol to a final concentration of about 30% PEG. The resulting plasma protein precipitate is then separated from the supernatant. The supernatant, which contains the undesired citrate and glycine salts, is discarded. The plasma protein precipitate is then redissolved in normal physiological saline, the pH is adjusted to from about 6.8 to about 7.2, and tribasic calcium carbonate is thoroughly mixed with the suspension to adsorb the coagulation factors. The resulting tribasic calcium phosphate adsorbed-protein precipitate is then thoroughly mixed with from about 0.05 M to about 0.2 M trisodium citrate followed by recovery of the resulting supernatant which contains the desired coagulation factors. The supernatant is subjected to a second precipitation with polyethylene glycol, first at a pH of from about 6 to about 7 and to a final concentration of from about 3% to about 4% polyethylene glycol by weight of the starting material followed by recovery of the resulting supernate, second at a pH of from about 6.4 to about 7.4 and to a final concentration of about 10% polyethylene glycol by weight of the supernate followed by the recovery of the resulting precipitate and then fractionated by precipitation of said recovered precipitate with an aqueous glycine solution having a molarity of about 1.8.

In the polyethylene glycol precipitation step, the AHF-containing precipitate is first redisolved and then the redisolved material is subjected to the above-described two successive precipitations with polyethylene glycol, followed by recovery and redis solución of the second precipitate. The redisolved polyethylene glycol precipitated fraction is then subjected to precipitation with glycine.

Recovery of the polyethylene glycol and glycine precipitated fractions for use in this invention can be accomplished, for example, by centrifugation or filtration of the respective precipitates or by similar such procedures. Redisolution of the recovered precipitates can be achieved by warming and agitating in citrated saline solution. In the case of the redisolution of the AHF-containing starting precipitate, it is preferred to use a glycine citrated saline solution and to increase the volume of the mixture to about one twentieth the volume of the original plasma which the starting precipitate represents. The polyethylene glycol- and glycine-precipitated fractions are preferably redisolved with citrated saline solution to increase the volume of the mixture to about one hundredth the volume of plasma which the respective precipitated fractions represent.

It is also preferred to purify each of the respective redisolved starting precipitate and polyethylene glycol- and glycine-precipitated fractions by clarifying with additional centrifugation and/or filtration to remove any insoluble matter.

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The above fractionation by successive precipitation with polyethylene glycol and glycine has been found to provide a highly soluble AHF concentrate of high potency which can be frozen and rendered stable, such as by lyophilization, and retained under ordinary refrigeration conditions for periods of a year or longer. The potency of each batch of material prepared by the above fractionation method can be precisely determined so that the administering physician can know exactly how much AHF his patient receives.

Since the redissolved AHF concentrate prepared by the above fractionation method has more than ten times the AHF activity of an equal volume of plasma, the hemophilic can be given a quantity of AHF which the heart could not otherwise tolerate. Even more importantly, the AHF activity in the above-prepared concentrate is contained in less than 2.5% the amount of protein present in plasma providing an equal amount of AHF activity. This lower protein content minimizes the likelihood of allergic reactions by the hemophilic recipient and reduces the possibility of overloading the circulatory system.

The concentrate of AHF which has been prepared by successive precipitation with polyethylene glycol and glycine can be further purified to a final concentration of 5% with ECTEOLA cellulose resin. This purification can be carried out either before or after the polyethylene glycol and glycine precipitation and may be done by column or batch techniques. The concentrate purified by this method has the additional advantage in that it can also be administered intramuscularly as well as by intravenous administration methods generally used in the case of the AHF concentrate which has not been treated with the ECTEOLA cellulose resin. The AHF concentrate purified with ECTEOLA cellulose resin has been found to be free of fibrinogen by the addition of thrombin and by immunoelectrophoresis.

As used herein, the term "ECTEOLA cellulose resin" refers to a modified cellulose which contains active basic substituents introduced into the cellulose molecule by reaction with epichlorohydrin and triethanolamine.


ECTEOLA cellulose resins are available commercially. However desirable, it is desired to initially treat these resins by recycling them with caustic soda before use in the herein-defined purification procedure.

In the purification procedure with ECTEOLA cellulose resin, the resin preferably is first equilibrated with a chloride buffer solution having a concentration of about 0.8% NaCl and then poured into a chromatographic glass column. The AHF concentrate which is desired to be purified is then applied to the column and finally eluted with a chloride buffer solution having a molarity of about 0.5.

