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(54) AUTOLOGOUS THROMBIN

AUTOLOGES THROMBIN
THROMBINE AUTOLOGUE

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Description**Technical Field**

5 [0001] The following invention relates generally to the preparation of a high specific activity thrombin enzyme from a given unit of plasma, which is sufficiently stable that it provides rapid clotting of a fibrinogen-rich solution of clotting and adhesive proteins for more than six hours when held at room temperature or lower.

Background Art

10 [0002] Formulation of a fibrin sealant mimics the last step of the coagulation cascade wherein the enzyme thrombin cleaves fibrinogen which is then cross-linked into a semi-rigid or flexible fibrin clot. This fibrin clot adheres to wound sites, forming a barrier to fluid leaks and generates adhesion between tissues, while providing hemostatic and healing properties to the treated site.

15 [0003] Presently marketed, applicant's CryoSeal™ system is a device which harvests cryoprecipitated, concentrated clotting and adhesive proteins, including fibrinogen and Factor XIII from a donor's plasma in approximately one hour. The one hour cryoprecipitation harvesting, provided by the CryoSeal™ system, compared to the 1 to 2 day cryoprecipitation process routinely practiced in Blood Banks, means that CryoSeal™ harvesting of clotting and adhesive proteins can occur right in the perioperative theater with the patient close by, thereby avoiding the need to initiate the process 20 days in advance.

25 [0004] These CryoSeal™ harvested clotting and adhesive proteins, when combined with bovine or human thrombin, forms a biological glue useful for surgical hemostasis and tissue adhesion. Commercially available thrombin, however, is generally sourced from bovine or human plasma pools, so the patient would still be at risk of negative immune reactions or contamination by infectious blood born viruses and, possibly Crutzfeld-Jacobs Disease (CJD) or new variants of CJD (NVCJD). An advantage of the Cryoseal™ cryoprecipitation invention is that the harvested clotting and adhesive proteins sourced from the patient's own blood eliminates the risk of contamination by infectious blood-borne disease when these clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

30 [0005] It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

35 [0006] The concept of utilizing thrombin and/or fibrinogen sourced from the patient in a medical procedure performed on that patient is not novel and was first described by Andrianova in 1974. Some twenty years later, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994 and its related U.S. Patent No. 5,795,780) describes an improved fibrin glue in which the thrombin component, which required thirteen steps, including centrifugation, and separation of intermediate precipitates and adjusting the ionic strength of the blood and pH of the plasma to prepare, would be combined with a fibrinogen component also sourced from the plasma of the same donor. However, these many preparation steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour. The present invention, *inter alia*, is distinguished in that it is undiluted by pH adjustment.

40 [0007] Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192 and its related WO96/31245) follows Cederholm-Williams by teaching another method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which most of the fibrinogen in the plasma is first precipitated and removed to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different and simpler than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides 45 clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes. The present invention, *inter alia*, is distinguished in that the plasma is unprocessed as for example by not precipitating out fibrinogen.

50 [0008] These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue, before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

55 [0009] US5795780 discloses a method of preparing thrombin and fibrinogen. The method includes acidification of the blood, separation of the plasma from the blood, introduction of the plasma in a chamber containing beads and ethanol, precipitation of the fibrinogen in an ice bath and removal of the fibrinogen using centrifuging steps-Once the euglobulin fraction has been formed via a number of additional steps, an alkalization step takes place and CaCl_2 is introduced into the chamber.

[0010] The following prior art reflects the state of the art of which applicant is aware and is included herewith to

discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

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[0011] The other prior art listed above, not all of which are specifically discussed catalog the prior art of which the applicant is aware. These undiscussed references diverge even more starkly from the instant invention specifically distinguished below.

30 **Disclosure of Invention**

[0012] The present invention provides a method for generating autologous thrombin, the steps comprising:

35 Obtaining plasma from a blood product;

Adding ethanol, a source of calcium ions and beads with the plasma to prepare a solution containing prothrombin; Converting the prothrombin in the solution to thrombin; and filtering the thrombin to remove particulate matter.

[0013] The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (e.g. six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological sealant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field (Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (i.e., less than 5 seconds) during only a very narrow four to five minute time period, or required so many steps and elapsed time it will provide fast clotting throughout even the longest surgeries. The thrombin produced by this invention can be diluted in saline, water and a dilute CaCl_2 solution (e.g. 125 mM CaCl_2) to provide precise, slower clotting times thereby allowing any preferred time from less than five seconds to longer than 2 minutes.

[0014] The procedure of the invention is preferably comprised of three steps, the first two of which should occur at the same time:

50 1. Preparing a fraction enriched in prothrombin by use of ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Fibrinogen and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent, activation to long-term functional thrombin.

55 2. Adding calcium ions and beads to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.

3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

Industrial Applicability

[0015] The industrial applicability of this invention shall be demonstrated through discussion of the following objects of the invention.

5 [0016] Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin from the donor's plasma.

[0017] It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

10 [0018] It is a further object of the present invention to provide thrombin as characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

[0019] It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

[0020] It is a further object of the present invention to provide a method for producing thrombin as characterized above which has a process time in as little as thirty minutes, up to seventy-five minutes.

15 [0021] It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

[0022] Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride ($CaCl_2$) to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

20 [0023] Viewed from a second vantage point, it is an object of the present invention to provide a method for generating autologous thrombin from a patient, the steps including: obtaining a blood product from the patient; sequestering plasma from the product; enriching the prothrombin in a plasma fraction; converting the prothrombin to thrombin, and filtering particulate from the thrombin.

25 [0024] Viewed from a third vantage point, it is an object of the present invention to provide a method for producing autologous thrombin which is stable for more than fifteen minutes, the steps including: sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

30 [0025] Viewed from a fourth vantage point, it is an object of the present invention to provide an autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

[0026] Viewed from a fifth vantage point, it is an object of the present invention to provide a composition for extracting thrombin from plasma consisting essentially of: Plasma; Ethanol (ETOH); $CaCl_2$.

35 [0027] Viewed from a sixth vantage point, it is an object of the present invention to provide a method for preparing thrombin comprising: obtaining plasma; adding ETOH and $CaCl_2$ and beads to the plasma, forming a composition: agitating the composition; incubating the composition in a static or rocking mode; filtering the composition of particulate, thereby passing the thrombin through the filter.

40 [0028] Viewed from a seventh vantage point, it is an object of the present invention to provide a device for preparing thrombin from plasma, comprising: a reaction chamber having beads, a solution of $CaCl_2$ and ETOH therein; means for admitting plasma into the reaction chamber; thrombin receiving syringe coupled to the reaction chamber to receive the thrombin; and a filter located between the reaction chamber and the thrombin receiving syringe.

45 [0029] Viewed from an eighth vantage point, it is an object of the present invention to provide an autologous biological glue processing device, comprising, in combination: a thrombin processing means, a clotting and adhesive proteins processing means operatively coupled to the thrombin processing means, means for receiving plasma via the operative coupling for subsequent conversion of the plasma to, respectively thrombin and clotting and adhesive proteins.

[0030] The present invention provides a method and apparatus that produces thrombin which is sufficiently stable that it can provide less-than-5-second clots for up to six hours, substantially more stable than demonstrated in all prior art. Further, the clot time can be modified at will through dilution with saline.

50 [0031] The present invention further provides an efficient method of preparation. Improved cryoprecipitation of clotting and adhesive proteins through the CryoSeal™ invention requires less than one hour. In this same time frame, the autologous human thrombin component can be manufactured with minimal materials and methods from the same source plasma. Both of the biological components of the biological glue are easily combined in a surgical setting, administered to the very same donor patient, and the resultant clotting provides hemostasis or tissue adhesion at the wound site.

55 [0032] The present invention additionally provides a method for sterile production of both components of the biological glue. The improved sterile manufacturing described herein provides a final product that is essentially free of contamination by non autologous microbes.

[0033] These and other objects will be made manifest when considering the following detailed specification when taken in conjunction with the appended drawing figures.

Brief Description Of Drawings**[0034]**

5 Figures 1A and 1B are perspective views of apparatuses for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins therefrom.

10 Figures 2A and 2B are plan views of the thrombin processing sets removed from the processing sets that extracts clotting and adhesive proteins.

15 Figures 3A and 3B are perspective views of the interior of the thrombin processing cases with the thrombin syringe shown in figures 2A and 2B removed therefrom.

Figures 4A and 4B are perspective views of the thrombin cases upper halves.

Figures 5A and 5B are perspective views of the thrombin cases lower halves.

15 Figures 6A and 6B are exploded parts views of the reaction chamber 26 shown in figures 3A and 3B along with the valving structure at opposed ends thereof.

Figures 7A and 7B are sectional views of the reaction chambers and valving structures depicted in figures 6A and 6B.

Figures 8A and 8B are detail of construction of that which is shown in figures 7A and 7B.

Figures 9A and 9B are exploded parts view of filter alternatives used in figures 3A and 3B.

20 Figure 10 is a perspective view of that which is shown in figure 9.

Figure 11 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations.

Figure 12 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations at different CaCl_2 concentrations.

Figure 13 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored on ice.

25 Figure 14 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored at room temperature.

Figure 15 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored on ice.

30 Figure 16 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored at room temperature.

Best Mode(s) for Carrying Out the Invention

35 **[0035]** Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figures 1A and 1B.

[0036] In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

40 **[0037]** More particularly, viewing both figures 1A and 1B, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has plural positions for stop valves 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60. The first valve branch 12 also includes a stop valve 6.

45 **[0038]** Viewing figure 1B, prior to the introduction of plasma through the first branch 12 thrombin processing unit 40, reagent from preloaded syringe 95 is injected pushing plunger mechanism 94 in the direction of A', into receiving system 20 through sterile barrier filter 92. The reagent passes through one way valve 91; Y connector 90, that merge coupling 18 and valve 91, through branch tubing 93; and finally into the interior of casing 22. Referring to figure 3B and 7B, a valve 24 initially directs the reagent to a reaction chamber 26.

50 **[0039]** Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. Although this system can be dimensioned for any size batch, the plasma required for the thrombin processing unit will typically be 9-10 ml so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and adhesive proteins from the clotting and adhesive proteins processing unit 60.

55 **[0040]** In the embodiments shown in figures 1A and 1B, sealed bags 16 and 78 overlie both the thrombin dispensing syringe 42 (and a lead in of conduit 64) and cryoprecipitate storage tube 76 to provide sterility until both storage containers are introduced into a sterile surgical field (e.g., operatory). Prior to that, the thrombin processing unit 40 operates as shown and described with reference to figures 2A through 10. Viewing figure 1B, after reagent is added, plasma enters

the first branch 12 and passes beyond a coupling 18, through tubing branch 93, and into an interior of the casing 22.

[0041] Referring back to figure 1A, the thrombin processing unit 40 operates as shown and described with reference to figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A and 10. As mentioned, fluid enters the first branch 12 and (figure 1A) passes beyond a coupling 18 and into an interior of a casing 22. Coupling 18 is preferably frictionally and/or adhesively attached to the first branch 12 yet the thrombin processing unit 40 can still be removed (e.g. figure 2A) from the processing set 10 (e.g., by merely detaching or severing branch 12 followed perhaps with heat sealing) after receiving the plasma as shown in figure 2. If adhesive is used, it is a sterile grade for use in an operatory.

[0042] Referring to figure 3A, a valve 24 initially directs the plasma to a reaction chamber 26 having an interior tube 28a (figure 6A) preferably formed from glass and capable of receiving a volume, for example 15 ml. Glass tube 28a is preferably shorter than and circumscribed by an overlying barrel 32 preferably formed from PVC. A window 31a in the PVC barrel 32 can be used to gauge and/or verify the contents within the glass tube 28a. Gauging may also include gradations 29, indicating a volume on the glass tube. The glass tube 28a of the reaction chamber 26 receives the plasma from the first branch 12 and into its interior for mixing with reagents preloaded in the glass tube 28a and described hereinafter. As shown in figure 7A, the interior of the glass tube is preferably prefilled only partially with beads 25 preferably formed from borosilicate, glass or ceramic to enhance the reaction and agitation.

[0043] Referring to figure 3B, a valve 24 initially directs the plasma to a reaction chamber 26 having tube 28b (figure 6B) preferably formed from clear polycarbonate and capable of receiving a volume, for example, 15 ml. Graduated lines 31b on the polycarbonate tube 28b can be used to gauge the contents within the tube 28b. The polycarbonate tube 28b of the reaction chamber 26 receives the plasma from the first branch 12 and into the interior for mixing with reagents previously added into the polycarbonate tube 28b and described hereinafter. As shown in figure 7B, the interior of the tube 28b is preferably prefilled only partially with beads 25 preferably formed from borosilicate or ceramic to enhance the reaction and agitation.

[0044] The reaction chamber 26 of the embodiment shown in figures 1A and 3A is formed with first and second end caps 34 detailed in figures 6A, 7A and 8A. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 is isolated from the beads 25 by a screen 23 nested within necked-down portion 48. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the glass tube 28a and through flow is controlled by the valves 24, 44. As shown in figure 8A, the cap 34 includes an annular necked-down portion 48a which frictionally and/or adhesively resides within an interior hollow of the PVC barrel 32. In this way, the necked-down portion 48 rests upon ends of the glass tube 28a in sealing engagement therewith, isolating the interior of the reaction chamber from the PVC barrel 32.

[0045] For the embodiment forming the reaction chamber 26 of the embodiment shown in figures 1B and 3B mainly out of polycarbonate tube 28 is detailed in figures 6B, 7B and 8B. This reaction chamber 26 is formed with first and second end caps 34 detailed in figure 8B. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 has interior obstructions preventing passage of beads 25 while allowing passage of fluid. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the polycarbonate tube 28b and through flow is controlled by the valves 24, 44. As shown in figure 8B, the cap 34 includes an annular interior recess portion 48b which adhesively resides on the interior surface of the polycarbonate tube 28b.

[0046] Preferably, ethanol and calcium chloride are the reagents which have been preloaded into the reaction chamber 26 or within reagent syringe 95. Initially, both valves 24 and 44 are oriented so that reagents will not pass therebeyond to seal the chamber for the embodiment of figure 1A. Viewing figure 1B, initially valve 24 is oriented so plasma will not enter reaction chamber 26, and valve 44 is oriented to allow passageway between the reaction chamber 26 and the draw plunger 56. Referring back to figure 1A, after the plasma has been pumped into processing unit 60, valve 44 is turned to allow access to the draw plunger 56 and valve 24 is oriented to allow access between the passageway 21 and the reaction chamber 26. Slide clip 6 is opened with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1A, syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. Referring back to figure 1B, the reagent syringe 95 is attached to open end of sterile barrier filter 92. Plunger 94 is depressed to transfer reagent syringe through sterile barrier filter and passageway 93 to reaction chamber 26. Likewise to the figure 1A embodiment, the figure 1B, with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1B, the syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. In both embodiments syringe 56 includes a filter 62 located in the flow path. More specifically, the path 43 between valve 44 and syringe 56 includes a filter 62 located in the flow path. The filter 62 provides an aseptic microbial barrier so that, upon subsequent delivery of the thrombin to the dispensing syringe 42 (figure 1), there is no contamination from around the seal 57 of plunger 58 delivered to syringe 42. Plasma will subsequently enter chamber 26 from conduit 4 to

replace air. Valve 24 is oriented to address filter 66. The reagents and plasma are briefly agitated assisted by beads 25 (and allowed to incubate for about 40 to 70 minutes). After incubation, thrombin processing unit 40 is agitated to loosen and break up gel formation. For the embodiment of figure 1B, the thrombin processing unit 40 is then returned to a motionless horizontal position for no less than 10 minutes. Afterwards the thrombin processing unit 40 is again agitated to loosen and break up gel formation. For both embodiments, the plunger of syringe 56 is pushed in the direction opposite arrow A to move thrombin from chamber 26 through filter 66 into syringe 42. Delivery of thrombin to syringe 42 can be enhanced by retracting plunger 43 of syringe 42, defining a push pull system. Filter 66 removes particulate matter from the thrombin, including gel.

[0047] By allowing the thrombin contained in the reaction chamber 26 to reside therein after agitation for no less than 10 minutes enhances the effectiveness of the filter 66 in removing particulate matter for subsequent utilization. The time span for conversion and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer, or expression through a thin tube.

[0048] Figures 9A, 9B and 10 reveal alternative embodiments of filter 66 which includes an outer cylindrical wall 65 with end caps 34 each having a cylindrical spout 37 circumscribed by an annular recess 39. The alternative embodiment shown in figure 9A shows the centrally disposed cylindrical filter element 67a is preferably formed from polyurethane foam. While as shown in figure 9B the centrally disposed cylindrical filter element 67b is preferably formed from rolled polyester. Also shown in figure 9B, are circular filters 68 preferably formed from glass fiber or polyester. In each alternative embodiment, filter 67a or 67b filters by weight, size and protein binding.

[0049] Referring back to figures 1A and 1B, attention is now directed to the clotting and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 72 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 74 of the chamber 72 for subsequent extraction by means of a clotting and adhesive protein collection tube or dispensing syringe 76 contained in a sterile pouch 78. Chamber 72 is protected during this process by a filter vent 82 preventing contamination. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive collection tube or dispensing syringe 76, the two storage containers 42, 76 can be decoupled from the processing set 10 (e.g. sterile disconnect device), and passed near the sterile, surgical arena. The overwrap bags are subsequently opened, and the storage containers 42, 76 are decoupled and transferred into the surgical area where the contents are dispensed into individual sterile 3cc plastic syringes which are subsequently loaded into the fibrin glue applicator for spraying or line and dot application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

[0050] Both dispensing syringes 42 and 76 are stored at room temperature, or preferably stored at their optimal conditions: cryoprecipitate 76 being stored at room temperature and thrombin 42, stored in an ice bath at 1°C to 5°C. Please see figures 13 through 16.

[0051] Assume 9-10 ml of room temperature plasma is introduced into the reaction chamber 26. Other plasma volumes have utility. Please see figures 15 and 16. Add 1.0 ml of 75 mM calcium chloride (CaCl_2) and 2.0 ml of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and added to comprise 18.9% volume/unit volume or 15.02% ethanol weight/unit volume). Other ratios of reagent volume, comprising of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and a stock solution of 75 mM calcium chloride (CaCl_2)), to plasma volume have utility phase. Please see figures 13 and 14. The thrombin life span is shown to have been at least 300 minutes while its clotting time is at 2.98 seconds. An ethanol final concentration range between 8.0% and 20.0% (volume/unit volume), however, still has utility. Please see figure 11.

[0052] When the ethanol is at a final concentration of 18.9% volume/unit volume (as above) and the calcium chloride final concentration is 5.7 mM (1 ml taken from a 75 mM stock solution of calcium chloride), the thrombin lifespan also extends to at least 360 minutes while maintaining a clot time of 5.98 seconds when thrombin is stored at room temperature. Storing thrombin in optimal 1°C to 5°C ice bath typically maintains lot times of 2 to 3 seconds at 360 minutes. Calcium chloride stock solution concentrations ranging between 50 mM and 250 mM, however, have utility. Please see figure 12. The final concentrations range from 4.5mM to 23 mM.

[0053] Solutions such as saline, dilute CaCl_2 (e.g. 40mM to 125 mM CaCl_2) or even sterile water added to the thrombin can alter both the clotting time and life span of the thrombin. Assume an ethanol final concentration of 18.9% and a final calcium chloride concentration of 5.7 mM was used in the reaction chamber 26. When the thrombin has been diluted 1 to 1.5 with water, the clot time has been extended to just less than 30 seconds, and has a life span of up to 150 minutes.

[0054] Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

Claims

1. A method for generating autologous thrombin, the steps comprising:
 - 5 Obtaining plasma from a blood product;
Adding ethanol, a source of calcium ions and beads with the plasma to prepare a solution containing prothrombin;
Converting the prothrombin in the solution to thrombin;
and filtering the thrombin to remove particulate matter.
 - 10 2. The method of claim 1 whereby subsequent to agitating the solution, the solution is incubated for an amount of time greater than or equal to ten minutes.
 - 15 3. The method of claim 1 wherein the beads are formed from borosilicate, glass or ceramic.
 - 15 4. The method of claim 1 wherein the source of calcium ions is CaCl_2 .
 - 15 5. The method of claim 1 wherein the time required for the thrombin to convert fibrinogen to a fibrin clot is less than 5 seconds.
 - 20 6. The method of claim 1 wherein the thrombin is stable for a period of time between about 15 minutes and three hundred and sixty minutes.
 - 20 7. The method of claim 1 wherein the time required to generate the thrombin is less than an hour.
 - 25 8. A composition for extracting thrombin from plasma comprising plasma; ethanol (ETOH), a source of calcium ions, and beads.
 - 25 9. The composition of claim 8 wherein the source of calcium ions is CaCl_2 .
 - 30 10. The composition of claim 9 wherein ETOH is present at 18.9% and CaCl_2 is present at 23.0mM both by volume in the final concentration.
 - 35 11. The composition of claim 9 wherein ETOH is present at 18.9% and CaCl_2 is present at 5.7mM both by volume in the final concentration.
 - 35 12. The composition of claim 9 wherein ETOH is present at a range between 8% and 20% and CaCl_2 is present at a range between 4.5mM and 23.0mM both by volume in the final concentration.
 - 40 13. The composition of claim 8 wherein the beads are formed from borosilicate, glass or ceramic.
 - 40 14. Apparatus to prepare thrombin from plasma comprising a reacting chamber containing beads to accept a source of calcium ions and ethanol, and means for delivery of plasma into said reacting chamber; a syringe connected to said reacting chamber to receive thrombin from said reacting chamber; and a filter between said reacting chamber and syringe which is to receive thrombin.
 - 45 15. The apparatus of claim 14 wherein the beads are formed from borosilicate, glass or ceramic.

