

## GLYCINE STABILIZED LYOPHILIZED PLASMA

### REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Application No. 60/707,526 entitled "Glycine Stabilized Lyophilized Plasma and Method for Making Same" filed Aug. 12, 2005, the entirety of which is hereby incorporated by reference.

### RIGHTS IN THE INVENTION

**[0002]** This invention was made with support from the United States Government, Department of the Army, and, accordingly, the United States has certain rights in the invention.

### BACKGROUND OF THE INVENTION

**[0003]** 1. Field of the Invention

**[0004]** The instant invention relates to lyophilized whole plasma and/or components thereof, and more specifically, to lyophilized whole plasma and/or components thereof that are stabilized by glycine, and which can be reconstituted with water to thereby exhibit physiological characteristics of control or untreated plasma.

**[0005]** 2. Description of the Background

**[0006]** Whole plasma and fresh frozen plasma (FFP) are the primary preparation forms for plasma storage. In locales where it can be problematic to readily obtain whole plasma, such as, for example, forward positions of a battlefield, FFP is the preferred storage form. While the use of FFP is preferred in such environs, there remain several limitations. For example, the use of FFP can be limited by the facilities required to transport, store and maintain FFP at temperatures of  $-25^{\circ}\text{C}$ . or below. Also, because FFP is often transported/stored using dry ice, it can be difficult to transport FFP given the hazards associated with the use of dry ice, i.e., an transport, and/or the amount of FFP that can be shipped is often limited. Finally, because FFP is frozen, it can take time to properly thaw FFP prior to being used.

**[0007]** Clearly, then, the physical and logistical limitations associated with FFP negatively effects the forward availability of plasma.

**[0008]** Accordingly, the ability to freeze dry plasma would effectively remedy the storage and shipment problems associated with the use of FFP- by converting liquid plasma into a solid, lightweight, stable at ambient temperature, product. While lyophilization of plasma would certainly be advantageous on the battlefield, it would also be beneficial to, for example, developing countries where facilities for preparation, transportation and storage of frozen blood products may be limited.

**[0009]** Pooled plasma was lyophilized for the first time during World War II. However, it was discovered that the process of lyophilization did not kill viruses in plasma. In addition, the use of plasma from large pools carried an unacceptable risk of transmitting pathogens. Therefore, the production of a stable lyophilized plasma product was abandoned.

**[0010]** In recent years several methods for pathogen inactivation in plasma have been introduced. Such methods are typically based on: solvent/detergent treatment; utilization of vitamin B2, Riboflavin, and light, and the application of psoralens and UV light. The current endeavor is to lyophilize

pathogen inactivated plasma products. These products will guarantee both unconstrained plasma availability and safety.

**[0011]** Several groups have reported stability results for lyophilized pathogen inactivated solvent/detergent (SD) treated plasma products. Hellstern et al. (Vox Sang; 63: 178-185 (1992)), describe the production of lyophilized and deep-frozen batches of human SD plasma and the in vitro characterization of the product. Clotting factor activities were found to decrease more markedly in the lyophilized plasmas than in the deep frozen batches, Storage stability data at ambient temperature are not reported in this study. The German Red Cross introduced a lyophilized pathogen inactivated SD plasma product in 1990. The product was examined to determine whether the quality is comparable to standard preparations. Several publications report these results. It was found, however, that lyophilized SD plasma did not fulfill basic requirements. The time required to reconstitute the lyophilized product was too long. The resultant pH values were close to alkaline range, and thus unsuitable for use without considerable changes in blood gas and electrolyte levels could be expected in the recipient. In a separate study, the quality of three conventional fresh-frozen plasma preparations and one lyophilized SD plasma preparation were compared. Coagulation activity was significantly reduced in the lyophilized SD plasma. Storage stability data at ambient temperature is not reported in these studies. In Thailand, lyophilized plasma has been used as an in home treatment of hemophilia patients since 1982. The chemical and coagulation properties of this product are nearly the same as FFP after reconstitution with sterile water. The clinical effectiveness of this product has been shown in hemophiliac patients with bleeding episodes. However, storage of the freeze-dried plasma product is still confined to  $4^{\circ}\text{C}$ .

**[0012]** Proteins are relatively unstable molecules and require protectants to improve their stability upon lyophilization and storage. Common compounds used for that purpose are the "polyols", such as sugars, and various hypotheses are being proposed to explain their stabilizing effect on solid proteins. The two disaccharides sucrose and trehalose are among the most commonly used protein stabilizers in lyophilized formulations. Their protective properties are well documented, including their ability to protect coagulation factor proteins and fibrinogen. A lyophilized recombinant factor IX formulation is developed, which contains 1% sucrose as a protectant. Sucrose is used to develop stable albumin-free lyophilized formulations of recombinant factor VIII-SQ (r-VIII SQ) and recombinant factor VIII (BDDrF VIII). Sucrose, trehalose, raffinose and arginine are listed as stabilizing agents in the albumin-free factor VIII formulations designed by Besman et al. (U.S. Pat. No. 6,586,573). Sucrose is also the protein stabilizer of choice used in formulating lyophilized hemostatic fibrinogen/thrombin sandwich bandages stable at ambient temperature.

**[0013]** Bulking agents are used in protein formulations to provide the lyophilized cake a pharmaceutically elegant (i.e., noncollapsed) structure or to support potent biopharmaceuticals used at low doses (mass) per vial. However, under specific conditions bulking agents may display a stabilizing effect as well. Whole plasma does not require a bulking agent to support the structure of the lyophilized cake and is often lyophilized without addition of any excipients.

**[0014]** Glycine is non-toxic, highly soluble, and has a high eutectic temperature when it crystallizes from frozen solution. The latter promotes efficient freeze-drying. The ability