Other methods of further purifying the AHF concentrate of the present invention will be apparent to those skilled in the art after reading the invention described herein.

The following examples further illustrate the present invention although the invention is not limited to these specific examples which are provided for purposes of illustration and not limitation. All parts and percentages herein are on a weight basis unless otherwise specified.

**EXAMPLE 1**

Fresh citrated plasma (from ACD preserved blood) is mixed with an aqueous solution of glycine to a concentration of 1.8 molar glycine and the resulting suspension is centrifuged to precipitate which contains a concentrate of AHF, is retained for subsequent treatment in Examples 4 and 5, below. The plasma supernatant (pH 6-7) is diluted with an equal volume of normal physiological saline and then mixed with polyethylene glycol having a molecular weight of about 4000 (PEG 4000) to a concentration of 30% PEG. The resulting plasma protein precipitate is separated from the supernatant by centrifugation at 400,000 g for 20 minutes. The resulting supernatant, which contains the undesired citrate and glycine salts, is discarded.

The plasma protein precipitate is suspended in normal physiological saline to a concentration of 10% (weight/volume) and the pH adjusted to 7.2 with 1 N NaOH. 500 grams of tribasic calcium phosphate is then added to 50 liters of the plasma protein suspension and the mixture stirred for about 30 minutes. The suspension is then centrifuged and the supernatant discarded. The retained precipitate is suspended in 0.1 M trisodium citrate to a final volume of 5 liters. The suspension is again centrifuged and the precipitate discarded. The pH of the retained supernatant (about 5 liters) is then adjusted to 7.2 with 1 N HCl, polyethylene glycol 4000 is added to the final concentration of 5%, and the suspension stirred for about 30 minutes. The suspension is clarified by centrifugation, with retention of the supernatant and discard of the precipitate. The pH of the retained supernatant is then adjusted to 5.2 with 1 N HCl, and polyethylene glycol 4000 is added to a final concentration of 20%. The suspension is centrifuged and the precipitate that is recovered is dissolved in citrated saline (1 part 0.1 M trisodium citrate to 4 parts 0.9% sodium chloride) to a final volume of 2 to 5 liters, which is equivalent to one twentieth to one tenth the volume of the original plasma protein suspension. Heparin is added in an amount of one unit per ml, and the solution is clarified and sterilized by passage through a combination of graded pore sizes of membrane filters. The solution is filled under aseptic conditions in 10 to 30 ml sterile bottles, freeze dried and capped with stoppers. The freeze-dried material can be reconstituted with sterile water and then administered intravenously, subcutaneously or intramuscularly to patients who are deficient in one or more of the above-mentioned coagulation factors, particularly Factor IX.

The Factor IX activity of the reconstituted product is about 20 times as great as an equal volume of normal whole plasma and is contained in about one eighteenth the amount of protein in normal whole plasma.

**EXAMPLE 2**

Example 1 is repeated up to the point of suspending the plasma protein precipitate in normal physiological saline. Tribasic calcium phosphate N.F. is added to suspension to a concentration of 1%. The resultant tribasic calcium phosphate precipitate is then suspended in 0.005 M trisodium citrate to a volume equal to one two-hundredth the original plasma volume. After mixing for an interval of thirty minutes at 5°C, the suspension is centrifuged to remove the tribasic calcium phosphate particles. The pH is adjusted to 6.8 with 2 N acetic acid and PEG 4000 is added to provide a final concentration of 10%. The precipitate that forms is discarded. The supernatant is then adjusted to pH 5.2 and sufficient PEG 4000 is added to provide a final concentration of 20%. The suspension is centrifuged and the precipitate that is recovered is dissolved in citrated saline (one part of 0.1 M sodium citrate to 9 parts of a 5% sodium chloride solution) to a volume to provide 40 units of Factor IX per ml. The pH is adjusted to 6.8 with 1 N sodium hydroxide. Heparin in an amount of 3 units per ml is added, and the solution is filtered through a series of one micron of graded pore sizes of Millipore filters. The solution is filled under aseptic conditions into sterile 100 ml. glass bottles in units of 20 ml of solution per bottle. After shelf-freezing
and drying from the frozen state under aseptic conditions, the bottles are closed with sterile stoppers under vacuum. The dried product prepared in accordance with this example can be used for intravenous or intramuscular injection after reconstitution with 20 ml of sterile water per each unit of dry product.