Patentansprüche

- 50 1. Verfahren zum Erzeugen von autologem Thrombin, das die folgenden Schritte beinhaltet:

Gewinnen von Plasma von einem Blutprodukt;
Hinzufügen von Ethanol, einer Quelle von Calciumionen und Kugelchen zum Plasma, um eine Lösung herzustellen, die Prothrombin enthält;
Umwandeln des Prothrombins in der Lösung zu Thrombin; und
Filtern des Thrombins, um partikuläre Materie zu entfernen.

2. Verfahren nach Anspruch 1, wobei die Lösung nach dem Rühren über einen Zeitraum inkubiert wird, der gleich oder größer als zehn Minuten ist.

5 3. Verfahren nach Anspruch 1, wobei die Kugelchen aus Borsilikat, Glas oder Keramik bestehen.

4. Verfahren nach Anspruch 1, wobei die Quelle von Calciumionen CaCl_2 ist.

5 5. Verfahren nach Anspruch 1, wobei die Zeit, die nötig ist, damit Thrombin Fibrinogen in ein Fibringerinnung umwandelt, weniger als 5 Sekunden beträgt.

10 6. Verfahren nach Anspruch 1, wobei das Thrombin über einen Zeitraum zwischen etwa 15 Minuten und dreihundert- und sechzig Minuten stabil ist.

15 7. Verfahren nach Anspruch 1, wobei die Zeit, die zum Erzeugen des Thrombins notwendig ist, weniger als eine Stunde beträgt.

8. Zusammensetzung zum Extrahieren von Thrombin aus Plasma, die Plasma, Ethanol (ETOH), eine Quelle von Calciumionen und Kugelchen umfasst.

20 9. Zusammensetzung nach Anspruch 8, wobei die Calciumionenquelle CaCl_2 ist.

10. Zusammensetzung nach Anspruch 9, wobei ETOH zu 18,9 % und CaCl_2 zu 23,0 mM (beides nach Volumen) in der Endkonzentration vorliegt.

25 11. Zusammensetzung nach Anspruch 9, wobei ETOH zu 18,9 % und CaCl_2 zu 5,7 mM (beides nach Volumen) in der Endkonzentration vorliegt.

12. Zusammensetzung nach Anspruch 9, wobei ETOH in einem Bereich zwischen 8 % und 20 % und CaCl_2 in einem Bereich zwischen 4,5 mM und 23,0 mM (beides nach Volumen) in der Endkonzentration vorliegt.

30 13. Zusammensetzung nach Anspruch 8, wobei die Kugelchen aus Borsilikat, Glas oder Keramik bestehen.

14. Vorrichtung zum Herstellen von Thrombin aus Plasma, die Folgendes umfasst:

35 eine Reaktionskammer, die Kugelchen für die Aufnahme einer Quelle von Calciumionen und Ethanol und ein Mittel zum Zuführen von Plasma in die genannte Reaktionskammer enthält; eine Spritze, die mit der genannten Reaktionskammer verbunden ist, um Thrombin von der genannten Reaktionskammer zu empfangen; und einen Filter zwischen der genannten Reaktionskammer und der Spritze, die Thrombin aufnehmen soll.

40 15. Vorrichtung nach Anspruch 14, wobei die Kugelchen aus Borsilikat, Glas und Keramik bestehen.

Revendications

45 1. Méthode pour produire de la thrombine autologue, les étapes comprenant :

l'obtention de plasma à partir d'un produit sanguin ;
l'addition d'éthanol, d'une source d'ions calcium et de perles avec le plasma pour préparer une solution contenant de la prothrombine ;
50 la conversion de la prothrombine dans la solution en thrombine ;
et le filtrage de la thrombine pour enlever les particules de matière.

2. Méthode selon la revendication 1 dans laquelle après avoir agité la solution, celle-ci est incubée pendant une durée supérieure ou égale à dix minutes.

55 3. Méthode selon la revendication 1, les perles étant formées de borosilicate, de verre ou de céramique.

4. Méthode selon la revendication 1, la source d'ions calcium étant CaCl_2 .

5. Méthode selon la revendication 1, le temps requis pour que la thrombine transforme le fibrinogène en un caillot de fibrine étant de moins de 5 secondes.
6. Méthode selon la revendication 1, la thrombine étant stable pendant une période de temps comprise entre 15 minutes et trois cent soixante minutes.
7. Méthode selon la revendication 1, le temps requis pour générer la thrombine étant de moins d'une heure.
- 10 8. Composition pour extraire la thrombine du plasma comprenant plasma, éthanol (ETOH), une source d'ions calcium et des perles.
9. Composition selon la revendication 8, la source d'ions calcium étant CaCl_2 .
- 15 10. Composition selon la revendication 9, ETOH étant présent à 18,9 % et CaCl_2 à 23,0 mM, dans les deux cas en volume, dans la concentration finale.
11. Composition selon la revendication 9, ETOH étant présent à 18,9 % et CaCl_2 à 5,7 mM, dans les deux cas en volume, dans la concentration finale.
- 20 12. Composition selon la revendication 9, ETOH étant présent à un pourcentage entre 8 % et 20 % et CaCl_2 étant présent à un taux entre 4,5 mM et 23,0 mM, dans les deux cas en volume, dans la concentration finale.
13. Composition selon la revendication 8, les perles étant formées de borosilicate, de verre ou de céramique.
- 25 14. Appareillage pour préparer la thrombine à partir de plasma comprenant une chambre de réaction contenant des perles pour accepter une source d'ions calcium et de l'éthanol, et des moyens pour acheminer le plasma dans ladite chambre de réaction ; une seringue connectée à ladite chambre de réaction pour recevoir la thrombine provenant de ladite chambre de réaction ; et un filtre entre ladite chambre de réaction et la seringue devant recevoir la thrombine.
- 30 15. Appareillage selon la revendication 14, les perles étant formées de borosilicate, de verre ou de céramique.

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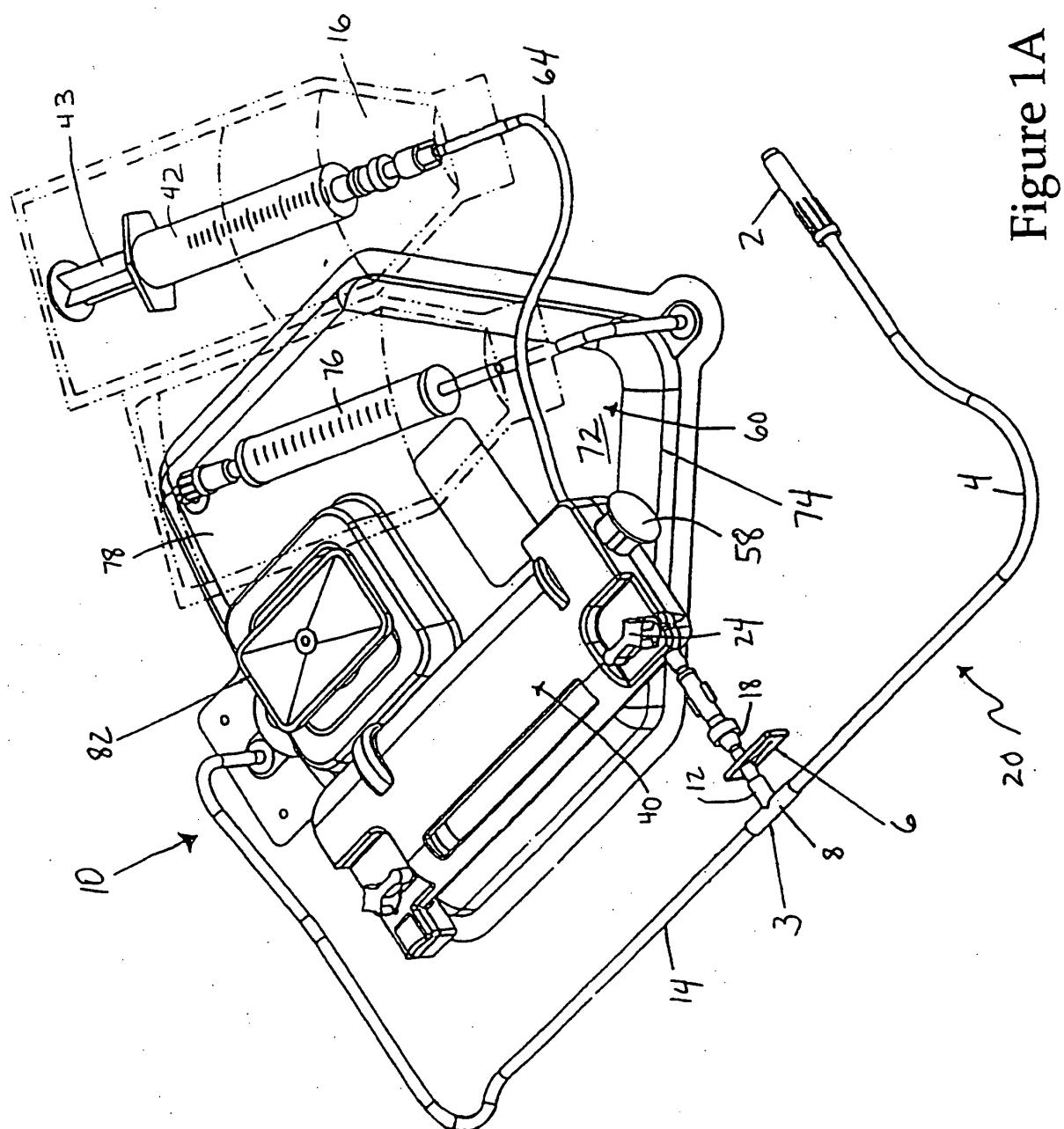
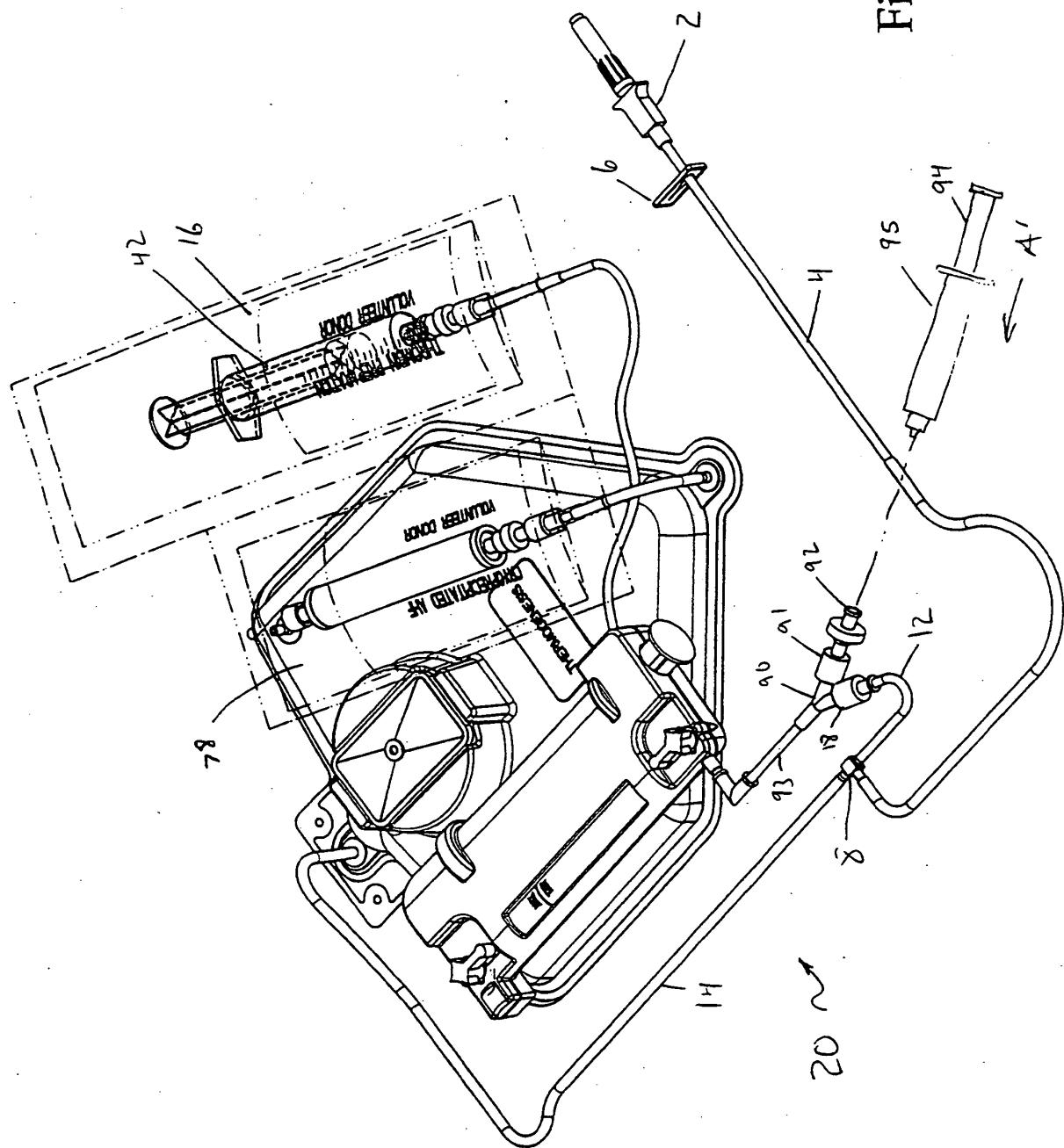


