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STABLE FIBRINOGEN SOLUTIONS AND METHOD FOR PRODUCING SAME

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This invention relates to and in general has for its object the provision of a highly soluble, stable and sterile fibrinogen product, and to a method for producing such product.

Presently, there is a serious need for fibrinogen of this form in connection with hypofibrinogenemia. For example, statistics indicate that in this country abruptio placentae occurs in about 0.5% of all births. Abruptio placentae results in the escape of thromboplastic enzymes from the placenta into the maternal circulation, thereby causing an immediate destruction of all of the fibrinogen in the maternal circulation, and, in turn, a massive and uncontrollable hemorrhage. The only known treatment for this is the actual replacement of the fibrinogen so lost from the blood. Hypofibrinogenemia also occurs as a result of certain drastic surgery, such as thoracic and pancreatic surgery wherein in some cases thromboplastins enter the circulatory system, destroy the patient's fibrinogen and cause an uncontrollable hemorrhage.

Hypofibrinogenemia can also exist as an inherited condition where the patient is devoid or substantially devoid of fibrinogen.

Although fibrinogen is currently available, the form in which it exists is subject to three major defects. Firstly, solutions thereof cannot be made sufficiently stable to withstand ultraviolet irradiation of sufficient intensity to destroy the virus of homologous serum jaundice which is often associated therewith, and here it is to be noted that this virus is largely concentrated in the fibrinogen fraction derived from plasma. Secondly, the lyophilized (freeze-dried) fibrinogen when reconstituted with water produces an insoluble residue in the amount of about five percent; and thirdly, it is impossible to reconstitute the dried material to make up a solution of fibrinogen containing as much as 2 grams of fibrinogen per 100 cc. of solution. A concentration of this order is particularly important in order to be able to introduce the required amount of fibrinogen into the circulatory system without the necessity of introducing too great a volume of solution.

Here it should be observed that it is impractical to treat abruptio placentae by the transfusion of whole blood, for whole blood contains only 0.16 grams of fibrinogen per 100 cc. Since in cases of this kind it is necessary to raise the fibrinogen level from 0.00 at least half of the normal level (0.087 gram per 100 cc. is the critical level) if clotting is to occur, simple arithmetic shows that it will require the transfusion of approximately 5.5 liters of whole blood to a person with a normal blood volume of 4.6 liters to reach this level. Even greater amounts are necessary if any fibrinogen is lost or destroyed during the long-drawn-out procedure of transfusion.

One expedient that has been resorted to in several cases of abruptio placentae consists of administering a fibrinogen solution containing about 0.7 gram per 100 cc. sterilized with nitrogen mustard. However, the toxicity of the residual nitrogen mustard was so great that the manufacture and sale of fibrinogen, sterilized in this manner,

was precluded by the federal regulatory body for biological products.

More specifically, one of the objects of this invention is the provision of a method for producing a fibrinogen material wherein a conventional Cohn Fraction I paste is dissolved in an aqueous diluent having an ionic strength of at least 0.30, a pH not greater than 5.85 and containing a small quantity of a carbohydrate selected from the group consisting of sugars, sugar alcohols and sugar acids.

A further object of this invention is the provision of a dry, sterile, stable, and highly soluble fibrinogen undamaged by ultraviolet irradiation and freeze-drying and which can be reconstituted with water to produce a solution containing at least 2 grams of fibrinogen per 100 cc. of solution.

More specifically and by way of one example, the objects of this invention may be attained by resorting to the following procedure:

Pooled plasma is cooled to 0° and a preliminary clarification and precipitation is conducted using an alcoholic buffer of the following compositions:

178 cc. of 53% ethanol at −5° + 1.5 cc. of acetate buffer of pH 4.0 and ionic strength of 0.8. For each liter of plasma at 0°, 2.5 cc. of the alcoholic buffer at −5° is added and the resultant mixture is clarified by continuous centrifugation at 0° to 1°.

Depending on the nature of the plasma, from 1.2 gms. to 2.3 gms. of paste is removed per liter of plasma.

Additional alcoholic buffer at −5° is then added to the supernatant in an amount sufficient to precipitate a conventional Cohn Fraction I paste at an ethanol concentration of 8% and ionic strength of 0.14, a pH of 7.2 and a temperature of −2.5° C. The Fraction I paste is removed by continuous centrifugation at −2.5°.

Following this or concurrently therewith, a solvent buffer of the following composition is prepared and maintained at 2° C.:

Sodium citrate .2 H_2O	gms.	12.3
Sodium chloride	gms.	11.6
Citric acid .2 H_2O	gms.	2.1
Dextrose	gms.	40
Distilled water q. s.	cc.	1000

The pH of this solvent buffer should be 5.40±.05.

Each kg. of Fraction I paste is suspended in 3130 cc. of the solvent buffer thus prepared at 2° C., using a rapid acting mechanical blendor and excluding access of air to the surface of the liquid so that all foaming is prevented. The pH of the solvent buffer containing the Dissolved Fraction I paste is approximately 5.6 and must be below pH 5.85. The solution of Fraction I paste in the solvent buffer contains approximately 3 gms. of fibrinogen for every 100 cc. of solution. The dissolved Fraction I paste is stirred gently and continuously, quickly warmed to 20° and maintained at 20° for thirty minutes, and is then filtered first through a coarse filter paper and then through a tight asbestos pad (for example, a C–2 pad) to produce a sparkling clear filtrate.