**EXAMPLE 3**

Example 2 is repeated except that frozen plasma is used instead of fresh plasma. The plasma is obtained from a plasma pool ranging in age from two weeks to two months and stored at -25° C. for various periods of time ranging up to nine weeks before processing in accordance with the procedure of Example 2. The prothrombin complex prepared in this manner is assayed after reconstitution with sterile water as follows:


(c) Thrombin activity: The presence of thrombin is tested for by determining the clotting time of recalified normal plasma at various dilutions according to the procedure of Bidwell and Dike, Treatment of Hemophilia and Other Coagulation Disorders, edited by Biggs and MacFarlane, published by F. A. Davis Co., Philadelphia, pp. 62-69 (1966).

(d) Total protein content: Total protein content is determined by ultraviolet absorption at a wavelength of 280 nm.

By the above assay procedures, the prothrombin complex of this example was found to be free of thrombin activity and to contain (to the average of many lots) about 8 mg. of protein per ml. The average Factor IX activity of these lots was about 20 to 40 times that of an equivalent volume of normal whole plasma; the average Factor II activity of these lots was about 5 to 15 times that of an equivalent volume of normal whole plasma. Since normal whole plasma contains about 70 mg. of protein per ml., the Factor IX activity in the prothrombin complex of this example is contained in only about one two-hundredth to one four-hundredth (1/200 to 1/400) the amount of protein present in plasma providing an equal amount of Factor IX activity and the Factor II activity is contained in only one fiftieth to one hundred fiftieth (1/50 to 1/100) the amount of protein present in plasma providing an equal amount of Factor II activity.

The prothrombin complex of the above example also contains high activity levels of Factors VII and X relative to the levels found in normal whole plasma. These factors are usually measured together in a determination of the prococonvertin-Stuart-Prower complex.

**EXAMPLE 4**

The AHF-containing precipitate from Example 1 is re-suspended in citrated saline solution to a volume equal to one-twentieth (1/20) the volume of the original plasma which the precipitate represents. The resulting suspension (pH 6-7) is mixed with PEG 4000 to a concentration of 3.5% PEG. The mixture is gently agitated at room temperature for ten minutes, and then centrifuged for fifteen minutes at 5000 r.p.m. The supernate is decanted and adjusted to pH 6.88 with 0.1 normal sodium hydroxide. Additional polyethylene glycol 4000 is added to the solution to make the final PEG concentration 10 percent. The mixture is gently agitated at room temperature for thirty minutes, and centrifuged at 5000 r.p.m. for one-half hour.

The supernate is decanted and the precipitate is washed in cold water (2° C.). Spin washing is then carried out for five minutes at 5000 r.p.m. at a temperature of -4° C. The supernate is decanted and the precipitate is redissolved in glycone citrated saline.

The redissolved precipitate is then adjusted to pH 6.88 with 0.1 normal acetic acid and centrifuged. By this means (for example, refrigeration or use of isopropanol Dry Ice bath) the solution is cooled to a temperature of from 6° C. to 10° C. To the cooled solution, sufficient glycone is added to make the solution 1.8 molal with respect to glycone. The mixture is gently agitated for 45 to 60 minutes at a temperature of from 2° C. to 10° C., and then centrifuged by continuous flow or bucket centrifugation. The resulting glycone precipitate is collected and gently washed with buffered water at a temperature of 0° C. to 4° C.

When the glycone precipitate has dissolved, it is preferable to clarify the solution by centrifugation and/or filtration using a 293 mm. Millipore filter (membranes used: 1.2 microns, 0.45 micron, and 0.3 micron).

The liquid human plasma product prepared in the above manner by the successive fractionation of the AHF-containing precipitate from Example 1, first by polyethylene glycol precipitation and then by glycone precipitation, has an AHF concentration of high potency. This liquid product is then frozen by shell freezing (-60° C.) and storing in a flash freezer (-20° C. to -30° C.) for at least three hours. The frozen product can then be retained under ordinary refrigeration conditions (-4° C., preferably at -20° C. to -30° C.) without loss of its AHF activity for periods of time of one year and longer. This product when reconstituted can be administered intravenously to hemophiliacs as required by conventional transfusion means.