Figure 1A

Figure 1B



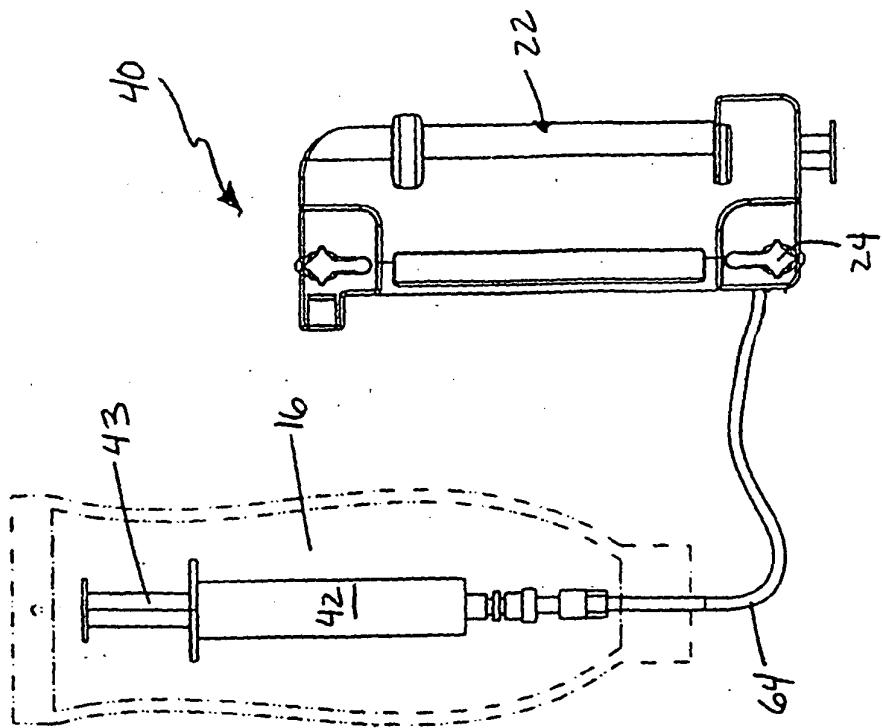


Figure 2B

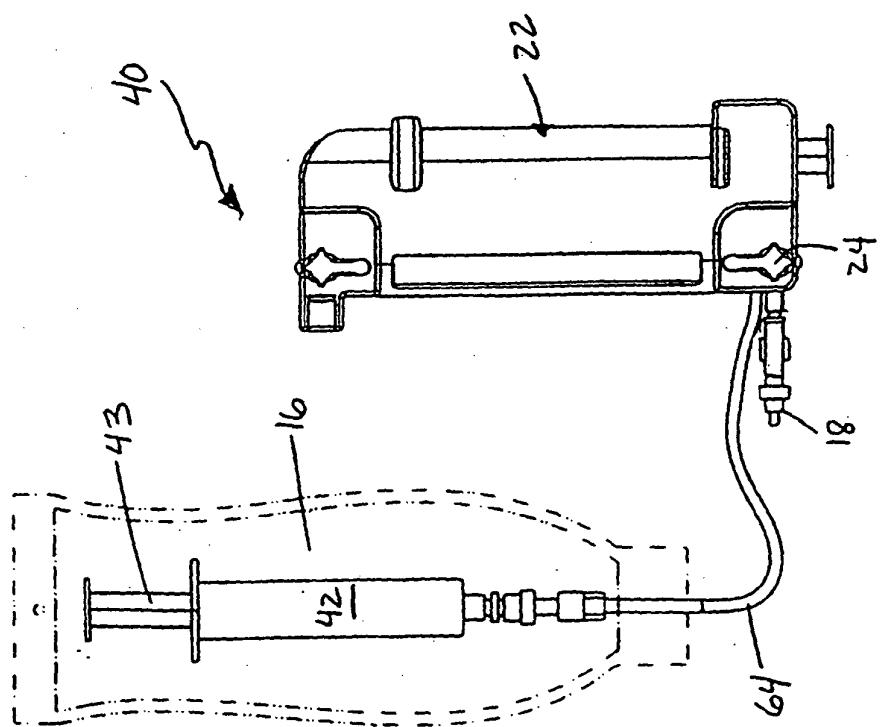


Figure 2A

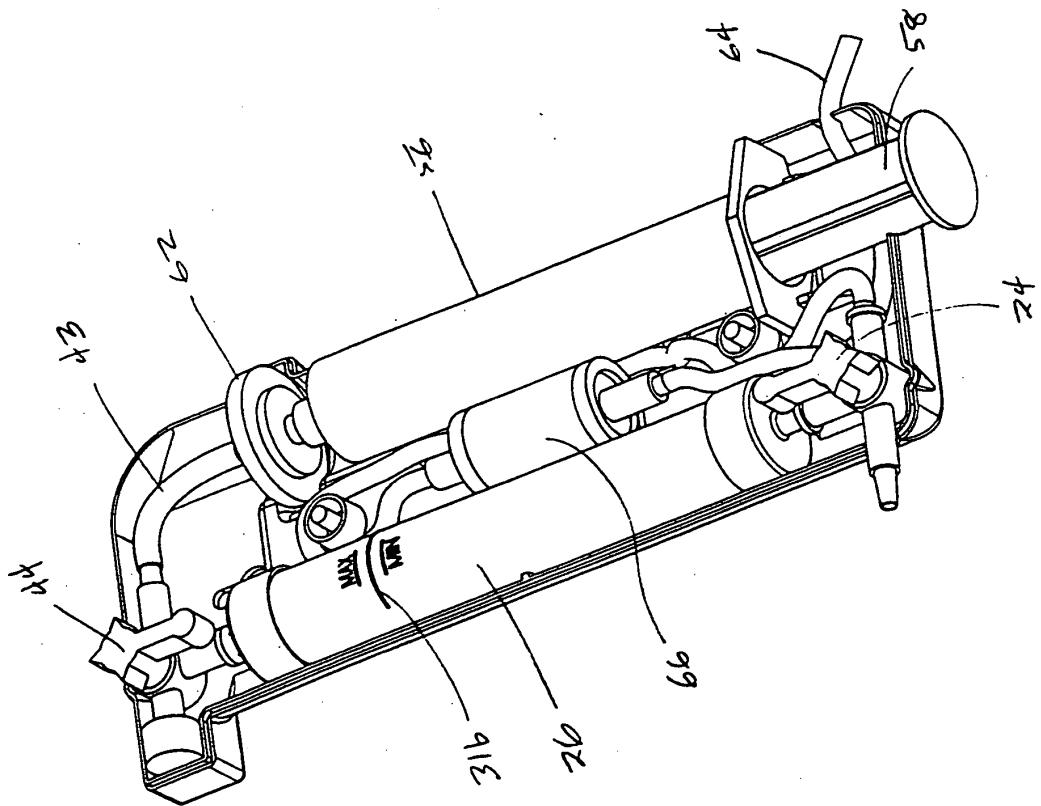


Figure 3B

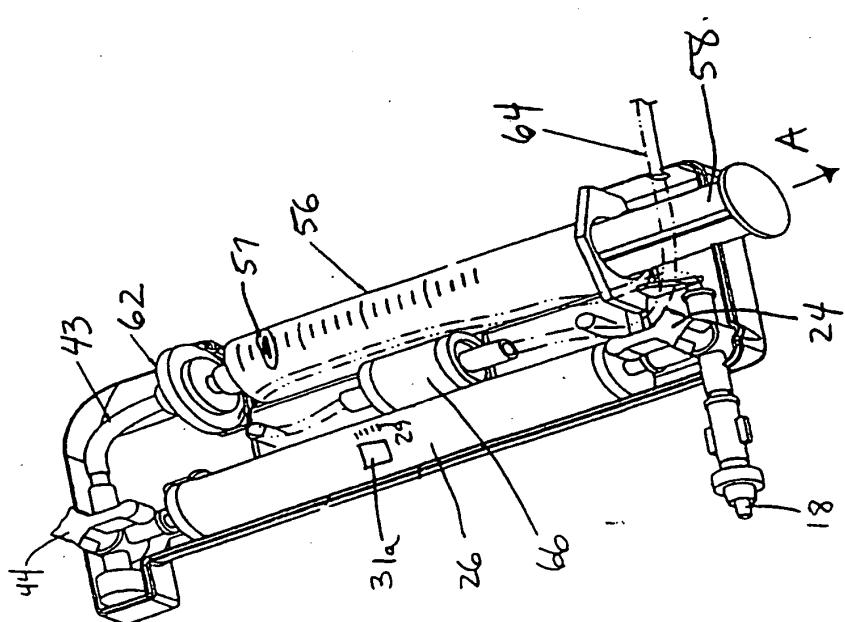


Figure 3A

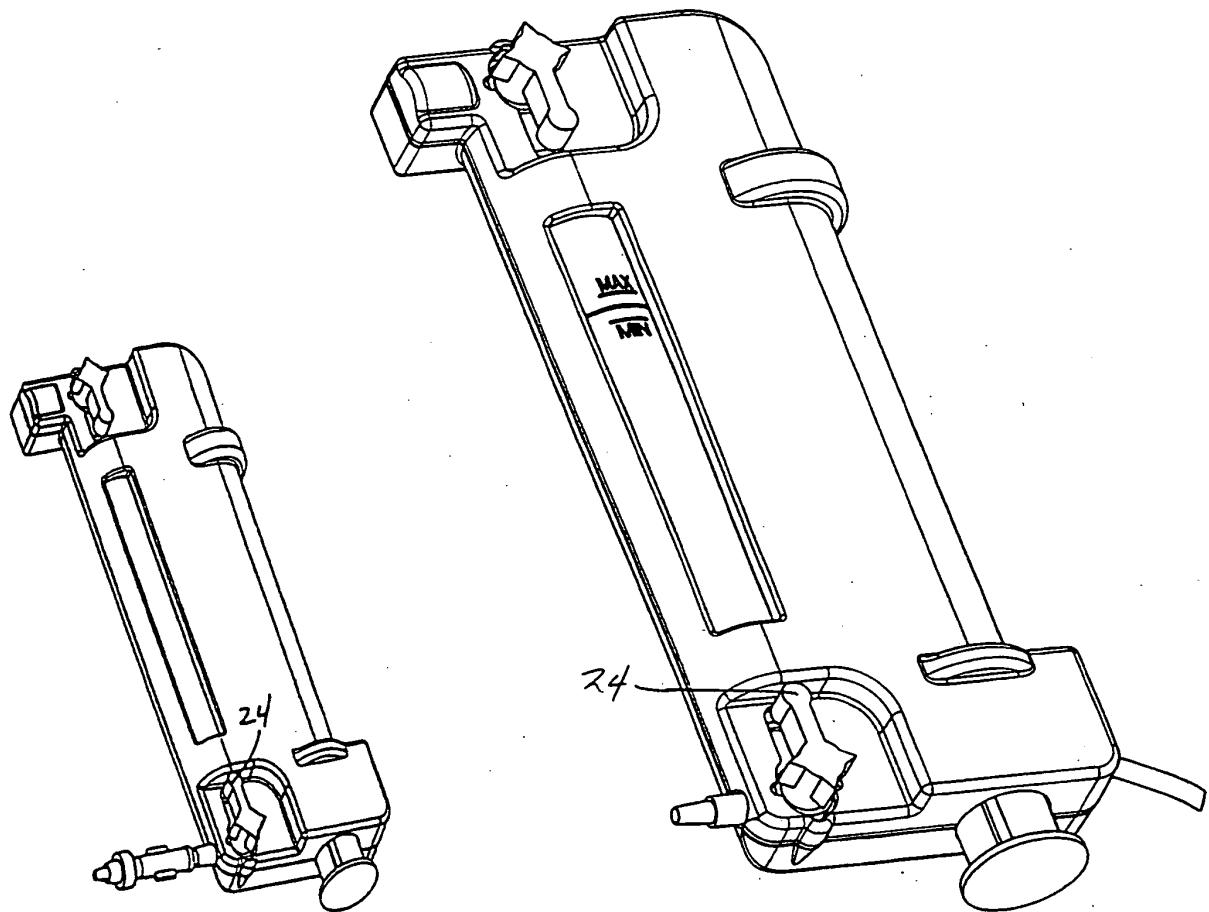


Figure 4A

Figure 4B

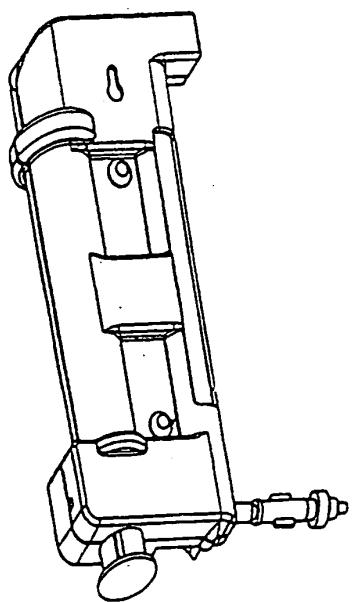


Figure 5A

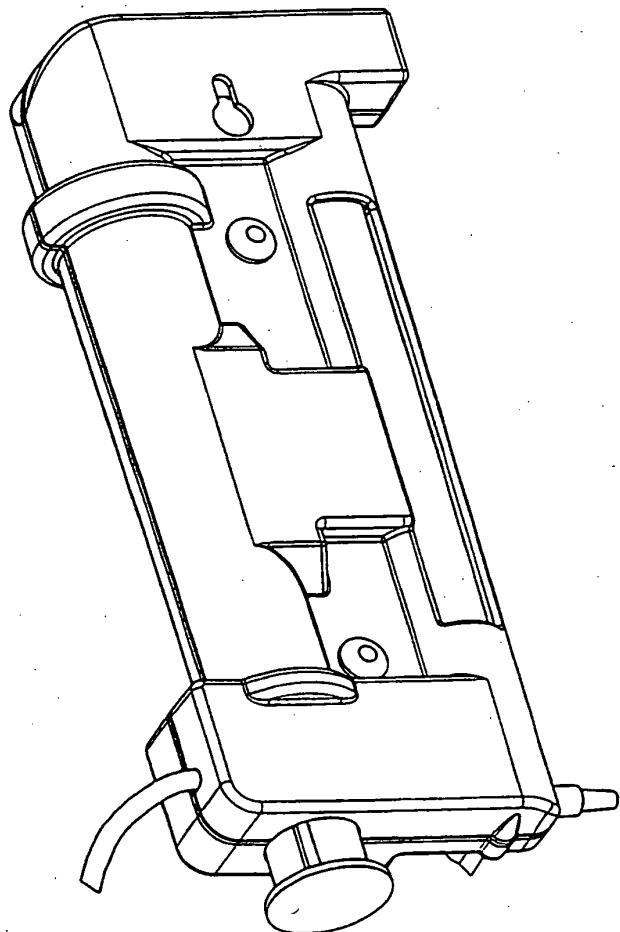


Figure 5B

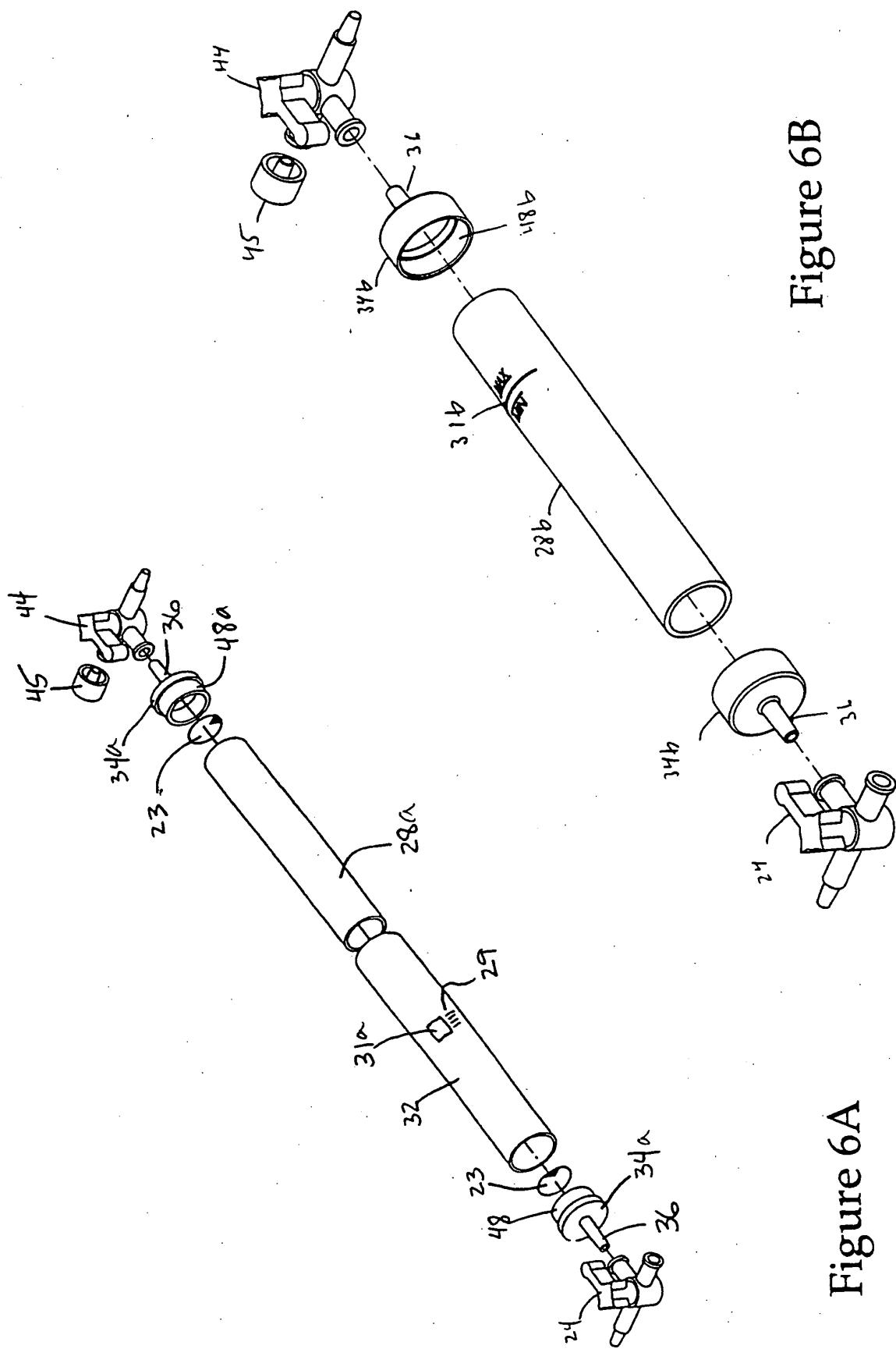


Figure 6A

Figure 6B

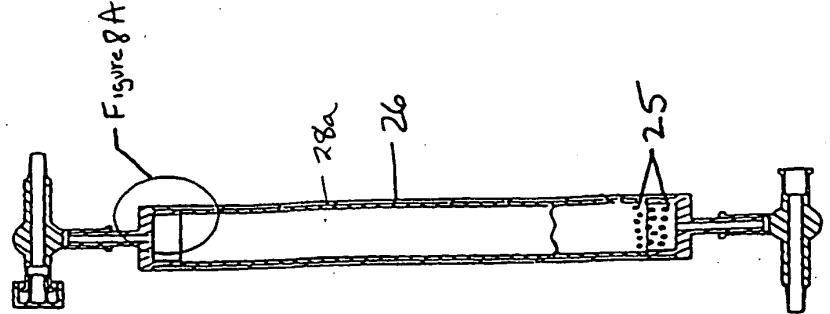
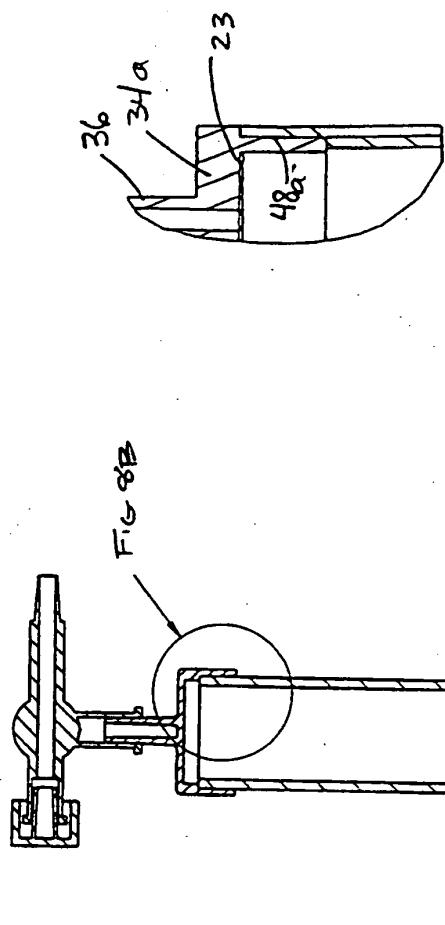