The clear fibrinogen filtrate is then immediately irradiated with ultraviolet using approximately twice the intensity of irradiation defined in the minimum requirements of the National Institutes of Health, entitled "Ultraviolet Irradiation for the Sterilization of Biological Products," dated January 15, 1953. The irradiated solution is immediately sterilized by filtration through sterilizing asbestos pads, and the sterile bulk is sampled for the immediate determination of the clottable protein content.

Following this, the solution is transferred under aseptic conditions and in unit dosage volumes into small vials

and there lyophilized, stoppered and sealed under vacuum, all in accordance with well known practice.

When sterile water is added to reconstitute the dry material to a fibrinogen content of at least 2%, the dry material completely dissolves within five minutes and will not clot spontaneously for periods in excess of 48 hours.

In carrying out this process any of the following carbohydrates can be substituted for the dextrose added to solvent buffer in which the Fraction I paste is suspended: pentose sugars, disaccharides, sorbitol, and gluconate.

The stabilized fibrinogen of this invention, in addition to being stable to ultraviolet irradiation, when reduced to the dry state, will withstand gamma radiation of doses up to 3×10^6, REP, without any evidence of damage. Doses of 4×10^6, REP, caused the destruction of 9.2% of the fibrinogen being irradiated.

The stability of reconstituted fibrinogen solutions containing 2% fibrinogen as measured by the spontaneous clotting time of the fibrinogen content thereof is indicated in the following table:

Exp. No.	pH before drying	Ionic Strength	Additive	Spontaneous Clotting in Hours
271—14-1	6.07	.15		1
271—14-4	6.07	.15	Dextrose, 8%	30
271—15-2	6.07	.15	Sorbitol, 8%	36
271—16-3	6.1	.15	NaGluconate, 5%	65
271—21-1	5.4	.15		<18
271—21-2	5.4	.25		18
271—21-3	5.4	.35		28
271—21-4	5.4	.45		63
271—21-5	5.8	.15		18
271—21-6	5.8	.25		>25 <53
271—21-7	5.8	.35		68
271—21-8	5.8	.45		108
271—25	6.05	.15	Dextrose, 4%	34
271—26	5.42	.45	do	192

From this table it will be noted that the stability of any particular solution depends on the three factors: pH, ionic strength and the carbohydrate additive used. More particularly, it is to be observed that the optimum results are obtained with a fibrinogen solution having a pH of $5.40\pm.05$ but at all events not greater than 5.85; having an ionic strength not less than 0.30 and containing not less than 3% of a carbohydrate additive.

A solution of fibrinogen having an ionic strength of 0.30 will begin to precipitate at a pH value of about 5.0 as will appear from the article by Morrison, Edsall and Miller, published during 1948 in the Journal of the American Chemical Society, vol. 70. The three dimensional curve appearing in the upper left corner of page 3105 of the article will indicate this condition where the ethanol content is zero. Thus the lower pH value contemplated herein can be expressed as that at which the fibrinogen would begin to precipitate.

Although the derivation and composition of a Cohn Fraction I solution and a Cohn Fraction I paste is well known in this field, if more detailed information concerning them is desired, reference can be made to the

Cohn Patent 2,390,074 of December 4, 1945, and to the publication in the Journal of the American Chemical Society, 68, 459 (1946).

It should be noted that during 1953, over thirty human cases, in which the administration of whole blood had failed and death was virtually certain, were treated intravenously with the fibrinogen solution of this invention in doses of from 3 g. to 5 g. of fibrinogen, and recovery was complete within 2 to 3 hours.

In conclusion, it will be seen that as a result of the procedure above set forth, I have provided a dry, stable, ultra-violet irradiated fibrinogen product capable of being reconstituted and completely dissolved in water within a relatively short time for making up a solution having a sufficiently high concentration of fibrinogen to serve as a therapeutic product.

I claim:

1. A method of preparing a stable solution of fibrinogen comprising: dissolving human fibrinogen in an aqueous diluent so that the resultant solution has an ionic strength of at least 0.30, a pH not greater than 5.85 and not less than a value at which the fibrinogen would begin to precipitate and containing at least 3% of a carbohydrate selected from the group consisting of sugars, sugar alcholos and sugar acids.

2. The method of preparing dry, sterile, human fibrinogen comprising: making up an aqueous solution of human fibrinogen; adjusting the ionic strength of such solution to at least 0.30; adjusting the pH of such solution within a range of 5.85 and such lower value at which the fibrinogen would begin to precipitate and adding to said solution at least 3% of a carbohydrate selected from the group consisting of sugars, sugar alcohols and sugar acids.

3. A dry, sterile, virus-free, stable composition of human fibrinogen containing at least 1.5 grams of a carbohydrate selected from the group consisting of sugars, sugar alcohols, sugar acids for each gram of fibrinogen, and containing an electrolyte in a quantity sufficient, when at least two grams of said composition are dissolved in 100 cc. of water, to bring the ionic strength of the resulting solution to at least 0.30 and its pH to a value in the order of from 5.85 to such lower value at which the fibrinogen would begin to precipitate.

4. An aqueous fibrinogen solution containing not less than 3 per cent of a carbohydrate selected from the group consisting of sugars, sugar alcohols and sugar acids, said solution having an ionic strength of not less than 0.30 and a pH within the range of 5.85 to such lower value at which the fibrinogen would begin to precipitate.

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