The AHF concentrate prepared by the procedure of this example contains less than 0.05% (generally as little as 0.01%) residual polyethylene glycol and is highly soluble in water.

**EXAMPLE 5**

Example 4 is repeated including the additional step of purification of the AHF fraction with ECTEOLA cellulose resin in the following manner:

**REAGENTS**

ECTEOLA cellulose resin
(1) Mix 60 grams NaOH with 150 ml. H₂O.
(2) Allow the mixture to cool.
(3) Place 60 grams cellulose (Whatman Cellulose Powder CF 11) in a beaker and mix thoroughly with the above NaOH solution.
(4) Allow the mixture to stand overnight (12 hours).
(5) On the next day prepare a solution of 35 ml. triethanolamine and 60 ml. of epichlorohydrin. Mix well under a hood.
(6) Add this solution quickly to the above cellulose and mix well. Place the reaction vessel out of a draft. (The reaction is exothermic and will heat to about 100° C.) The mixture will turn brown in one to two hours.
(7) Cool the mixture at room temperature under a hood.
(8) Add 350 ml. 2 M NaCl in small portions.
(9) Filter this mixture through a coarse sintered glass filter.
(10) Wash the precipitate twice with 500 ml. of 1 N NaOH. (This removes deep discoloration.)
(11) Suspend the precipitate in 350 ml. 1 N HCl in filter funnel. Apply a vacuum.
(12) Repeat step 11 with 250 ml. 1 N NaOH.
(13) Repeat step 11 with 250 ml. 1 N HCl.
(14) Repeat step 11 with 250 ml. 1 N NaOH.
(15) Transfer the precipitate to a 3 liter beaker.
(16) Add 250 ml. 1 N NaOH. Mix.
(17) Add distilled water to the mixture to fill beaker; mix. Cover and allow to stand overnight.
(18) Decant the supernatant.
(19) Add water to the precipitate to fill beaker and mix.
(20) Wash the mixture on the filter with water. (Four liters or more, until a negative test for alkali with 1% alcoholic phenolphthalein is obtained.)

(21) Make a final wash with two 250 ml. portions absolute ethanol.

(22) Place on filter paper. Mash and spread and place it to dry overnight.

Chloride buffer:
- 0.8% NaCl (8 gm./l.)
- 0.02 M imidazole (1.36 gm./l.)

Adjust pH to 6.9 with 1 N HCl.

Eluting buffer:
- 0.5 M NaCl
- 0.02 M imidazole (1.36 gm./l.)

Adjust pH to 6.9 with 1 N HCl.

A commercially available ECTEOLA cellulose resin which is first recycled with NaOH, for example, as in the following manner, can be used in place of the above-prepared ECTEOLA cellulose resin.

Recycled commercial ECTEOLA cellulose resin

(1) Mix 60 grams commercial ECTEOLA cellulose resin with 250 ml. 2 M NaCl.

(2) Filter this mixture through a coarse sintered glass filter.

(3) Wash the precipitate two times with 500 ml. 1 N NaOH.

(4) Wash one time with 350 ml. 1 N HCl.

(5) Wash one time with 250 ml. 1 N NaOH.

(6) Wash one time with 250 ml. 1 N HCl.

(7) Wash one time with 250 ml. 1 N NaOH.

(8) Transfer the precipitate to a 3 liter beaker and add 250 ml. 1 N NaOH. Mix.

(9) Add distilled water to the mixture to fill beaker. Mix.

(10) Decant the liquid; add 3 liters water to the precipitate; mix. Filter.

(11) Wash the precipitate with water until phenolphthalein test is negative.

(12) Wash the precipitate two times with 500 ml. absolute ethanol.

(13) Air-dry precipitate on filter paper.