Figure 8A

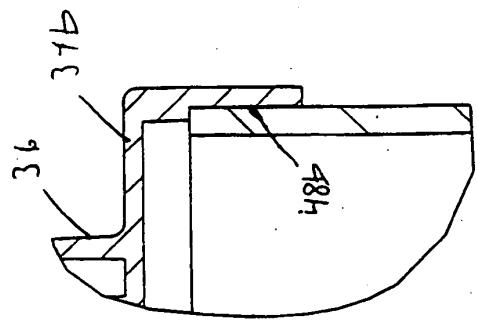


Figure 8B

Figure 10

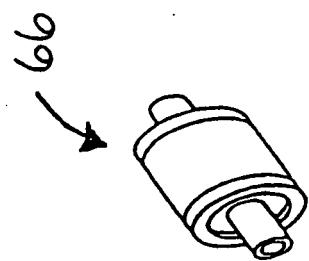


Figure 9B

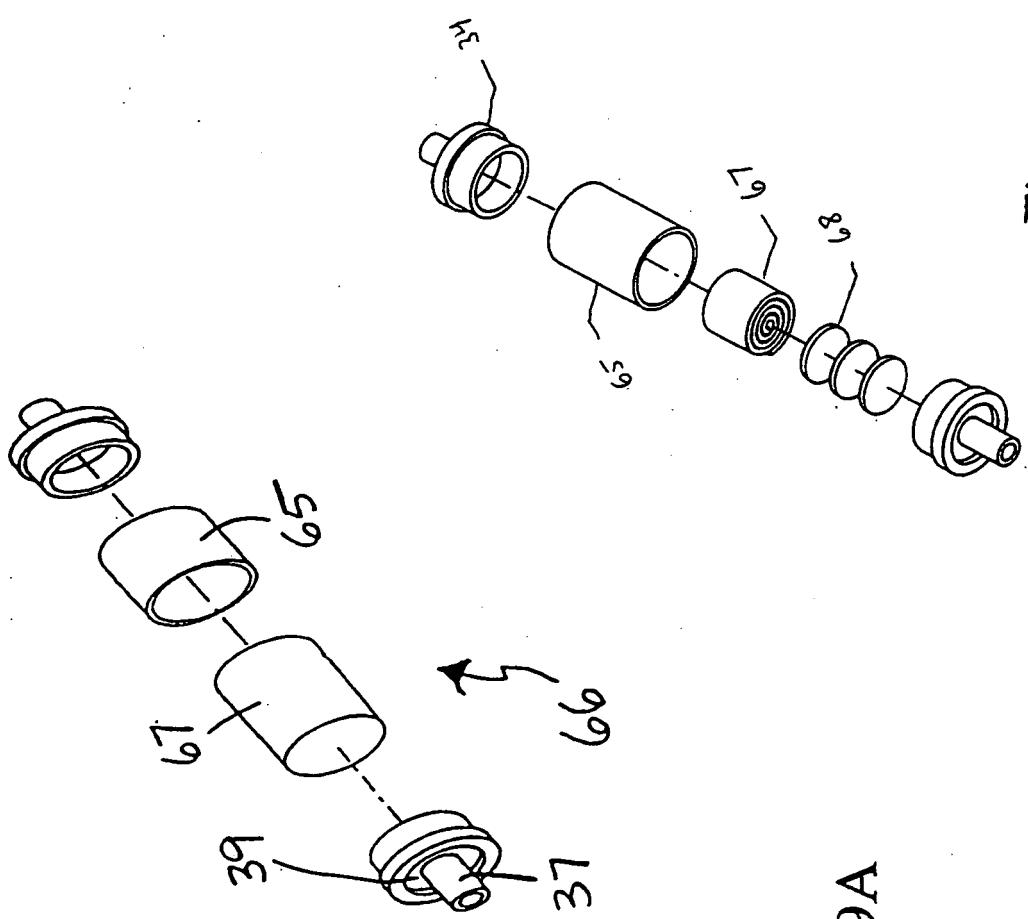


Figure 9A

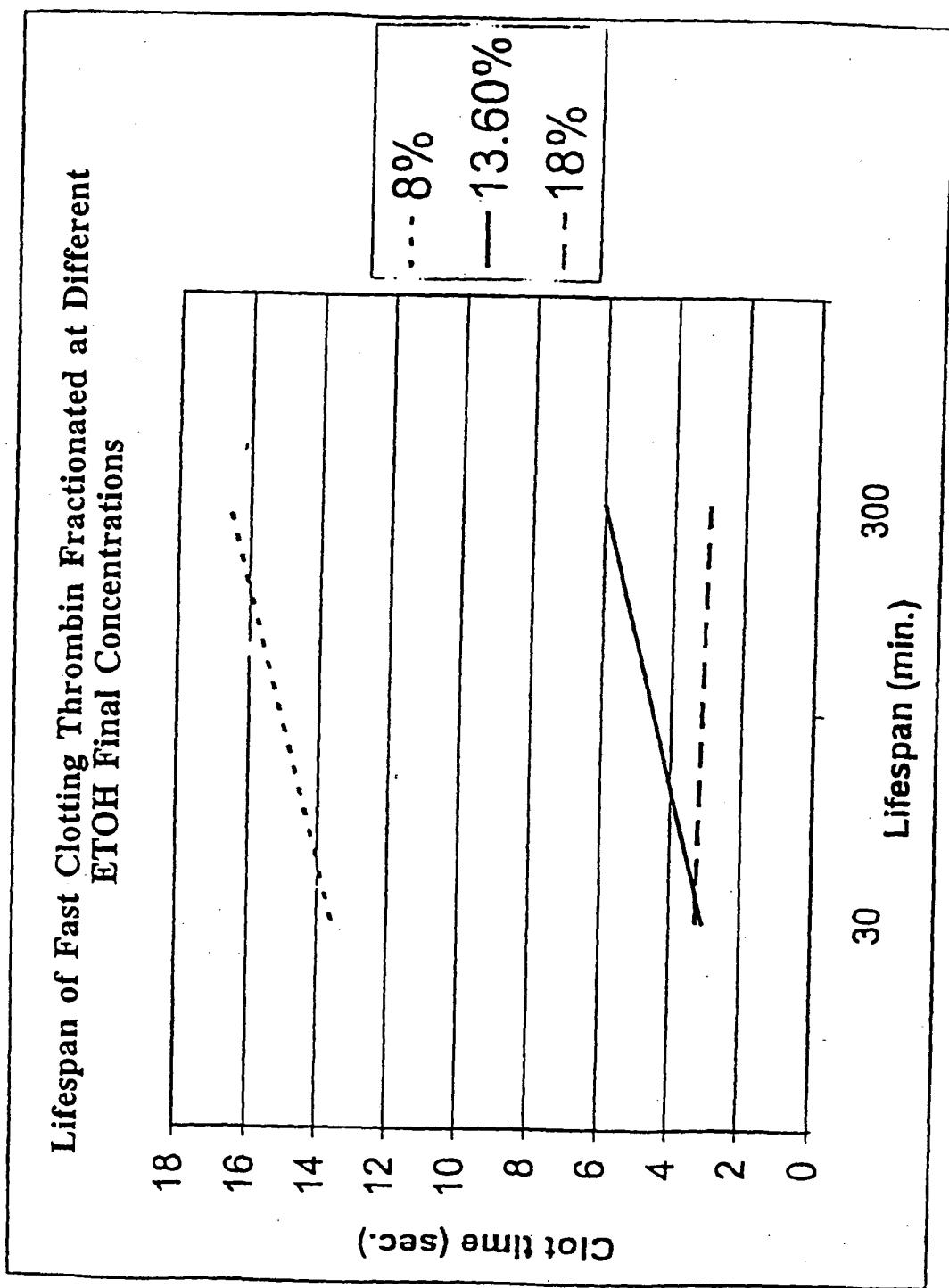


Figure 11

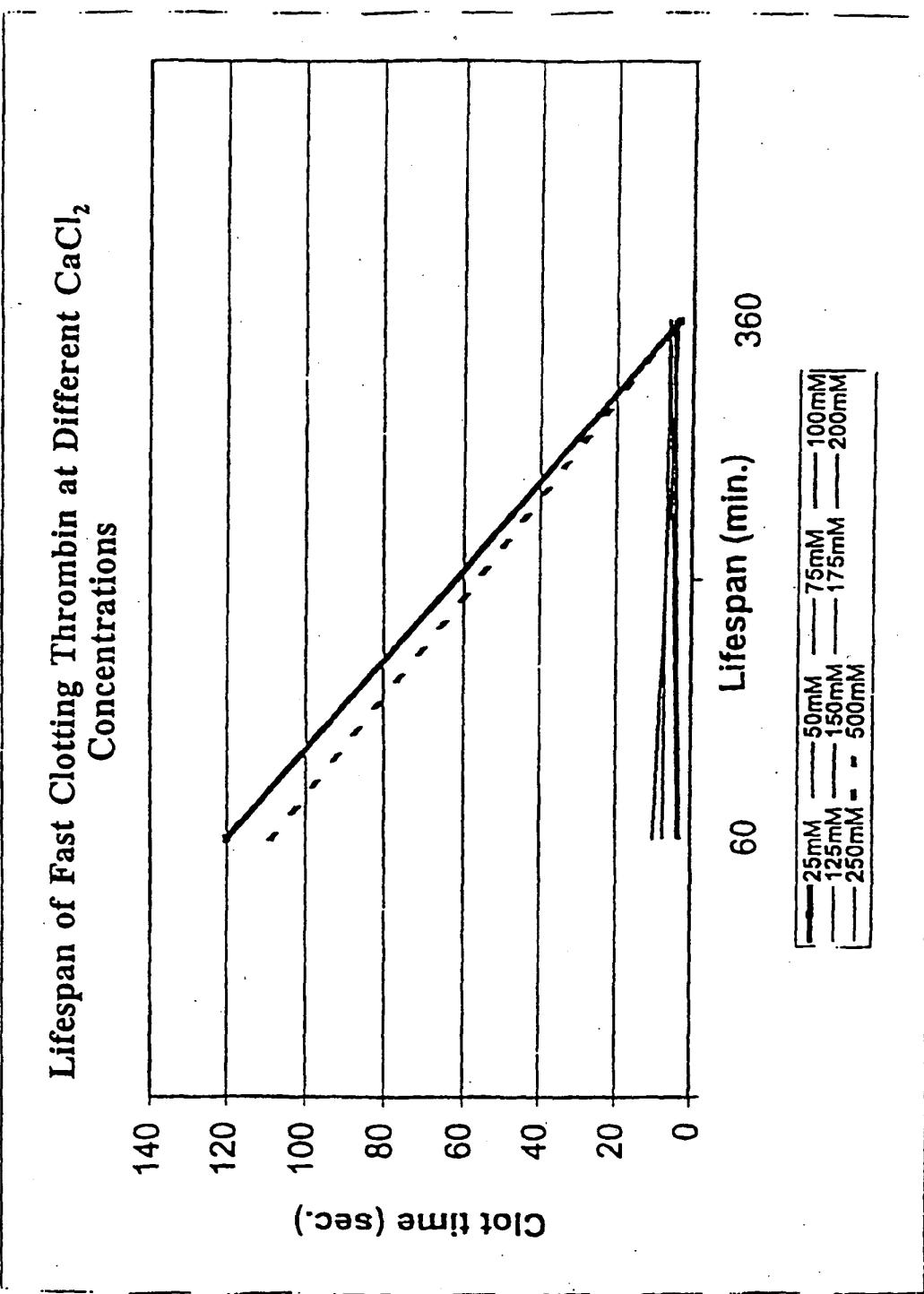


Figure 12

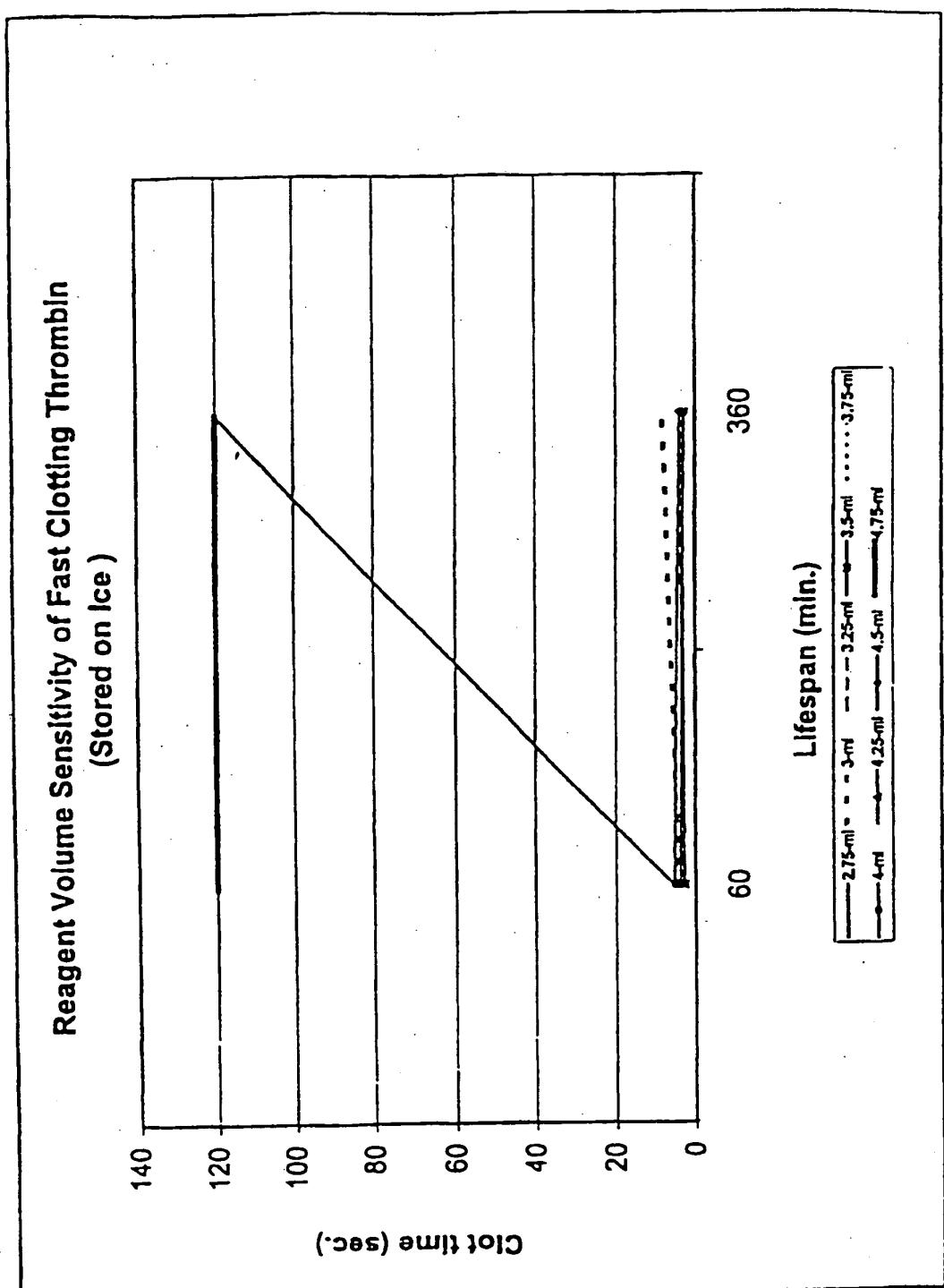


Figure 13

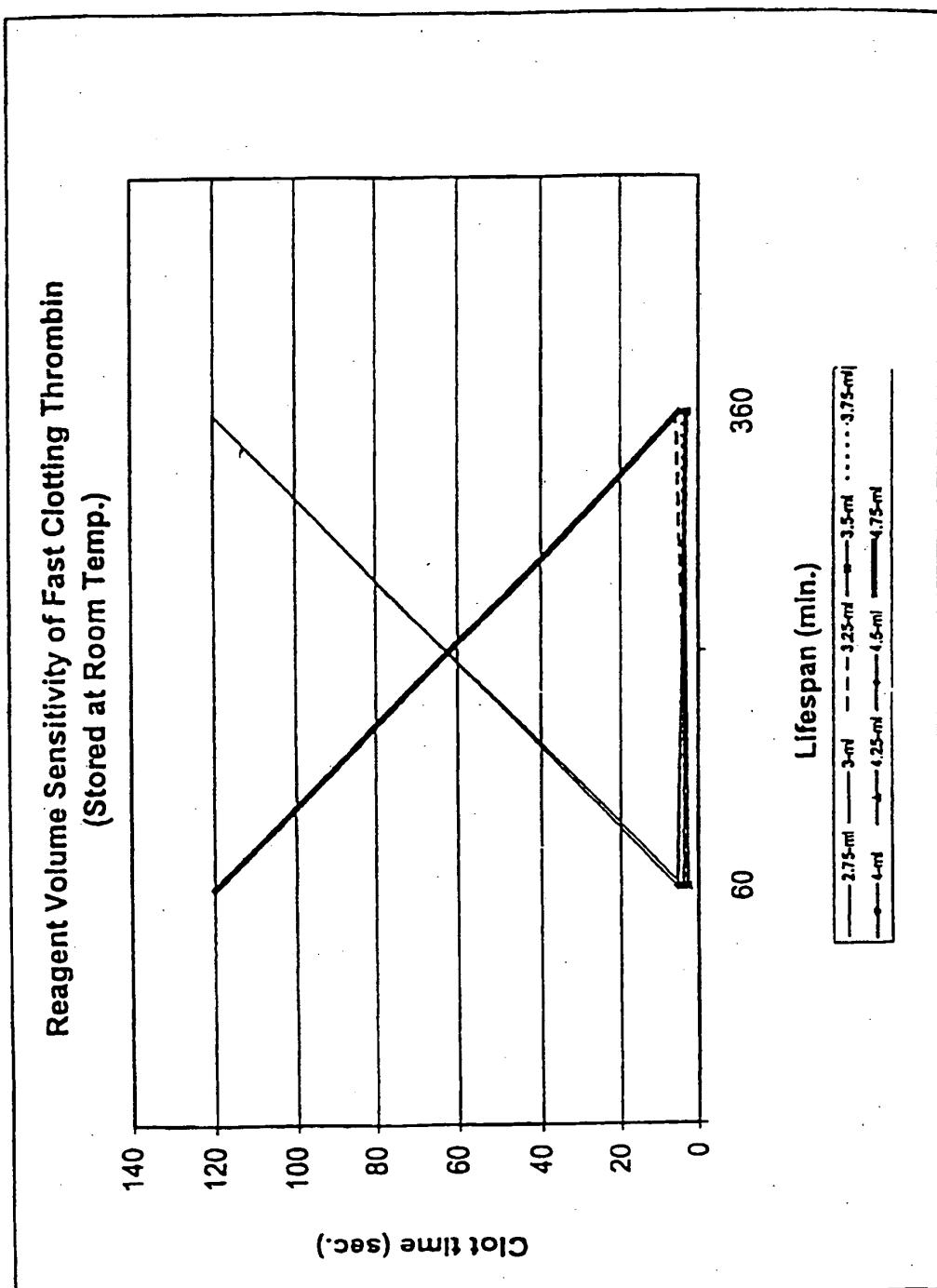


Figure 14

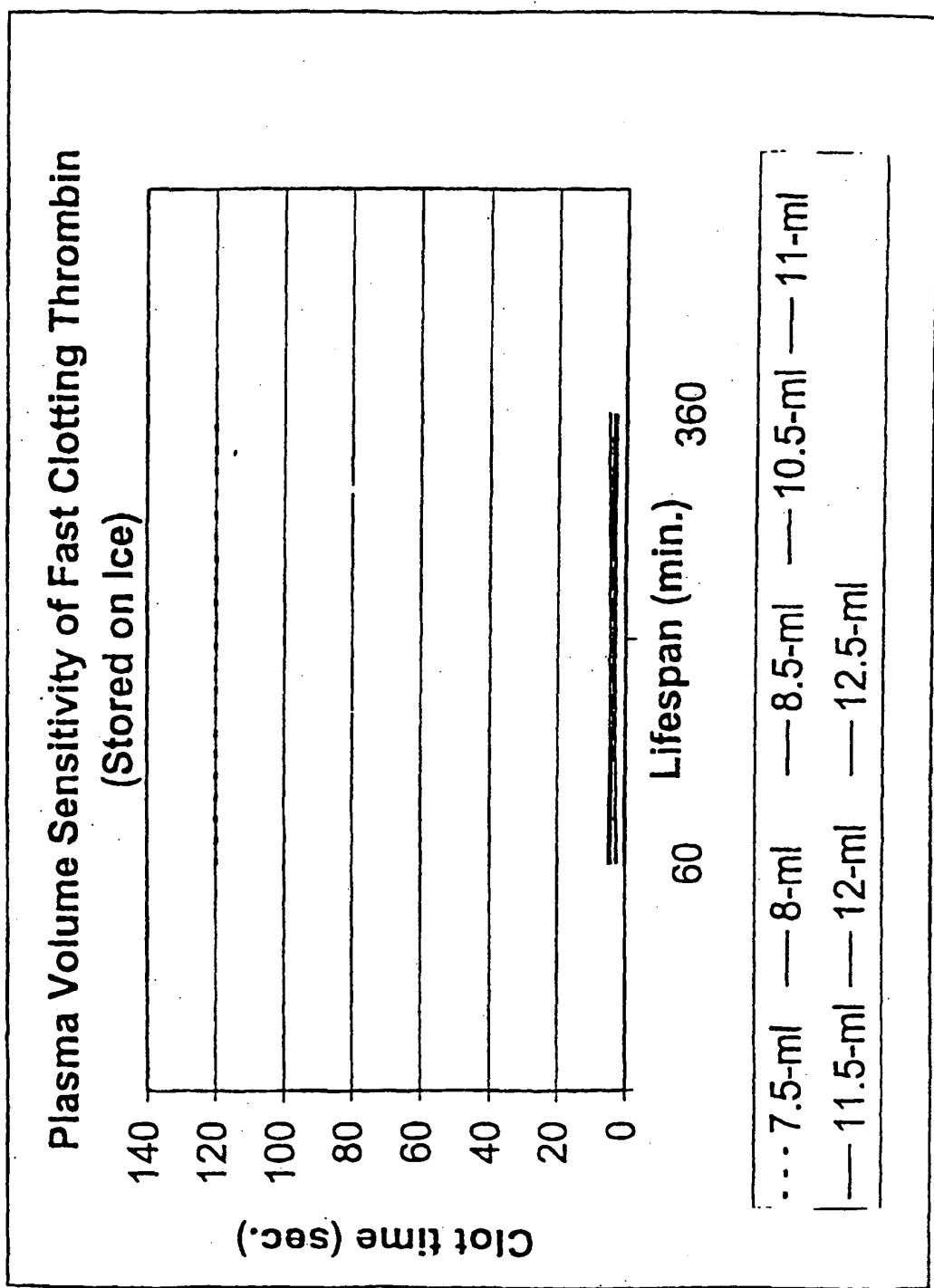


Figure 15

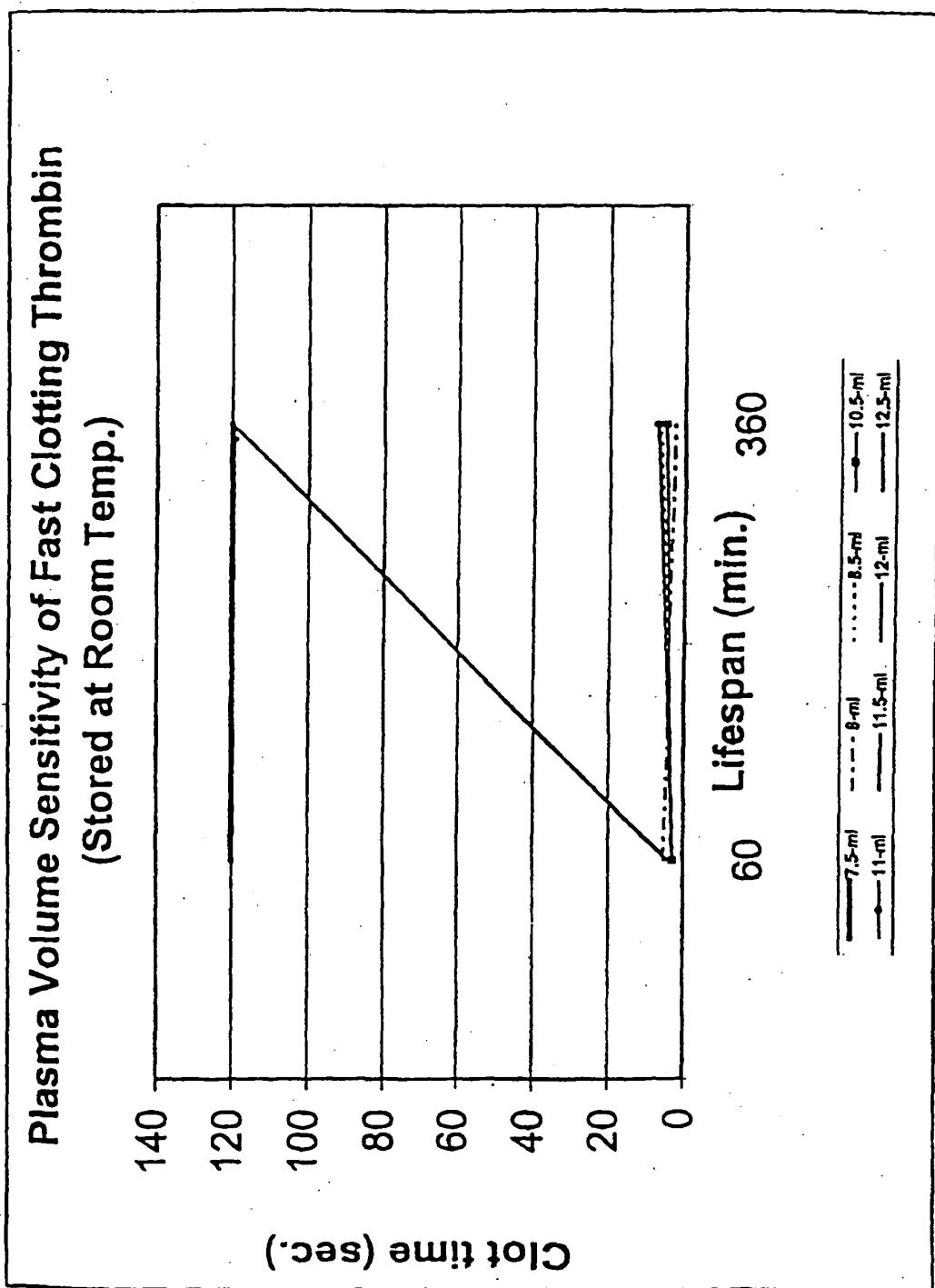


Figure 16

REFERENCES CITED IN THE DESCRIPTION

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Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description**Technical Field**

5 [0001] The following invention relates generally to the preparation of a high specific activity thrombin enzyme from a given unit of plasma, which is sufficiently stable that it provides rapid clotting of a fibrinogen-rich solution of clotting and adhesive proteins for more than six hours when held at room temperature or lower.

Background Art

10 [0002] Formulation of a fibrin sealant mimics the last step of the coagulation cascade wherein the enzyme thrombin cleaves fibrinogen which is then cross-linked into a semi-rigid or flexible fibrin clot. This fibrin clot adheres to wound sites, forming a barrier to fluid leaks and generates adhesion between tissues, while providing hemostatic and healing properties to the treated site.

15 [0003] Presently marketed, applicant's CryoSeal™ system is a device which harvests cryoprecipitated, concentrated clotting and adhesive proteins, including fibrinogen and Factor XIII from a donor's plasma in approximately one hour. The one hour cryoprecipitation harvesting, provided by the CryoSeal™ system, compared to the 1 to 2 day cryoprecipitation process routinely practiced in Blood Banks, means that CryoSeal™ harvesting of clotting and adhesive proteins can occur right in the perioperative theater with the patient close by, thereby avoiding the need to initiate the process 20 days in advance.

25 [0004] These CryoSeal™ harvested clotting and adhesive proteins, when combined with bovine or human thrombin, forms a biological glue useful for surgical hemostasis and tissue adhesion. Commercially available thrombin, however, is generally sourced from bovine or human plasma pools, so the patient would still be at risk of negative immune reactions or contamination by infectious blood born viruses and, possibly Crutzfeld-Jacobs Disease (CJD) or new variants of CJD (NVCJD). An advantage of the Cryoseal™ cryoprecipitation invention is that the harvested clotting and adhesive proteins sourced from the patient's own blood eliminates the risk of contamination by infectious blood-borne disease when these clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

30 [0005] It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

35 [0006] The concept of utilizing thrombin and/or fibrinogen sourced from the patient in a medical procedure performed on that patient is not novel and was first described by Andrianova in 1974. Some twenty years later, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994 and its related U.S. Patent No. 5,795,780) describes an improved fibrin glue in which the thrombin component, which required thirteen steps, including centrifugation, and separation of intermediate precipitates and adjusting the ionic strength of the blood and pH of the plasma to prepare, would be combined with a fibrinogen component also sourced from the plasma of the same donor. However, these many preparation steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour. The present invention, *inter alia*, is distinguished in that it is undiluted by pH adjustment.

40 [0007] Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192 and its related WO96/31245) follows Cederholm-Williams by teaching another method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which most of the fibrinogen in the plasma is first precipitated and removed to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different and simpler than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides 45 clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes. The present invention, *inter alia*, is distinguished in that the plasma is unprocessed as for example by not precipitating out fibrinogen.

50 [0008] These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue, before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

55 [0009] US5795780 discloses a method of preparing thrombin and fibrinogen. The method includes acidification of the blood, separation of the plasma from the blood, introduction of the plasma in a chamber containing beads and ethanol, precipitation of the fibrinogen in an ice bath and removal of the fibrinogen using centrifuging steps-Once the euglobulin fraction has been formed via a number of additional steps, an alkalization step takes place and CaCl_2 is introduced into the chamber.

[0010] The following prior art reflects the state of the art of which applicant is aware and is included herewith to

discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

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25 **[0011]** The other prior art listed above, not all of which are specifically discussed catalog the prior art of which the applicant is aware. These undiscussed references diverge even more starkly from the instant invention specifically distinguished below.

Disclosure of Invention

30 **[0012]** The present invention provides a method for generating autologous thrombin, the steps comprising:

Obtaining plasma from a blood product;

Adding ethanol, a source of calcium ions and beads with the plasma to prepare a solution containing prothrombin; Converting the prothrombin in the solution to thrombin; and filtering the thrombin to remove particulate matter.

35 **[0013]** The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (e.g. six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological scalant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field (Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (i.e., less than 5 seconds) during only a very narrow four to five minute time period, or required so many steps and elapsed time it would not be suitable for perioperative preparation, both totally impractical for the broad range of surgeries.

40 **[0014]** The present invention provides a stable thrombin enzyme which can cause precise, repeatable fast or slow polymerization of clotting and adhesive proteins over a duration of up to six hours - throughout even a long surgery. Further, the user of clotting and adhesive proteins and thrombin all sourced from a single donor will eliminate various disease risks posed from the user of commercial fibrin glues where the fibrinogen is sourced from plasma pooled from thousands of donors and the thrombin is either sourced from a similar pool of human plasma or of bovine origin. The speed and simplicity of the production of stable thrombin by use of this invention allows it to be prepared just prior to or during operative procedures and it will provide fast clotting throughout even the longest surgeries. The thrombin produced by this invention can be diluted in saline, water and dilute CaCl_2 solution (e.g. 125 mM CaCl_2) to provide precise, slower clotting times thereby allowing any preferred time from less than five seconds to longer than 2 minutes.

45 **[0015]** The procedure of the invention is preferably comprised of three steps, the first two of which should occur at the same time:

55 1. Preparing a fraction enriched in prothrombin by use of ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Fibrinogen and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent activation

to long-term functional thrombin.

2. Adding calcium ions and beads to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.
3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

Industrial Applicability

[0016] The industrial applicability of this invention shall be demonstrated through discussion of the following objects of the invention.

[0017] Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin from the donor's plasma.

[0018] It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

[0019] It is a further object of the present invention to provide thrombin as characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

[0020] It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

[0021] It is a further object of the present invention to provide a method for producing thrombin as characterized above which has a process time in as little as thirty minutes, up to seventy-five minutes.

[0022] It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

[0023] Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride ($CaCl_2$) to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

[0024] Viewed from a second vantage point, it is an object of the present invention to provide a method for generating autologous thrombin from a patient, the steps including: obtaining a blood product from the patient; sequestering plasma from the product; enriching the prothrombin in a plasma fraction; converting the prothrombin to thrombin, and filtering particulate from the thrombin.

[0025] Viewed from a third vantage point, it is an object of the present invention to provide a method for producing autologous thrombin which is stable for more than fifteen minutes, the steps including: sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

[0026] Viewed from a fourth vantage point, it is an object of the present invention to provide an autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

[0027] Viewed from a fifth vantage point, it is an object of the present invention to provide a composition for extracting thrombin from plasma consisting essentially of: Plasma; Ethanol (ETOH); $CaCl_2$.

[0028] Viewed from a sixth vantage point, it is an object of the present invention to provide a method for preparing thrombin comprising: obtaining plasma; adding ETOH and $CaCl_2$ and beads to the plasma, forming a composition; agitating the composition; incubating the composition in a static or rocking mode; filtering the composition of particulate, thereby passing the thrombin through the filter.

[0029] Viewed from a seventh vantage point, it is an object of the present invention to provide a device for preparing thrombin from plasma, comprising: a reaction chamber having beads, a solution of $CaCl_2$ and ETOH therein; means for admitting plasma into the reaction chamber; thrombin receiving syringe coupled to the reaction chamber to receive the thrombin; and a filter located between the reaction chamber and the thrombin receiving syringe.

[0030] Viewed from an eighth vantage point, it is an object of the present invention to provide an autologous biological glue processing device, comprising, in combination: a thrombin processing means, a clotting and adhesive proteins processing means operatively coupled to the thrombin processing means, means for receiving plasma via the operative coupling for subsequent conversion of the plasma to, respectively thrombin and clotting and adhesive proteins.

[0031] The present invention provides a method and apparatus that produces thrombin which is sufficiently stable that it can provide less-than-5-second clots for up to six hours, substantially more stable than demonstrated in all prior art. Further, the clot time can be modified at will through dilution with saline.

[0032] The present invention further provides an efficient method of preparation. Improved cryoprecipitation of clotting and adhesive proteins through the CryoSeal™ invention requires less than one hour. In this same time frame, the autologous human thrombin component can be manufactured with minimal materials and methods from the same source plasma. Both of the biological components of the biological glue are easily combined in a surgical setting, administered

to the very same donor patient, and the resultant clotting provides hemostasis or tissue adhesion at the wound site.

[0033] The present invention additionally provides a method for sterile production of both components of the biological glue. The improved sterile manufacturing described herein provides a final product that is essentially free of contamination by non autologous microbes.

5 **[0034]** These and other objects will be made manifest when considering the following detailed specification when taken in conjunction with the appended drawing figures.

Brief Description Of Drawings

10 **[0035]**

Figures 1A and 1B are perspective views of apparatuses for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins therefrom.

15 Figures 2A and 2B are plan views of the thrombin processing sets removed from the processing sets that extracts clotting and adhesive proteins.

Figures 3A and 3B are perspective views of the interior of the thrombin processing cases with the thrombin syringe shown in figures 2A and 2B removed therefrom.

20 Figures 4A and 4B are perspective views of the thrombin cases upper halves.

Figures 5A and 5B are perspective views of the thrombin cases lower halves.

25 Figures 6A and 6B are exploded parts views of the reaction chamber 26 shown in figures 3A and 3B along with the valving structure at opposed ends thereof.

Figures 7A and 7B are sectional views of the reaction chambers and valving structures depicted in figures 6A and 6B.

Figures 8A and 8B are detail of construction of that which is shown in figures 7A and 7B.

25 Figures 9A and 9B are exploded parts view of filter alternatives used in figures 3A and 3B.

Figure 10 is a perspective view of that which is shown in figure 9.

Figure 11 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations.

Figure 12 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations at different CaCl_2 concentrations.

30 Figure 13 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored on ice.

Figure 14 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored at room temperature.

35 Figure 15 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored on ice.

Figure 16 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored at room temperature.

Best Mode(s) for Carrying Out the Invention

40 **[0036]** Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figures 1A and 1B.

[0037] In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

45 **[0038]** More particularly, viewing both figures 1A and 1B, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has plural positions for stop valves 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60. The first valve branch 12 also includes a stop valve 6.

50 **[0039]** Viewing figure 1B, prior to the introduction of plasma through the first branch 12 thrombin processing unit 40, reagent from preloaded syringe 95 is injected pushing plunger mechanism 94 in the direction of A', into receiving system 20 through sterile barrier filter 92. The reagent passes through one way valve 91; Y connector 90, that merge coupling 18 and valve 91, through branch tubing 93; and finally into the interior of casing 22. Referring to figure 3B and 7B, a valve 24 initially directs the reagent to a reaction chamber 26.

55 **[0040]** Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. Although this system can be dimensioned for any

size batch, the plasma required for the thrombin processing unit will typically be 9-10 ml so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and adhesive proteins from the clotting and adhesive proteins processing unit 60.

[0041] In the embodiments shown in figures 1A and 1B, sealed bags 16 and 78 overlie both the thrombin dispensing syringe 42 (and a lead in of conduit 64) and cryoprecipitate storage tube 76 to provide sterility until both storage containers are introduced into a sterile surgical field (e.g., operatory). Prior to that, the thrombin processing unit 40 operates as shown and described with reference to figures 2A through 10. Viewing figure 1B, after reagent is added, plasma enters the first branch 12 and passes beyond a coupling 18, through tubing branch 93, and into an interior of the casing 22.

[0042] Referring back to figure 1A, the thrombin processing unit 40 operates as shown and described with reference to figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A and 10. As mentioned, fluid enters the first branch 12 and (figure 1A) passes beyond a coupling 18 and into an interior of a casing 22. Coupling 18 is preferably frictionally and/or adhesively attached to the first branch 12 yet the thrombin processing unit 40 can still be removed (e.g. figure 2A) from the processing set 10 (e.g., by merely detaching or severing branch 12 followed perhaps with heat sealing) after receiving the plasma as shown in figure 2. If adhesive is used, it is a sterile grade for use in an operatory.

[0043] Referring to figure 3A, a valve 24 initially directs the plasma to a reaction chamber 26 having an interior tube 28a (figure 6A) preferably formed from glass and capable of receiving a volume, for example 15 ml. Glass tube 28a is preferably shorter than and circumscribed by an overlying barrel 32 preferably formed from PVC. A window 31a in the PVC barrel 32 can be used to gauge and/or verify the contents within the glass tube 28a. Gauging may also include gradations 29, indicating a volume on the glass tube. The glass tube 28a of the reaction chamber 26 receives the plasma from the first branch 12 and into its interior for mixing with reagents preloaded in the glass tube 28a and described hereinafter. As shown in figure 7A, the interior of the glass tube is preferably prefilled only partially with beads 25 preferably formed from borosilicate, glass or ceramic to enhance the reaction and agitation.

[0044] Referring to figure 3B, a valve 24 initially directs the plasma to a reaction chamber 26 having tube 28b (figure 6B) preferably formed from clear polycarbonate and capable of receiving a volume, for example, 15 ml. Graduated lines 31b on the polycarbonate tube 28b can be used to gauge the contents within the tube 28b. The polycarbonate tube 28b of the reaction chamber 26 receives the plasma from the first branch 12 and into the interior for mixing with reagents previously added into the polycarbonate tube 28b and described hereinafter. As shown in figure 7B, the interior of the tube 28b is preferably prefilled only partially with beads 25 preferably formed from borosilicate or ceramic to enhance the reaction and agitation.

[0045] The reaction chamber 26 of the embodiment shown in figures 1A and 3A is formed with first and second end caps 34 detailed in figures 6A, 7A and 8A. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 is isolated from the beads 25 by a screen 23 nested within necked-down portion 48. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the glass tube 28a and through flow is controlled by the valves 24, 44. As shown in figure 8A, the cap 34 includes an annular necked-down portion 48a which frictionally and/or adhesively resides within an interior hollow of the PVC barrel 32. In this way, the necked-down portion 48 rests upon ends of the glass tube 28a in sealing engagement therewith, isolating the interior of the reaction chamber from the PVC barrel 32.

[0046] For the embodiment forming the reaction chamber 26 of the embodiment shown in figures 1B and 3B mainly out of polycarbonate tube 28 is detailed in figures 6B, 7B and 8B. This reaction chamber 26 is formed with first and second end caps 34 detailed in figure 8B. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 has interior obstructions preventing passage of beads 25 while allowing passage of fluid. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the polycarbonate tube 28b and through flow is controlled by the valves 24, 44. As shown in figure 8B, the cap 34 includes an annular interior recess portion 48b which adhesively resides on the interior surface of the polycarbonate tube 28b.

[0047] Preferably, ethanol and calcium chloride are the reagents which have been preloaded into the reaction chamber 26 or within reagent syringe 95. Initially, both valves 24 and 44 are oriented so that reagents will not pass therebeyond to seal the chamber for the embodiment of figure 1A. Viewing figure 1B, initially valve 24 is oriented so plasma will not enter reaction chamber 26, and valve 44 is oriented to allow passageway between the reaction chamber 26 and the draw plunger 56. Referring back to figure 1A, after the plasma has been pumped into processing unit 60, valve 44 is turned to allow access to the draw plunger 56 and valve 24 is oriented to allow access between the passageway 21 and the reaction chamber 26. Slide clip 6 is opened with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1A, syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. Referring back to figure 1B, the reagent syringe 95 is attached to open end of sterile barrier filter 92. Plunger 94 is

depressed to transfer reagent syringe through sterile barrier filter and passageway 93 to reaction chamber 26. Likewise to the figure 1A embodiment, the figure 1B, with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1B, the syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. In both embodiments syringe 56 includes a filter 62 located in the flow path. More specifically, the path 43 between valve 44 and syringe 56 includes a filter 62 located in the flow path. The filter 62 provides an aseptic microbial barrier so that, upon subsequent delivery of the thrombin to the dispensing syringe 42 (figure 1), there is no contamination from around the seal 57 of plunger 58 delivered to syringe 42. Plasma will subsequently enter chamber 26 from conduit 4 to replace air. Valve 24 is oriented to address filter 66. The reagents and plasma are briefly agitated assisted by beads 25 (and allowed to incubate for about 40 to 70 minutes). After incubation, thrombin processing unit 40 is agitated to loosen and break up gel formation. For the embodiment of figure 1B, the thrombin processing unit 40 is then returned to a motionless horizontal position for no less than 10 minutes. Afterwards the thrombin processing unit 40 is again agitated to loosen and break up gel formation. For both embodiments, the plunger of syringe 56 is pushed in the direction opposite arrow A to move thrombin from chamber 26 through filter 66 into syringe 42. Delivery of thrombin to syringe 42 can be enhanced by retracting plunger 43 of syringe 42, defining a push pull system. Filter 66 removes particulate matter from the thrombin, including gel.

[0048] By allowing the thrombin contained in the reaction chamber 26 to reside therein after agitation for no less than 10 minutes enhances the effectiveness of the filter 66 in removing particulate matter for subsequent utilization. The time span for conversion and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer, or expression through a thin tube.

[0049] Figures 9A, 9B and 10 reveal alternative embodiments of filter 66 which includes an outer cylindrical wall 65 with end caps 34 each having a cylindrical spout 37 circumscribed by an annular recess 39. The alternative embodiment shown in figure 9A shows the centrally disposed cylindrical filter element 67a is preferably formed from polyurethane foam. While as shown in figure 9B the centrally disposed cylindrical filter element 67b is preferably formed from rolled polyester. Also shown in figure 9B, are circular filters 68 preferably formed from glass fiber or polyester. In each alternative embodiment, filter 67a or 67b filters by weight, size and protein binding.

[0050] Referring back to figures 1A and 1B, attention is now directed to the clotting and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 72 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 74 of the chamber 72 for subsequent extraction by means of a clotting and adhesive protein collection tube or dispensing syringe 76 contained in a sterile pouch 78. Chamber 72 is protected during this process by a filter vent 82 preventing contamination. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive collection tube or dispensing syringe 76, the two storage containers 42, 76 can be decoupled from the processing set 10 (e.g. sterile disconnect device), and passed near the sterile, surgical arena. The overwrap bags are subsequently opened, and the storage containers 42, 76 are decoupled and transferred into the surgical area where the contents are dispensed into individual sterile 3cc plastic syringes which are subsequently loaded into the fibrin glue applicator for spraying or line and dot application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

[0051] Both dispensing syringes 42 and 76 are stored at room temperature, or preferably stored at their optimal conditions: cryoprecipitate 76 being stored at room temperature and thrombin 42, stored in an ice bath at 1°C to 5°C. Please see figures 13 through 16.

[0052] Assume 9-10 ml of room temperature plasma is introduced into the reaction chamber 26. Other plasma volumes have utility. Please see figures 15 and 16. Add 1.0 ml of 75 mM calcium chloride (CaCl_2) and 2.0 ml of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and added to comprise 18.9% volume/unit volume or 15.02% ethanol weight/unit volume). Other ratios of reagent volume, comprising of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and a stock solution of 75 mM calcium chloride (CaCl_2)), to plasma volume have utility phase. Please see figures 13 and 14. The thrombin life span is shown to have been at least 300 minutes while its clotting time is at 2.98 seconds. An ethanol final concentration range between 8.0% and 20.0% (volume/unit volume), however, still has utility. Please see figure 11.

[0053] When the ethanol is at a final concentration of 18.9% volume/unit volume (as above) and the calcium chloride final concentration is 5.7 mM (1 ml taken from a 75 mM stock solution of calcium chloride), the thrombin lifespan also extends to at least 360 minutes while maintaining a clot time of 5.98 seconds when thrombin is stored at room temperature. Storing thrombin in optimal 1°C to 5°C ice bath typically maintains lot times of 2 to 3 seconds at 360 minutes. Calcium chloride stock solution concentrations ranging between 50 mM and 250 mM, however, have utility. Please see figure 12. The final concentrations range from 4.5mM to 23 mM.

[0054] Solutions such as saline, dilute CaCl_2 (e.g. 40mM to 125 mM CaCl_2) or even sterile water added to the thrombin can alter both the clotting time and life span of the thrombin. Assume an ethanol final concentration of 18.9% and a final calcium chloride concentration of 5.7 mM was used in the reaction chamber 26. When the thrombin has been diluted 1

to 1.5 with water, the clot time has been extended to just less than 30 seconds, and has a life span of up to 150 minutes. [0055] Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

5

Claims

1. A method for generating autologous thrombin, the steps comprising:
 - 10 Obtaining plasma from a blood product;
 - Adding ethanol, a source of calcium ions and beads with the plasma to prepare a solution containing prothrombin;
 - Converting the prothrombin in the solution to thrombin;
 - and filtering the thrombin to remove particulate matter.
- 15 2. The method of claim 1 whereby subsequent to agitating the solution, the solution is incubated for an amount of time greater than or equal to ten minutes.
- 20 3. The method of claim 1 wherein the beads are formed from borosilicate, glass or ceramic.
4. The method of claim 1 wherein the source of calcium ions is CaCl_2 .
- 25 5. The method of claim 1 wherein the time required for the thrombin to convert fibrinogen to a fibrin clot is less than 5 seconds.
6. The method of claim 1 wherein the thrombin is stable for a period of time between about 15 minutes and three hundred and sixty minutes.
- 30 7. The method of claim 1 wherein the time required to generate the thrombin is less than an hour.
8. A composition for extracting thrombin from plasma comprising plasma; ethanol (ETOH), a source of calcium ions, and beads.
- 35 9. The composition of claim 8 wherein the source of calcium ions is CaCl_2 .
10. The composition of claim 9 wherein ETOH is present at 18.9% and CaCl_2 is present at 23.0mM both by volume in the final concentration.
- 40 11. The composition of claim 9 wherein ETOH is present at 18.9% and CaCl_2 is present at 5.7mM both by volume in the final concentration.
12. The composition of claim 9 wherein ETOH is present at a range between 8% and 20% and CaCl_2 is present at a range between 4.5mM and 23.0mM both by volume in the final concentration.
- 45 13. The composition of claim 8 wherein the beads are formed from borosilicate, glass or ceramic.
14. Apparatus to prepare thrombin from plasma comprising a reacting chamber containing beads to accept a source of calcium ions and ethanol, and means for delivery of plasma into said reacting chamber; a syringe connected to said reacting chamber to receive thrombin from said reacting chamber; and a filter between said reacting chamber and syringe which is to receive thrombin.
- 50 15. The apparatus of claim 14 wherein the beads are formed from borosilicate, glass or ceramic.

55 Patentansprüche

1. Verfahren zum Erzeugen von autologem Thrombin, das die folgenden Schritte beinhaltet:

Gewinnen von Plasma von einem Blutprodukt;
Hinzufügen von Ethanol, einer Quelle von Calciumionen und Kugelchen zum Plasma, um eine Lösung herzustellen, die Prothrombin enthält;
Umwandeln des Prothrombins in der Lösung zu Thrombin; und
5 Filtern des Thrombins, um partikuläre Materie zu entfernen.