PROCEDURE

When the starting material for the purification with ECTEOLA cellulose resin is a polyethylene glycol precipitated fraction of AHF, the AHF fraction is first dissolved in chloride buffer. When the starting material is a glycine precipitated fraction of AHF, the AHF fraction is first diazoyed against the chloride buffer for one hour to remove glycine and reduce its ionic strength. Purification of the buffered AHF fraction by column technique with the above-prepared ECTEOLA cellulose resin proceeds as follows:

The ECTEOLA cellulose resin is equilibrated overnight (12 hours) in a 5°C. C. box by mixture with chloride buffer in proportions of 15 grams of resin to 600 ml. buffer. The resulting resin slurry is poured on a column of from one to one and one-half (1½) inches in diameter x 18 inches high. After the buffer comes down to the level of the resin, the AHF fraction is adjusted to two and one-half (2½) ml. per minute and the amount of AHF fraction applied to a single column is from 1000 to 3500 units of AHF. (one unit of AHF is equal to the AHF activity in one cc. of normal human blood plasma.) After the AHF has been applied to the column, from 200 to 500 ml. chloride buffer is washed through the resin. When the chloride buffer comes down to the level of the resin, the eluting buffer is applied to the column. The eluate is collected in ten ml. portions and analyzed for protein, fibrogen and AHF activity.

The eluate portions having the most active AHF activity are retained and stabilized by the addition of 1% albumin. The stabilized solution is then filtered using a 293 mm. Millipore filter as in Example 4. A silver filter of the same size can also be used in place of the Millipore filter. This final liquid product can then be frozen by shelf freezing, followed by storage in a flash freezer for at least three hours, and then retained under ordinary refrigeration conditions in the manner of the final product of Example 4.

Human whole blood plasma, and bovine and porcine plasma and AHF-containing plasma fractions can be fractionated by the successive polyethylene glycol and glycine precipitation procedures described herein to produce AHF concentrates similar to those described above in Examples 4 and 5.

Various modifications and adaptations of the present invention can be devised, after reading the foregoing specification and the claims appended hereto, by the person skilled in the art without departing from the spirit and scope of the invention. All such variations and modifications are included within the scope of the invention as defined in the following claims.

What is claimed is:

1. A method for the preparation of a prothrombin complex-I from citrated blood plasma comprising fractionating citrated blood plasma by mixing with glycine having a molarity of from about 1.3 to about 1.8 to produce an AHF-containing precipitate and a prothrombin complex-containing supernatant, fractionating said prothrombin complex-containing supernatant by mixing with polyethylene glycol having a molecular weight of from about 200 to about 20,000 to a concentration of about 30% by weight and then separating the resulting prothrombin complex-containing precipitate from the undesired citrate and glycine salt-containing supernatant, suspending said prothrombin complex-containing precipitate in normal physiological saline, adjusting the suspension to a pH of from about 6.8 to about 7.2, thoroughly mixing the supernatant with tribasic calcium phosphate to adsorb the coagulation factors, thoroughly mixing the tri-calcium phosphate adsorbed protein precipitate with from about 0.05 M to about 0.2 M trisodium citrate followed by recovery of the resulting supernatant, and then subjecting the recovered supernatant to a succession of two precipitations with polyethylene glycol having a molecular weight of from about 200 to about 20,000, first at a pH of from about 6.8 to about 8.0 and a final concentration of from about 5% to about 10% polyethylene glycol with retention of the resulting supernatant, and then at a pH of from about 5.0 to about 5.4 and a final concentration of at least about 20% polyethylene glycol by weight of said retained supernatant with retention of the resulting precipitate as the active prothrombin complex.

2. The method of claim 1 in which the polyethylene glycol has an average molecular weight of from about 400 to about 6,000.

3. The method of claim 1 in which the polyethylene glycol has an average molecular weight of about 4,000.

4. A method for the preparation of a concentrate of AHF from citrated blood plasma comprising fractionating citrated blood plasma by mixing with glycine having a molarity of from about 1.3 to about 1.8 to produce an AHF-containing precipitate and a prothrombin complex-containing supernatant, fractionating said AHF-containing precipitate by mixing with polyethylene glycol having a molecular weight of from about 200 to about 20,000 to a concentration of from about 5% to about 10% polyethylene glycol by weight, mixing the AHF-containing supernatant with said polyethylene glycol to a concentration of about 10% by weight, mixing the resulting AHF-containing precipitate with glycine having a molarity of about 1.8 and recovering the remaining precipitate as the active concentrate of AHF.

5. The method of claim 1 in which the polyethylene glycol has an average molecular weight of from about 400 to about 6,000.
6. The method of claim 1 in which the polyethylene glycol has an average molecular weight of about 4,000.

7. The method of claim 1 including the additional step of purification with triethanolamino-ethylated cellulose resin.

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U.S. Cl. X.R.