2. Verfahren nach Anspruch 1, wobei die Lösung nach dem Rühren über einen Zeitraum inkubiert wird, der gleich oder größer als zehn Minuten ist.

10 3. Verfahren nach Anspruch 1, wobei die Kugelchen aus Borsilikat, Glas oder Keramik bestehen.

4. Verfahren nach Anspruch 1, wobei die Quelle von Calciumionen CaCl_2 ist.

15 5. Verfahren nach Anspruch 1, wobei die Zeit, die nötig ist, damit Thrombin Fibrinogen in ein Fibringerinnsel umwandelt, weniger als 5 Sekunden beträgt.

6. Verfahren nach Anspruch 1, wobei das Thrombin über einen Zeitraum zwischen etwa 15 Minuten und dreihundert- undsechzig Minuten stabil ist.

20 7. Verfahren nach Anspruch 1, wobei die Zeit, die zum Erzeugen des Thrombins notwendig ist, weniger als eine Stunde beträgt.

8. Zusammensetzung zum Extrahieren von Thrombin aus Plasma, die Plasma, Ethanol (ETOH), eine Quelle von Calciumionen und Kugelchen umfasst.

25 9. Zusammensetzung nach Anspruch 8, wobei die Calciumionenquelle CaCl_2 ist.

10. Zusammensetzung nach Anspruch 9, wobei ETOH zu 18,9 % und CaCl_2 zu 23,0 mM (beides nach Volumen) in der Endkonzentration vorliegt.

30 11. Zusammensetzung nach Anspruch 9, wobei ETOH zu 18,9 % und CaCl_2 zu 5,7 mM (beides nach Volumen) in der Endkonzentration vorliegt.

35 12. Zusammensetzung nach Anspruch 9, wobei ETOH in einem Bereich zwischen 8 % und 20 % und CaCl_2 in einem Bereich zwischen 4,5 mM und 23,0 mM (beides nach Volumen) in der Endkonzentration vorliegt.

13. Zusammensetzung nach Anspruch 8, wobei die Kugelchen aus Borsilikat, Glas oder Keramik bestehen.

40 14. Vorrichtung zum Herstellen von Thrombin aus Plasma, die Folgendes umfasst:

eine Reaktionskammer, die Kugelchen für die Aufnahme einer Quelle von Calciumionen und Ethanol und ein Mittel zum Zuführen von Plasma in die genannte Reaktionskammer enthält; eine Spritze, die mit der genannten Reaktionskammer verbunden ist, um Thrombin von der genannten Reaktionskammer zu empfangen; und einen Filter zwischen der genannten Reaktionskammer und der Spritze, die Thrombin aufnehmen soll.

45 15. Vorrichtung nach Anspruch 14, wobei die Kugelchen aus Borsilikat, Glas und Keramik bestehen.

Revendications

50 1. Méthode pour produire de la thrombine autologue, les étapes comprenant :

l'obtention de plasma à partir d'un produit sanguin ;

l'addition d'éthanol, d'une source d'ions calcium et de perles avec le plasma pour préparer une solution contenant de la prothrombine ;

la conversion de la prothrombine dans la solution en thrombine ;

et le filtrage de la thrombine pour enlever les particules de matière.

2. Méthode selon la revendication 1 dans laquelle après avoir agité la solution, celle-ci est incubée pendant une durée supérieure ou égale à dix minutes.
3. Méthode selon la revendication 1, les perles étant formées de borosilicate, de verre ou de céramique.
4. Méthode selon la revendication 1, la source d'ions calcium étant CaCl_2 .
5. Méthode selon la revendication 1, le temps requis pour que la thrombine transforme le fibrinogène en un caillot de fibrine étant de moins de 5 secondes.
10. Méthode selon la revendication 1, la thrombine étant stable pendant une période de temps comprise entre 15 minutes et trois cent soixante minutes.
15. Méthode selon la revendication 1, le temps requis pour générer la thrombine étant de moins d'une heure.
8. Composition pour extraire la thrombine du plasma comprenant plasma, éthanol (ETOH), une source d'ions calcium et des perles.
9. Composition selon la revendication 8, la source d'ions calcium étant CaCl_2 .
20. 10. Composition selon la revendication 9, ETOH étant présent à 18,9 % et CaCl_2 à 23,0 mM, dans les deux cas en volume, dans la concentration finale.
25. 11. Composition selon la revendication 9, ETOH étant présent à 18,9 % et CaCl_2 à 5,7 mM, dans les deux cas en volume, dans la concentration finale.
12. Composition selon la revendication 9, ETOH étant présent à un pourcentage entre 8 % et 20 % et CaCl_2 étant présent à un taux entre 4,5 mM et 23,0 mM, dans les deux cas en volume, dans la concentration finale.
30. 13. Composition selon la revendication 8, les perles étant formées de borosilicate, de verre ou de céramique.
14. Appareillage pour préparer la thrombine à partir de plasma comprenant une chambre de réaction contenant des perles pour accepter une source d'ions calcium et de l'éthanol, et des moyens pour acheminer le plasma dans ladite chambre de réaction ; une seringue connectée à ladite chambre de réaction pour recevoir la thrombine provenant de ladite chambre de réaction ; et un filtre entre ladite chambre de réaction et la seringue devant recevoir la thrombine.
35. 15. Appareillage selon la revendication 14, les perles étant formées de borosilicate, de verre ou de céramique.

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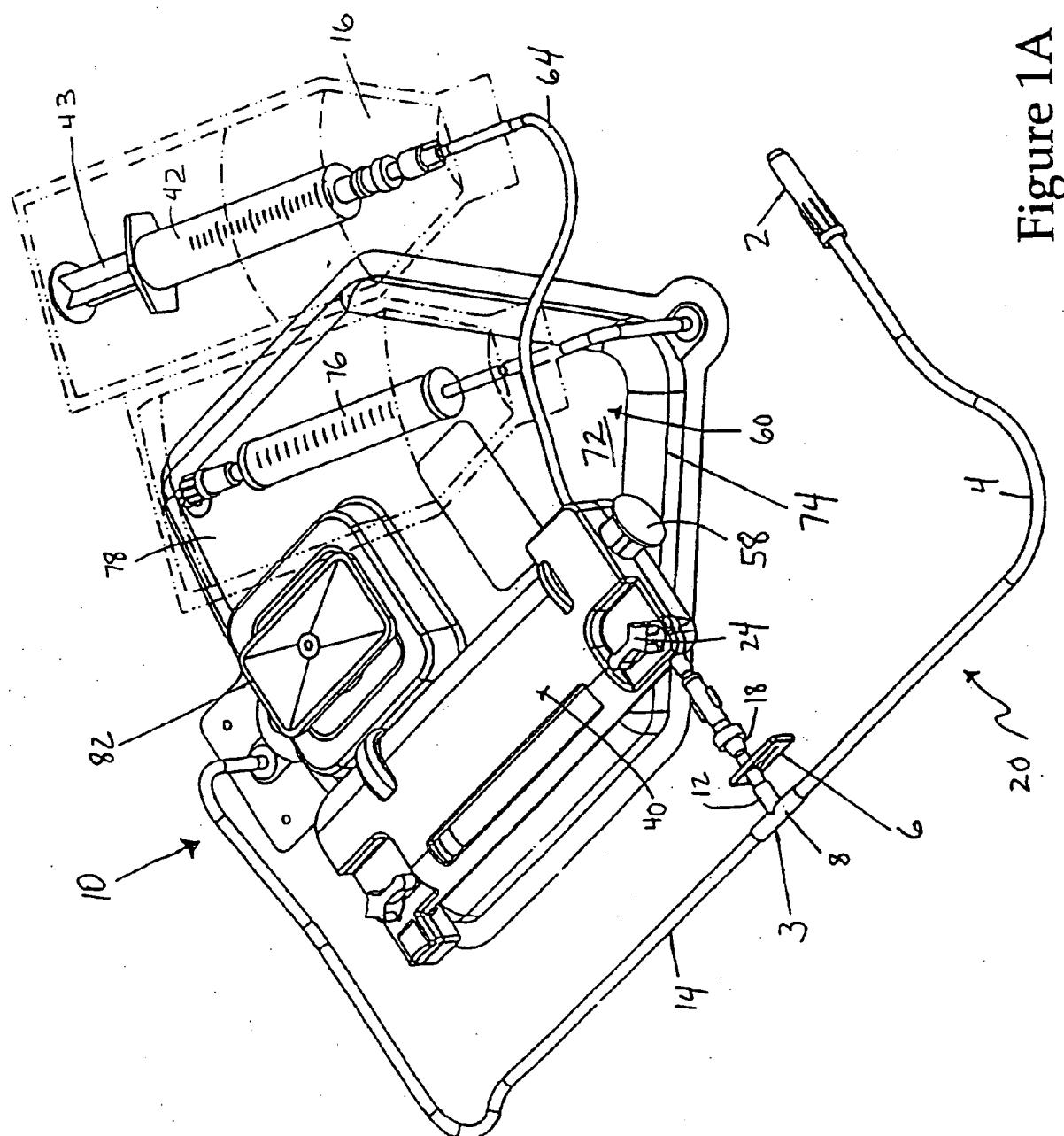
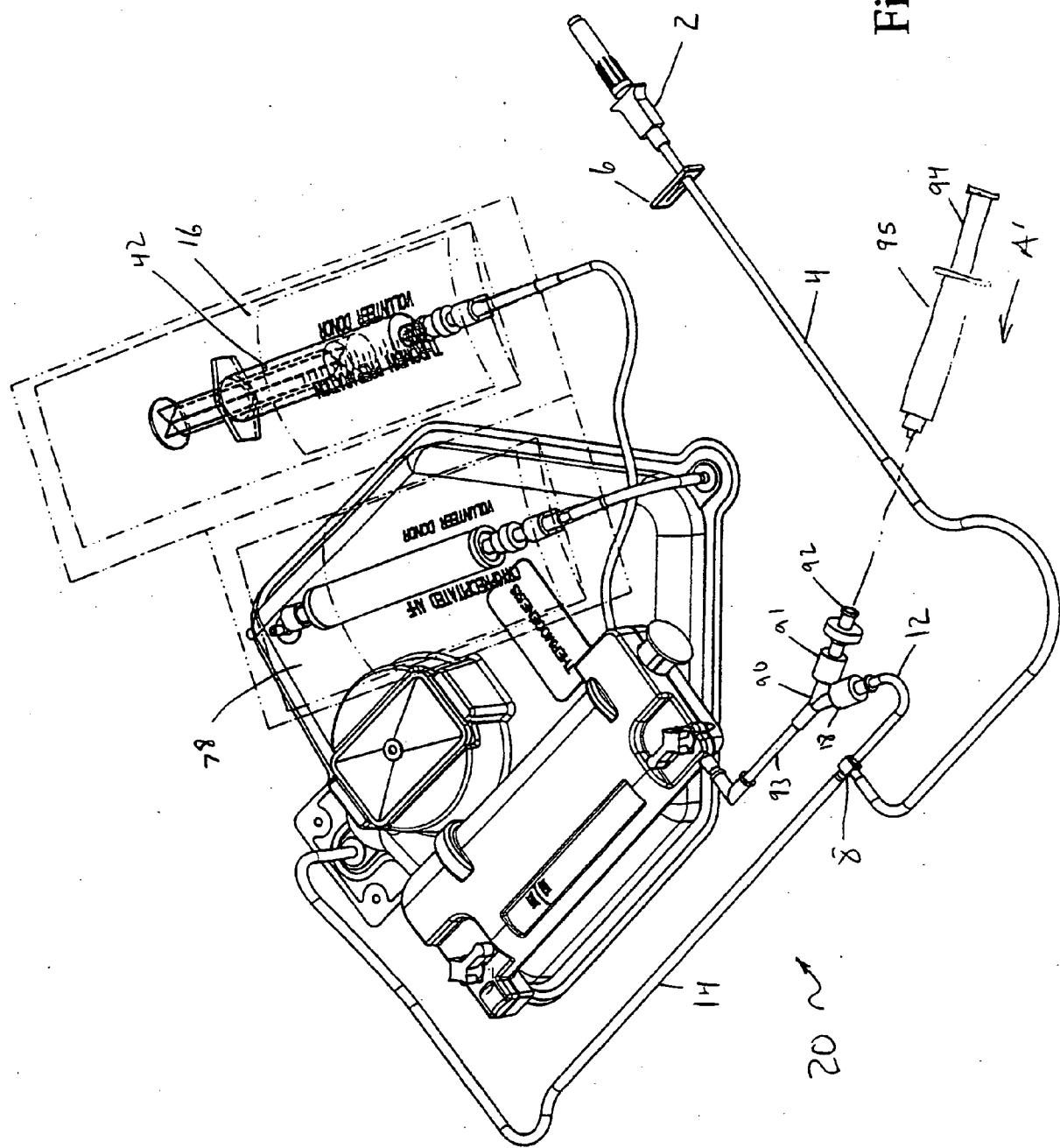


Figure 1B



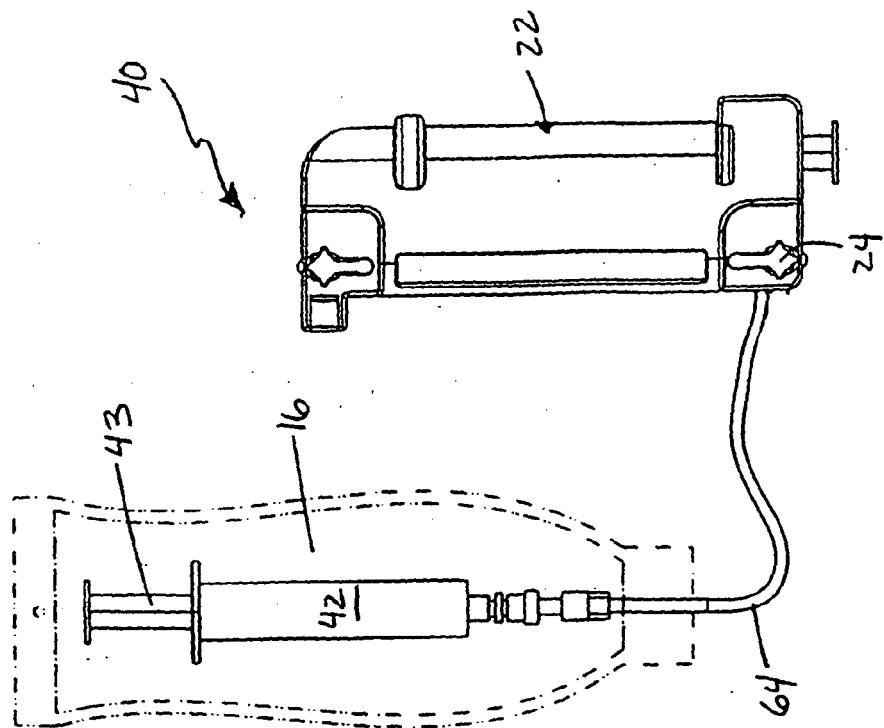


Figure 2B

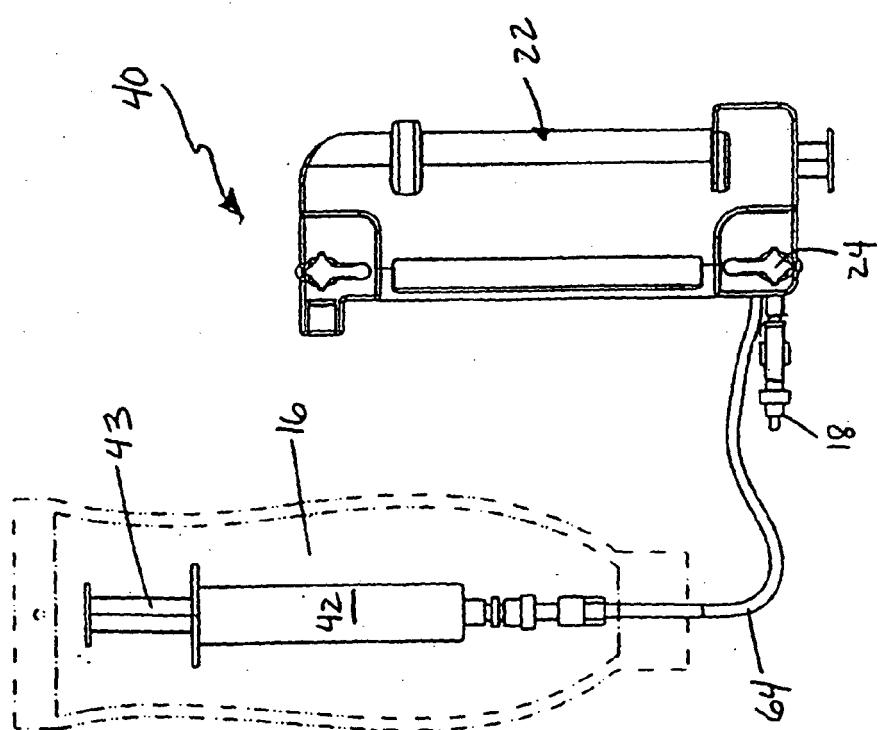


Figure 2A

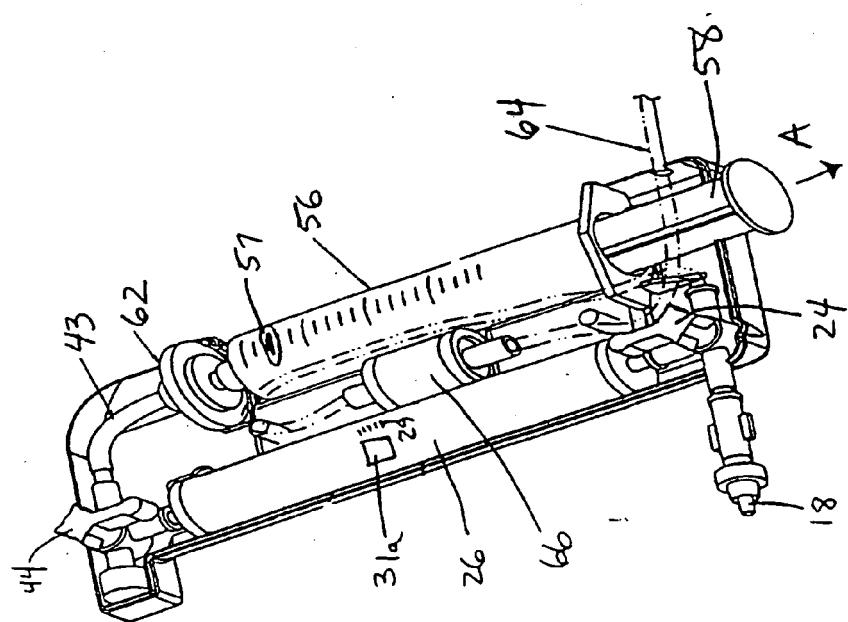
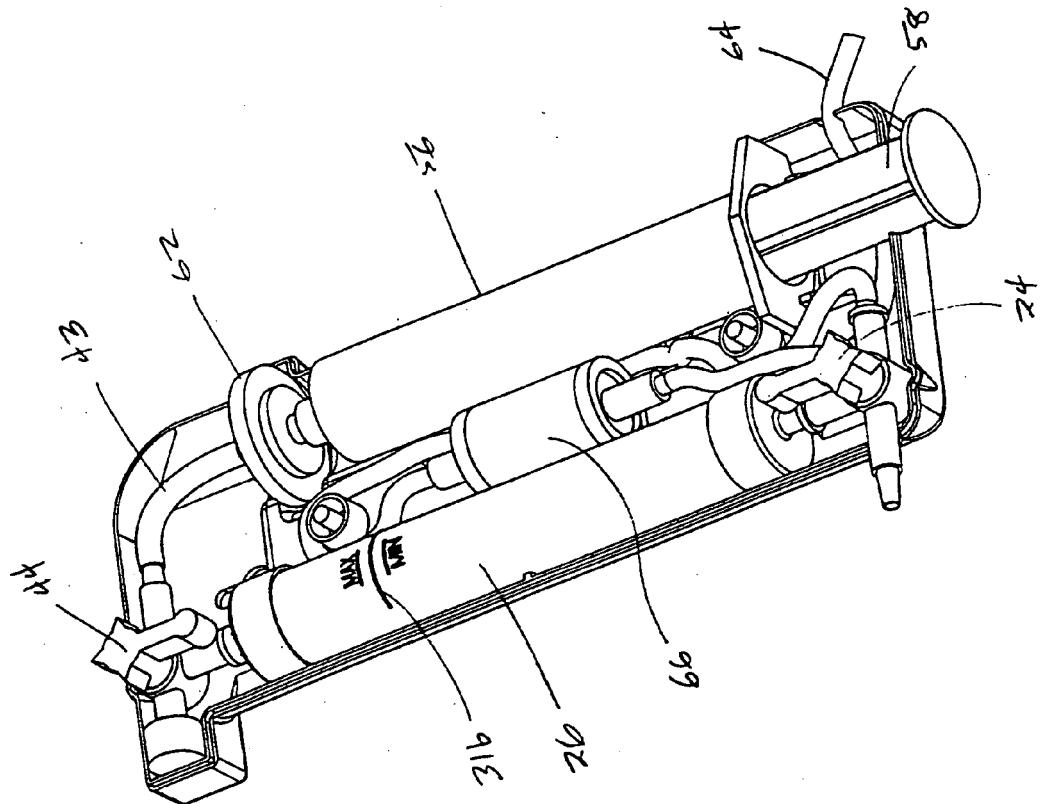


Figure 3A

Figure 3B

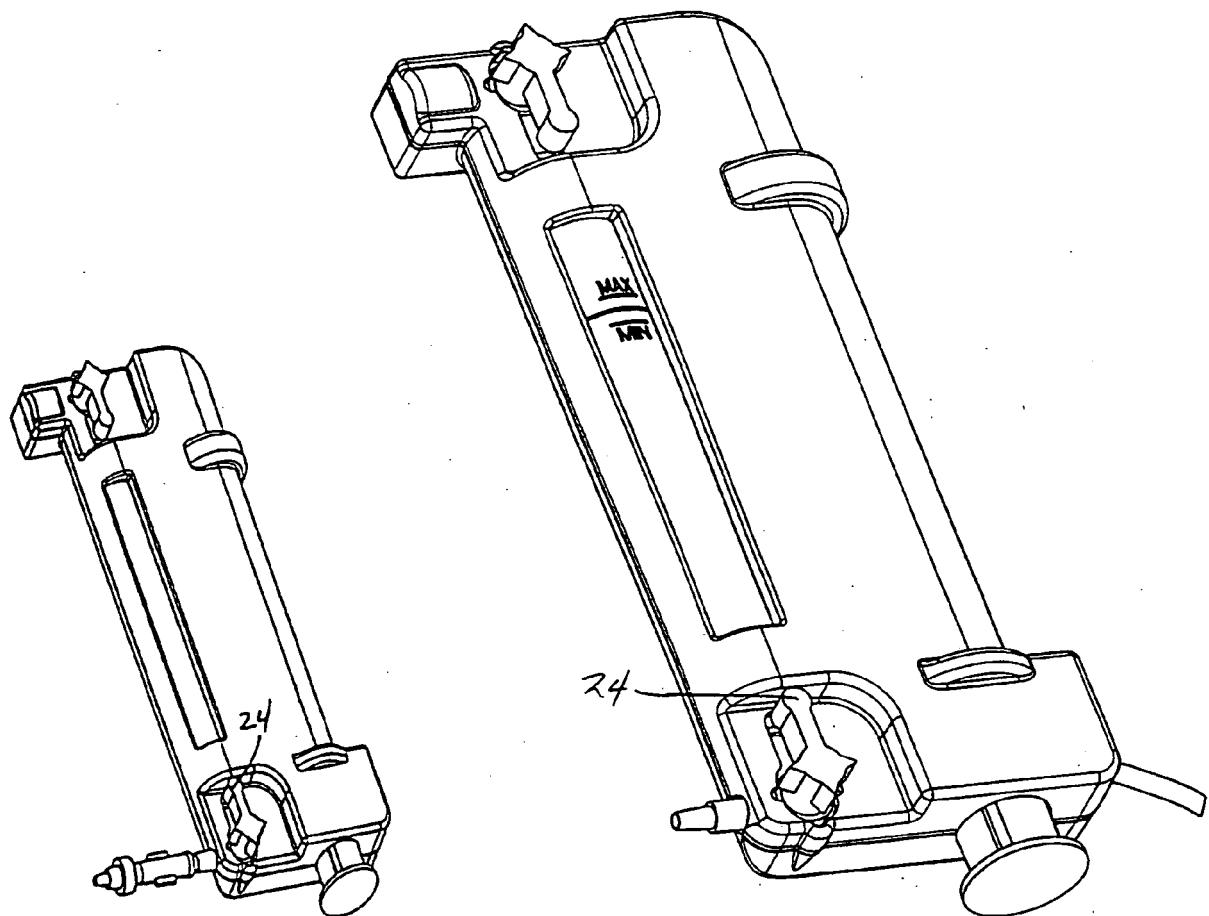


Figure 4A

Figure 4B

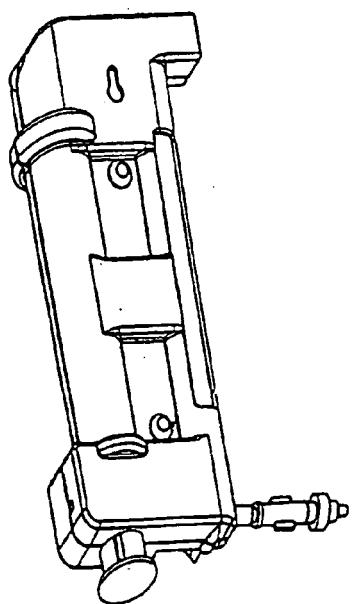


Figure 5A

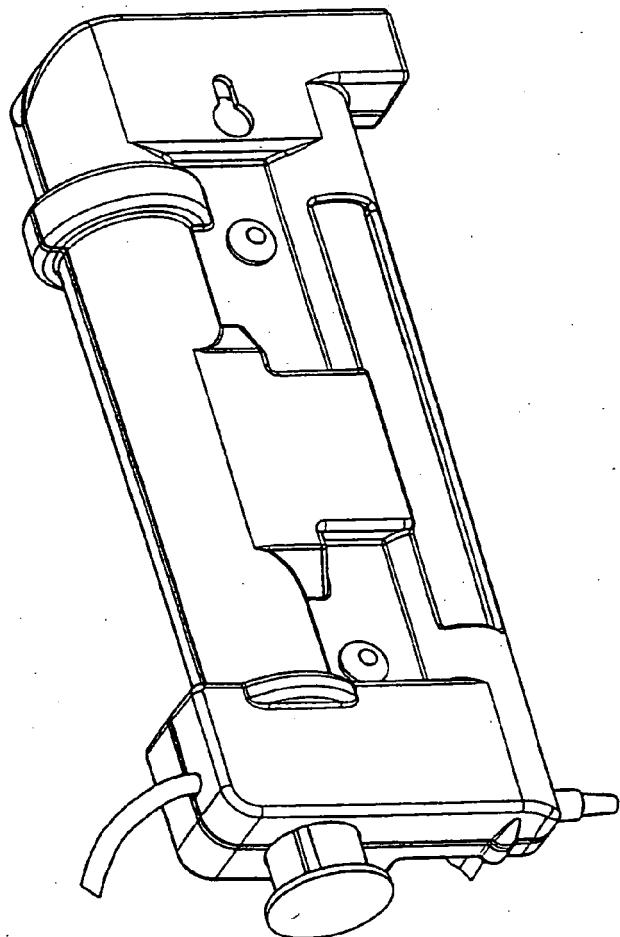


Figure 5B

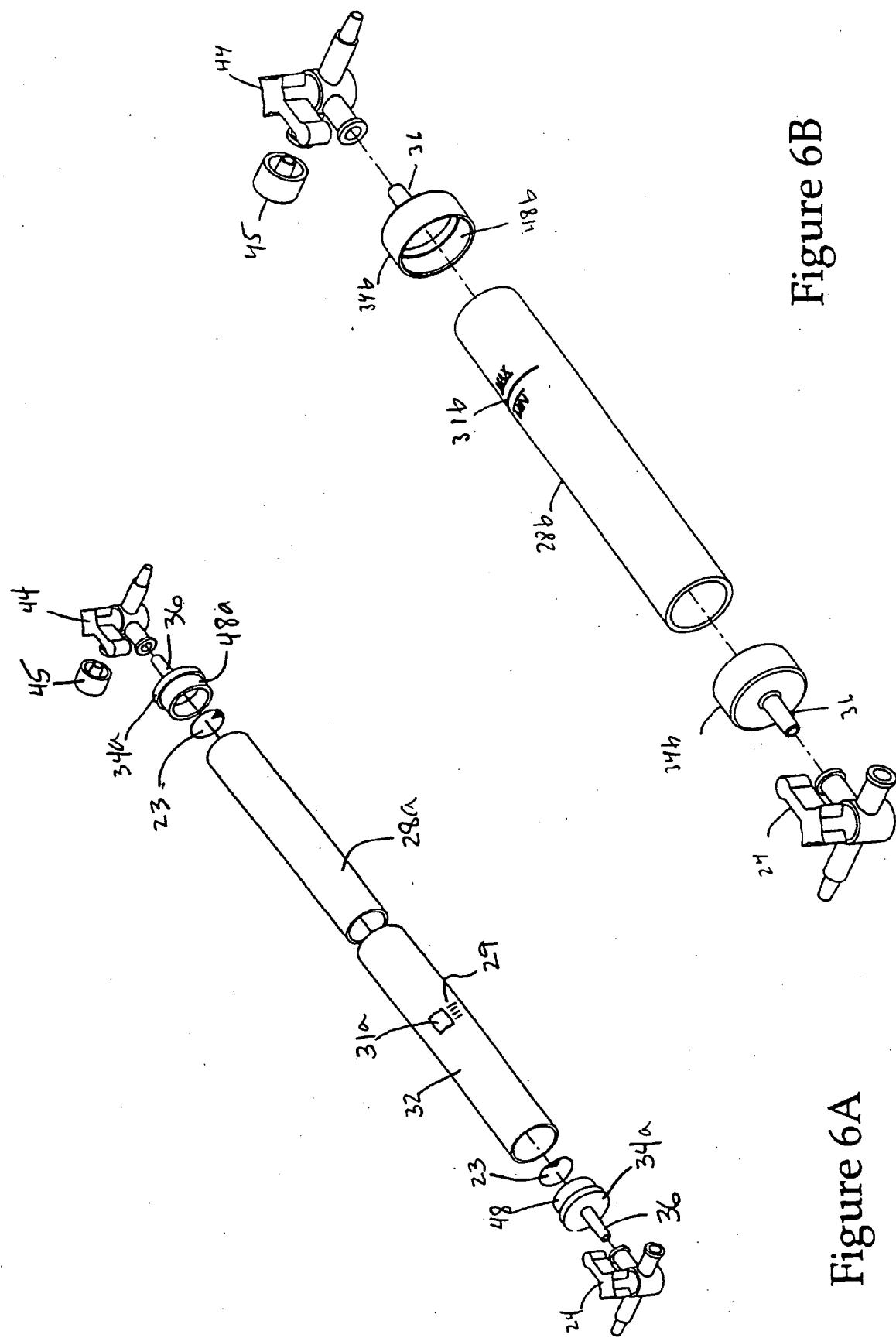


Figure 6A

Figure 6B

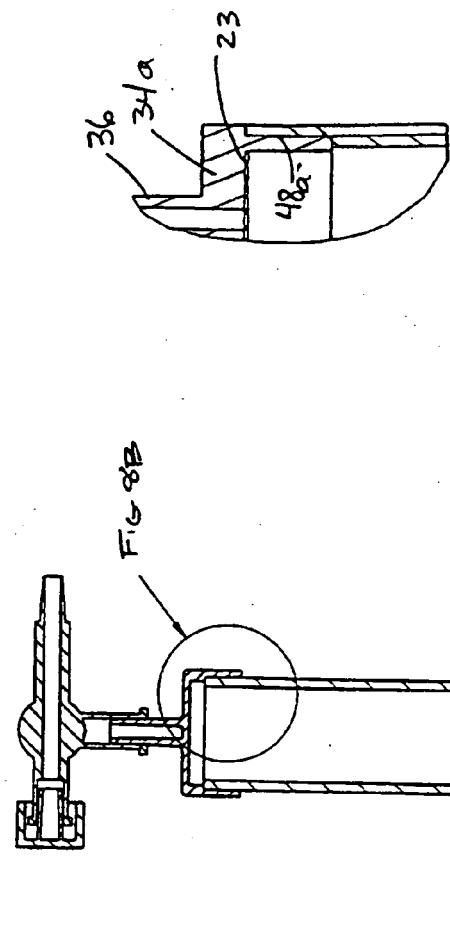


Figure 8A

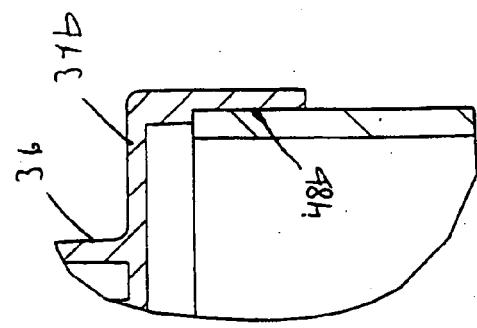


Figure 8B

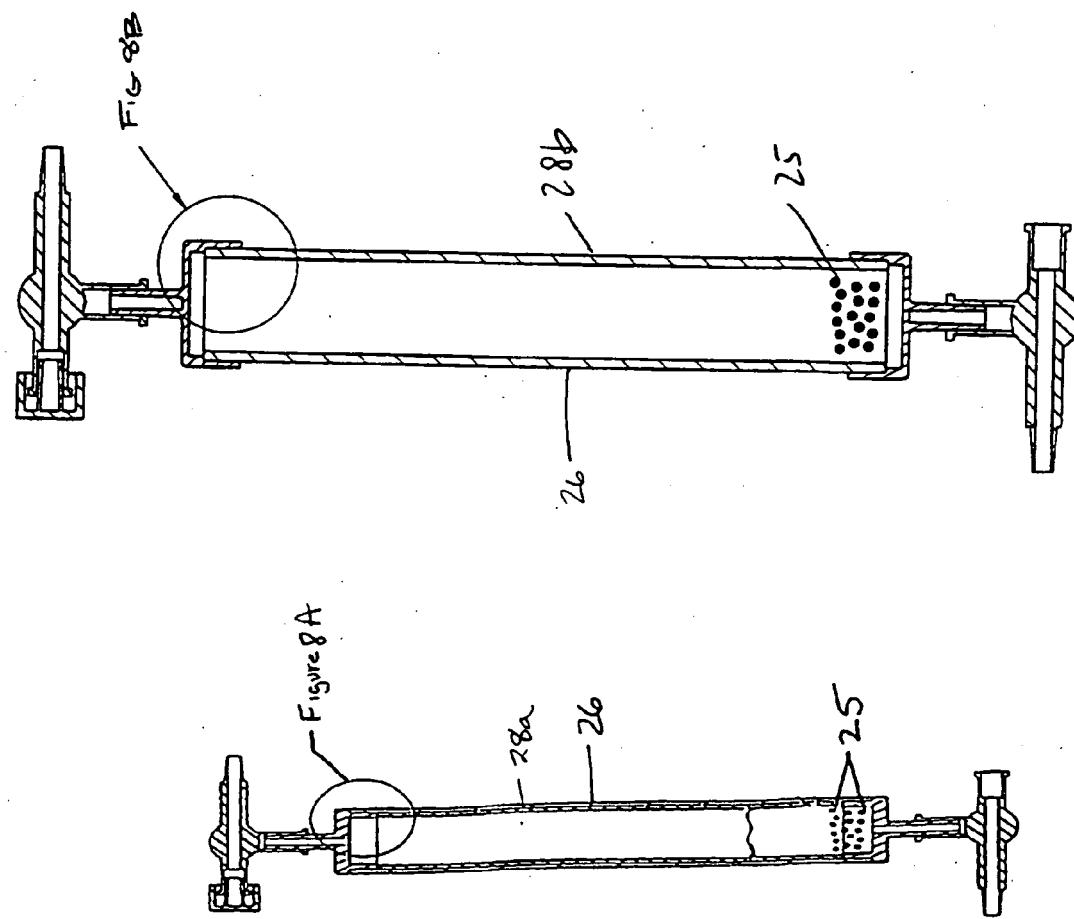


Figure 7A

Figure 7B

Figure 10

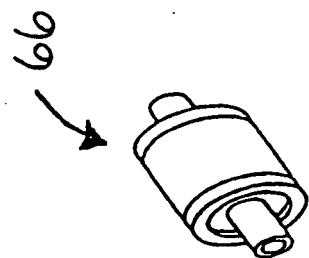


Figure 9B

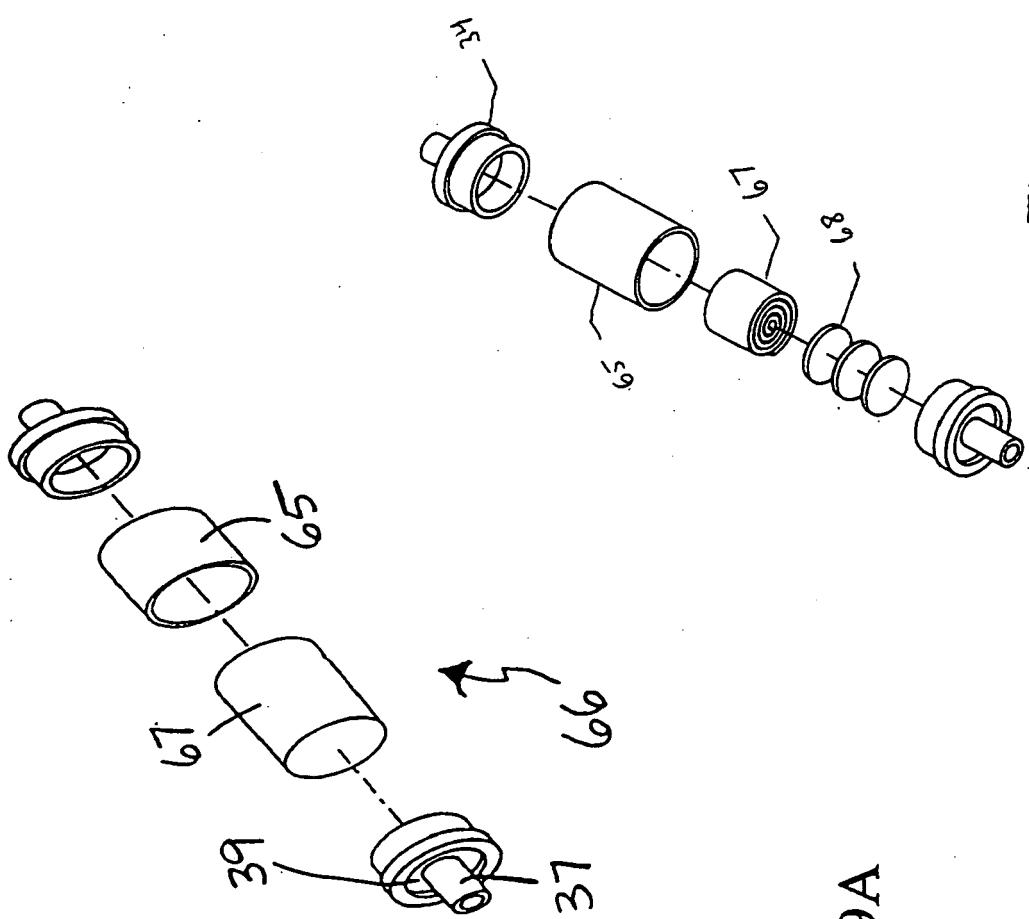


Figure 9A

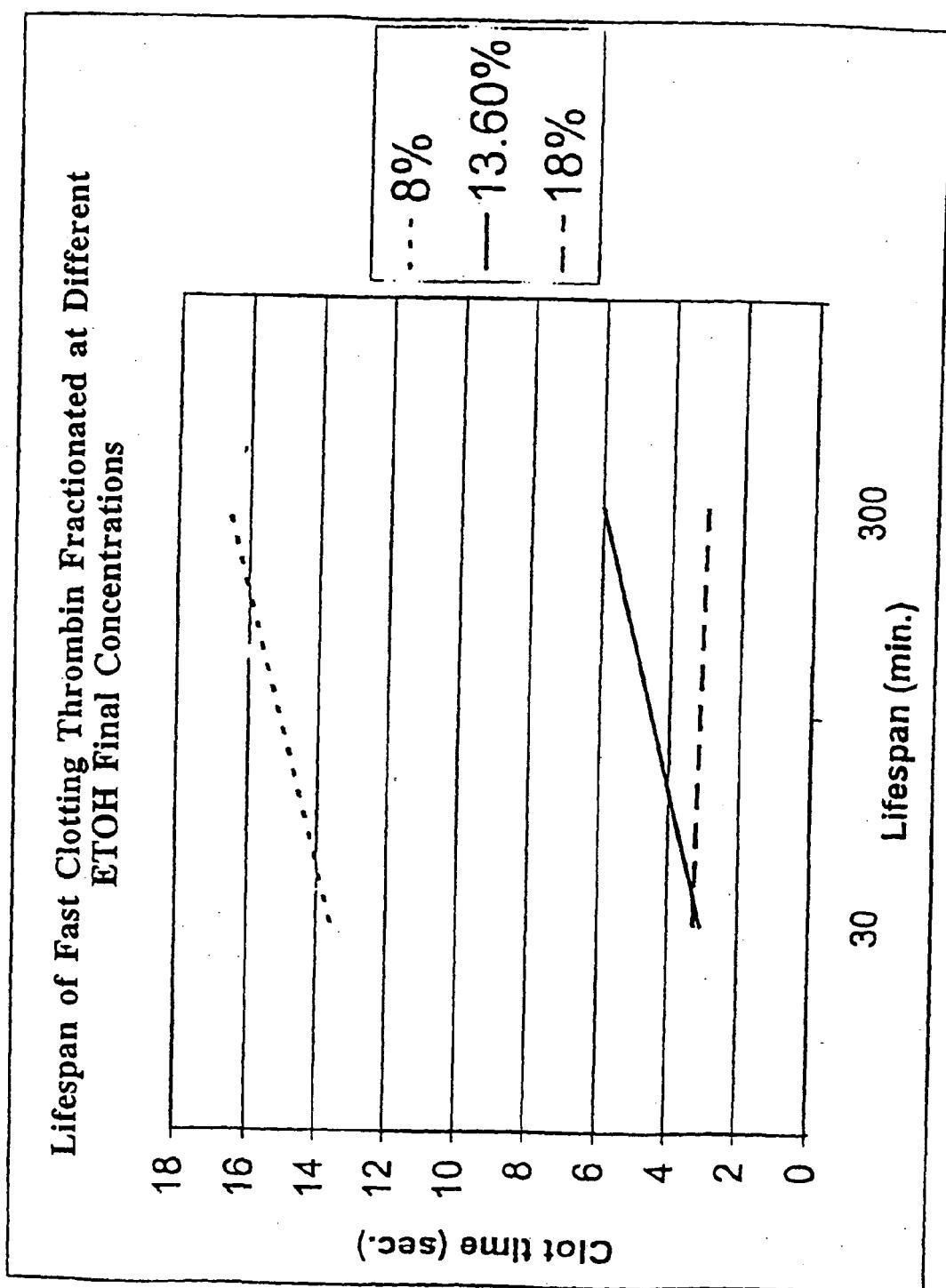


Figure 11

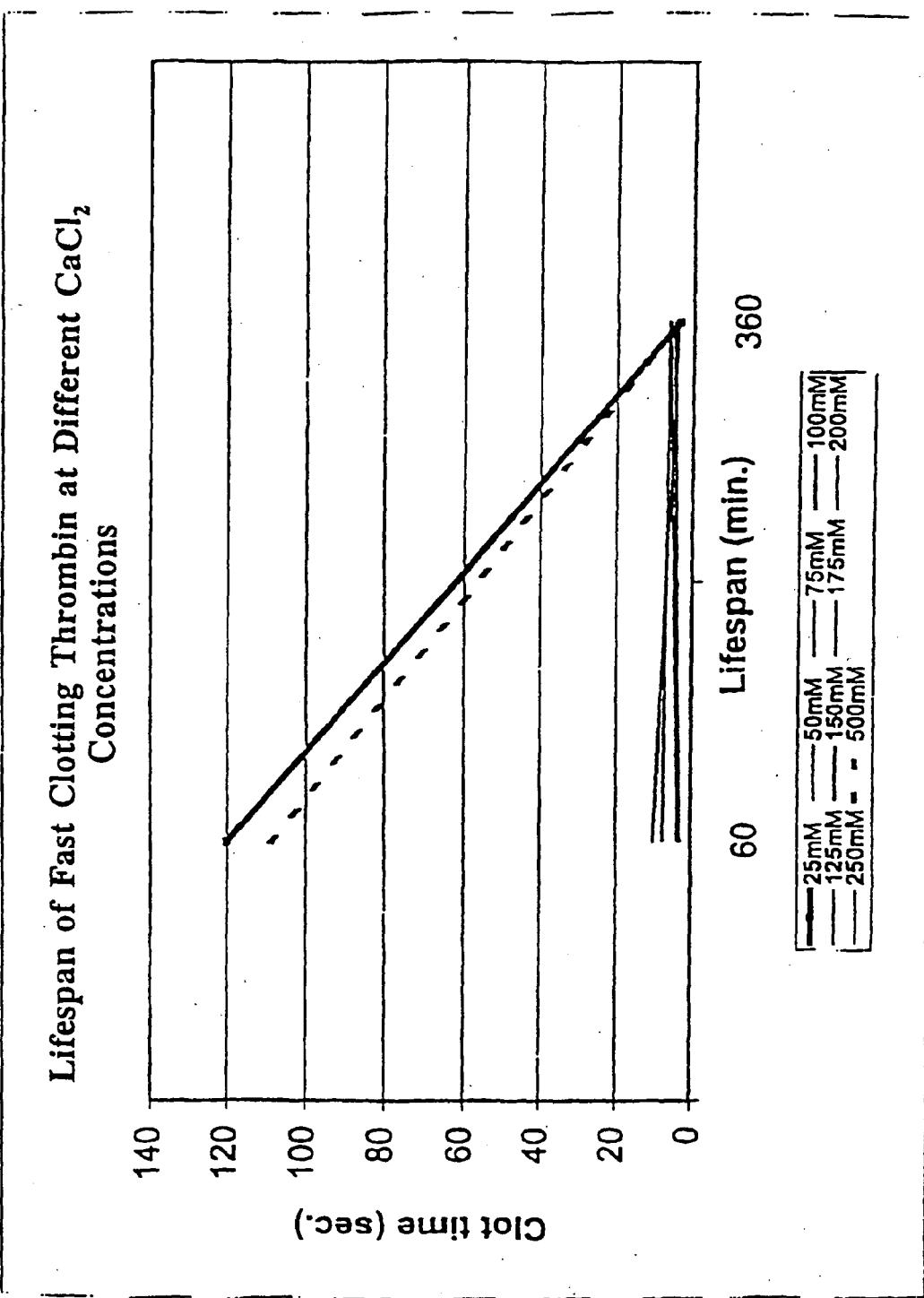


Figure 12

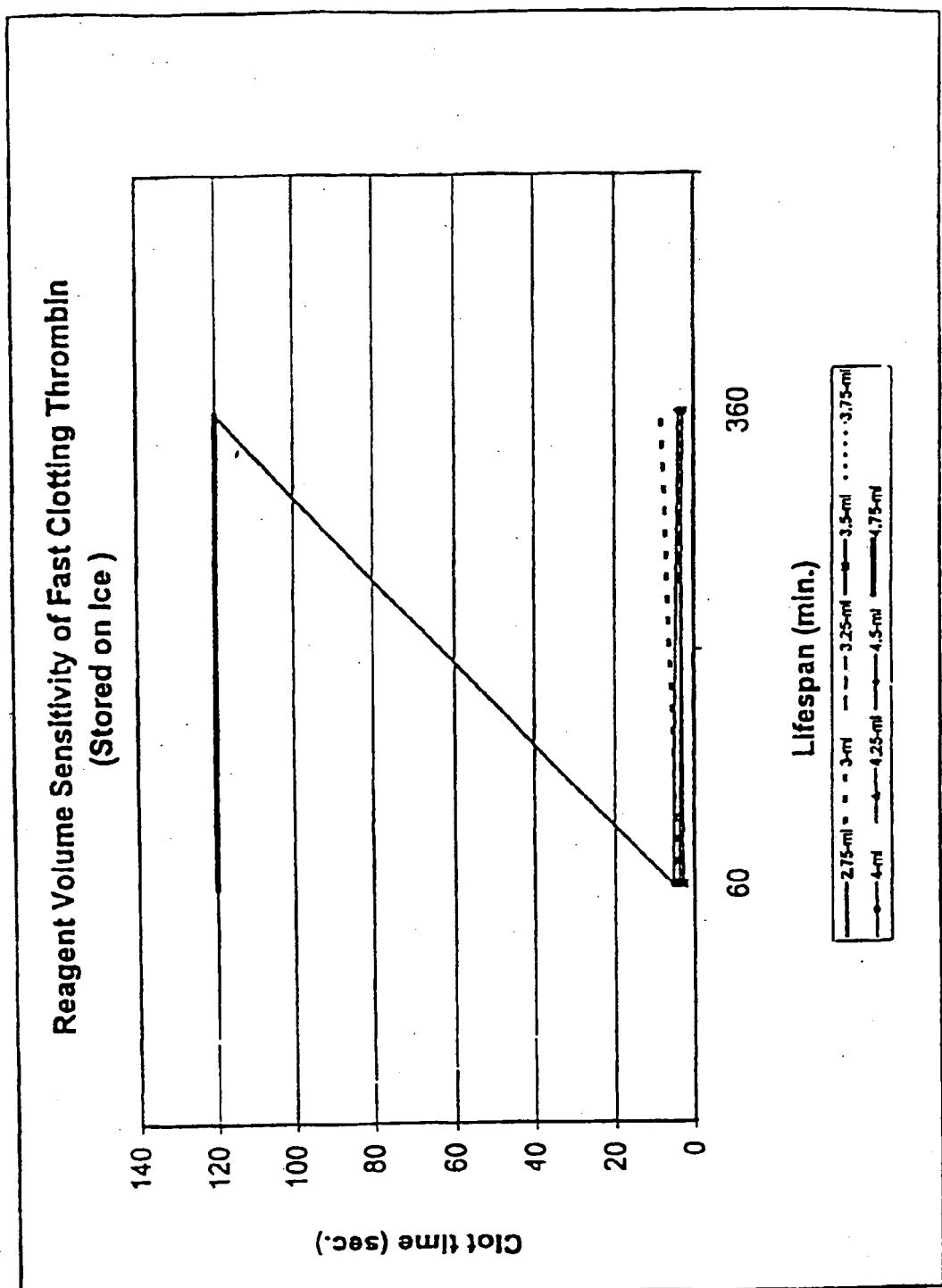


Figure 13

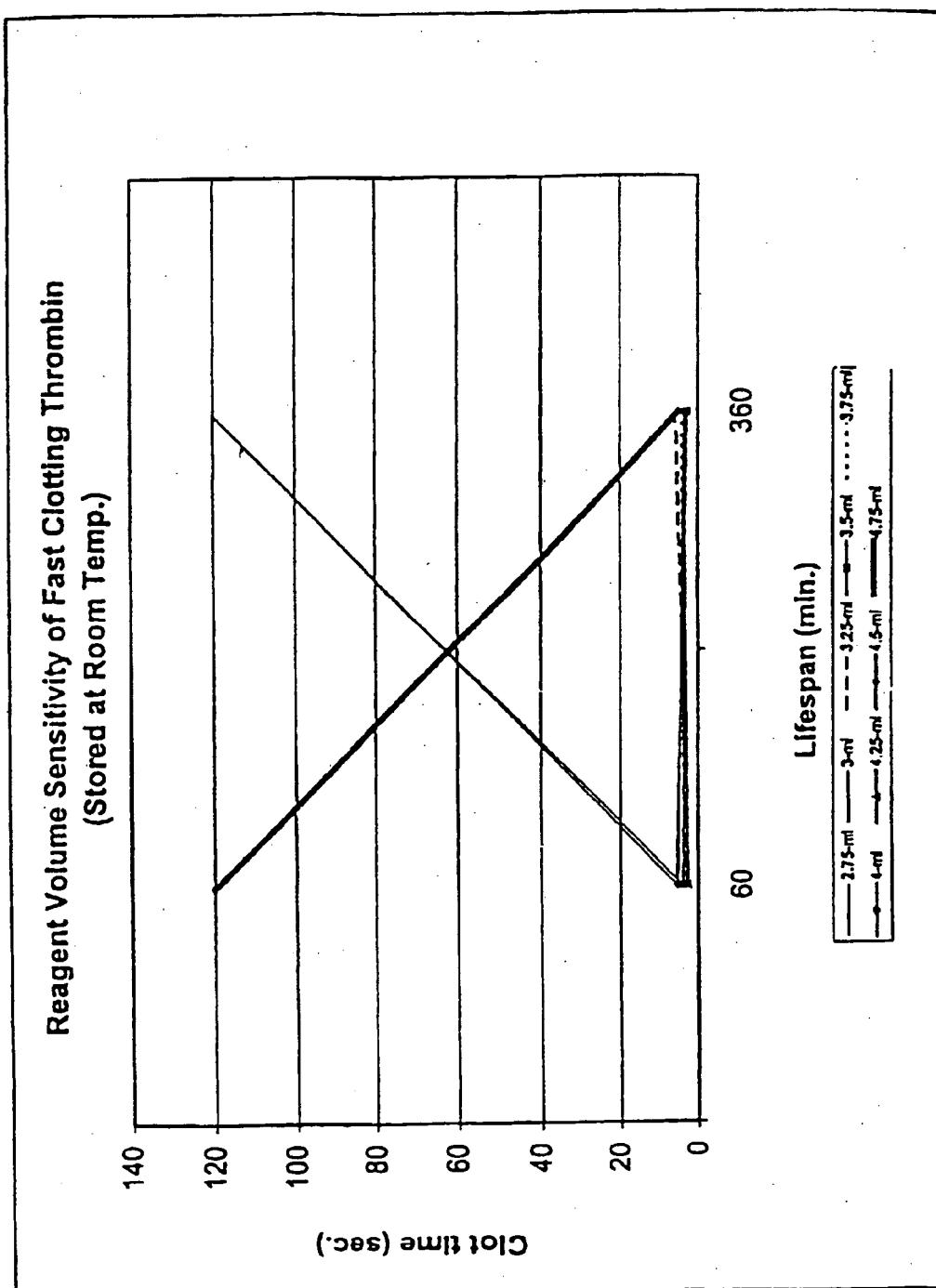


Figure 14

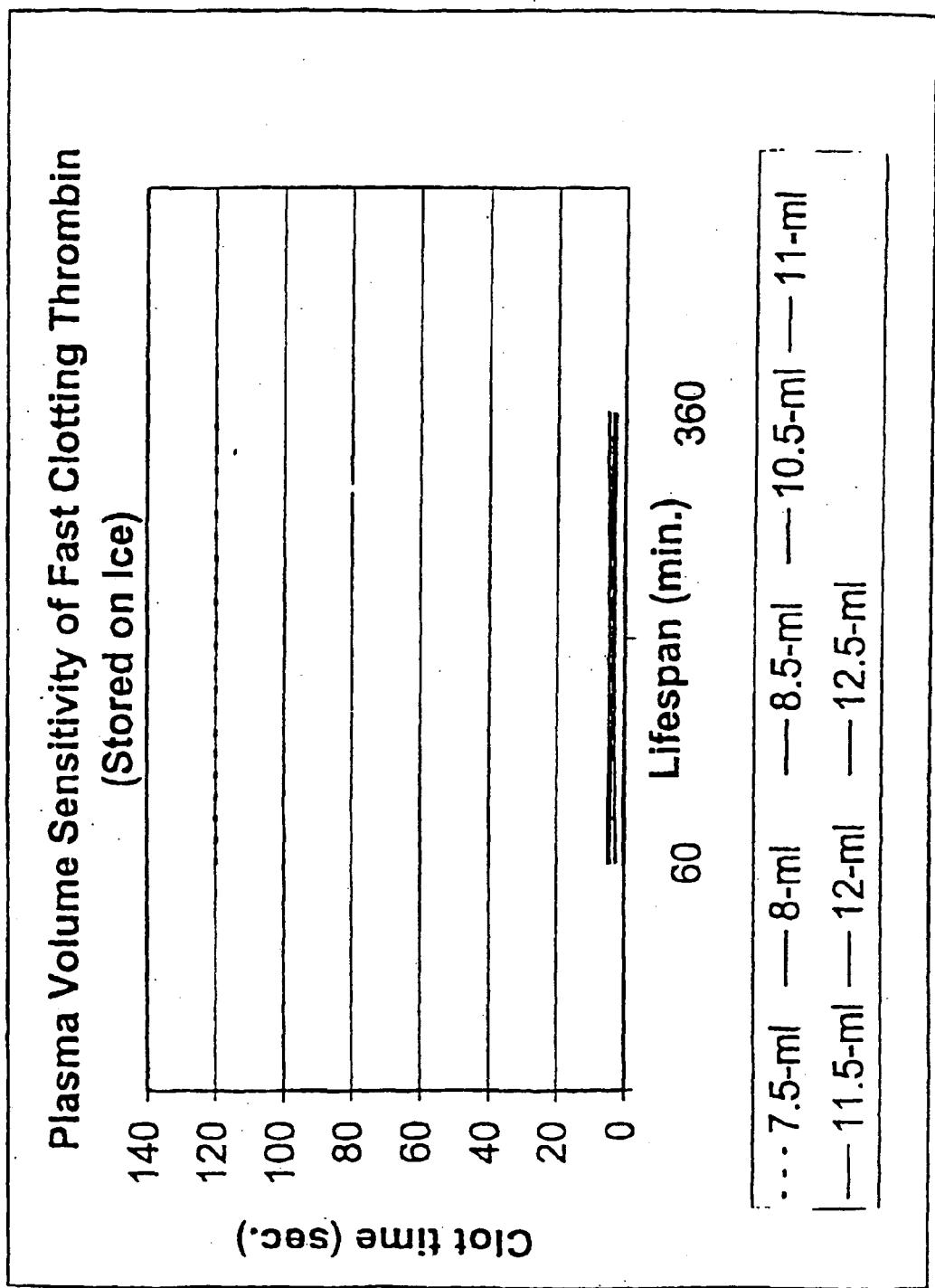


Figure 15

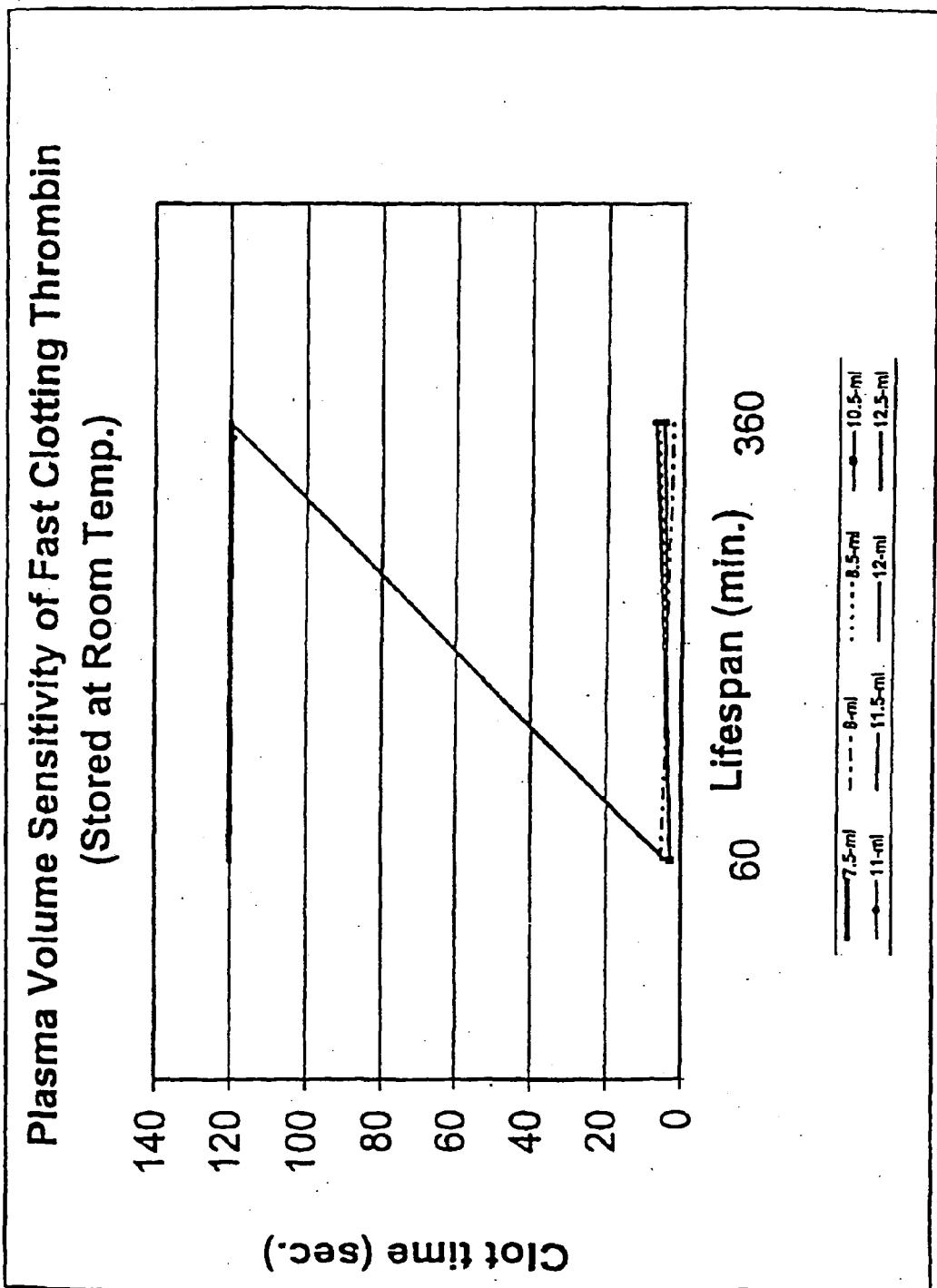


Figure 16

REFERENCES CITED IN THE DESCRIPTION

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