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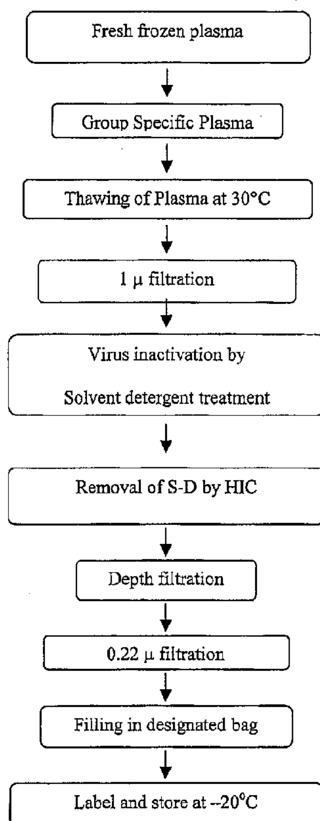
(54) Title: PROCESS FOR THE PREPARATION OF VIRUS-SAFE BIOLOGICAL FLUIDS



(57) **Abstract:** The present invention relates to a process of disinfecting biological materials. In particular, the invention relates to a novel process for removing detergent and/or solvent added to biological material. This present invention provides a safe, efficient, and economical method of removing virucidal agents viz. solvent and detergent from virus inactivated pooled plasma by hydrophobic interaction chromatography.

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PROCESS FOR VIRUS-SAFE BIOLOGICAL FLUIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from provisional Indian provisional patent application filed June 29,2004 under Application No. 695/MUM/2004.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to processes for safe removal of virucidal materials from biological samples. In particular, the invention relates to processes for removal of detergents and/or solvents from biological materials.

BACKGROUND OF THE INVENTION

Human plasma serves as a source for deriving valuable proteins. Therapeutic proteins derived from human plasma have been used in the treatment of a broad range of diseases including primary immune deficiencies (immunoglobulin G), critical care involving hypovolemia (albumin), wound healing (fibrinogen), and hereditary deficiencies such as hemophilia A (Factor VIII), hemophilia B (Factor IX), von Willebrand's disease (vWF), and congenital emphysema resulting from a deficiency of alpha-1 Proteinase Inhibitor (A1PI). Besides its application as a raw material for isolating very valuable proteins, whole human plasma continues to be major source of coagulation factor replacement therapy for patients with clotting factor deficiency. Human plasma and therapeutic proteins derived therefrom are used to treat about more than one million patients globally each year. The global demand of such therapeutics is significantly greater than the current level of supply.

For patients in the need of whole plasma for therapeutic purposes, it is available as either fresh frozen plasma or liquid plasma. Fresh frozen plasma (FFP) is the plasma removed from a unit of whole blood and frozen at or below -18°C within eight hours of blood collection as a single-donor plasma unit. Liquid plasma is stored at temperatures of 4-8°C within 4 hours of blood collection and separated from the red blood within 48 hours of blood collection. Each of these plasma units are from single donors and individually tested for viral markers and, in regards to

viral transmittance, single donors are considered to be reasonably safe. There continues, however, to be a small but defined risk of viral transmission, because such plasma units usually do not undergo a process of viral inactivation to kill viruses like HIV, hepatitis B, hepatitis C, and other lesser known viruses which could potentially cause disease.

For the purpose of deriving the therapeutic proteins, a large number of fresh frozen plasma units are pooled together from various donors. Human plasma proteins for therapeutic use have been manufactured from large pools of plasma for over 50 years. One of the important concerns of single donor or pooled plasma, however, is viral safety. Though every donor who contributes to the pool of plasma is tested individually for viruses, including HIV, HBV, HCV, etc. before blood or plasma donation, there remains a small risk of infection with viruses due to "window period donations," that is donations made between the initial acquisition of infection and the detection of a positive test result with existing diagnostics due to inherent technical limitations. Even a single donor infected with a pathogen, which remained undetected after screening, can potentially contaminate an entire pool of plasma and infect many or all recipients exposed to the pool. Therefore, there is a need to address the viral safety of pooled plasma or the therapeutic proteins derived therefrom.

To render plasma or plasma-derived therapeutic proteins virus-safe, various methods have been attempted to remove or inactivate viruses. For virus inactivation, biological fluids of interest are subjected to physical treatments like pasteurization, wherein the pooled plasma is subjected to wet heat at a temperature of about 60 °C for periods of about 10 hours, or treated with dry heat during which the product of interest is treated at higher temperature of about 80°C for prolonged periods as long as about 72 hours. Such treatments often are found to damage, denature, or vary valuable protein factors, especially labile blood-coagulating components under conditions to which the biological samples are subjected for inactivating the viruses efficiently. During such inactivation processes, the labile coagulation components of the mammalian blood plasma may get inactivated or denatured as much as up to the extent of 50-90% or more present in the untreated plasma. The coagulating components, which may be lost during such treatment include valuable plasma factors like factors II, V, VII, VIII IX, X; plasma fibrinogen (factor I), IgM, hemoglobin, interferon, etc. Therefore, attempts have been made to incorporate suitable steps to protect the proteins of interest.

Other methods for viral inactivation involve treatment with ß-propiolactone, formaldehyde, sodium hypochlorite, and the like. However, these methods are generally not considered to be very safe. These methods not only tend to denature the valuable protein components, but also pose difficulties in complete removal of agents such as ß-propiolactone, which is deleterious and has shown to be carcinogenic in animals and is dangerous even to personnel handling it.

One of the most commonly employed methods for viral inactivation of plasma or plasma derived protein products is solvent detergent treatment. Solvent detergent treated plasma has been approved for use in the treatment of patients with documented deficiencies of coagulation factors for which there are no concentrate preparations available, including congenital single-factor deficiencies of factors I, V, VII, XI and XIII, and acquired multiple coagulation factor deficiencies; reversals of warfarin effect; and treatment of patients with thrombotic thrombocytopenic purpura (TTP). A cost-effectiveness analysis for solvent-detergent–treated frozen plasma (SDFP), calculated a cost of $289,300 per quality-adjusted life year (QALY) saved. (Jackson et al. JAMA.1999; 282: 329). Solvent-detergent treatment is particularly effective for enveloped viruses such as Vesicular Stomatitis Virus (VSV), Pseudorabiesvirus (PRV), Semliki Forest Virus (SFV) and Bovine Diarrhoea Virus (BVDV). (Seitz et al. Biologicals, 30(3):197-205(9) (2002)). Solvent detergent treatment is primarily employed to reduce the already-low risk of viral transmitted fresh frozen plasma from donors in the infectious, seronegative window period of currently known viral infections and the risk of transmission of lipid enveloped viruses not currently recognized as a risk to transfusion safety may very well could still be a potential risk in the future.

In a solvent detergent treatment for virus inactivation, the protein-containing composition is contacted with dialkyl- or trialkylphosphate, preferably with mixtures of trialkylphosphate, and detergent, usually followed by removal of the dialkyl- or trialkylphosphate (see U.S. Patent No. 4,540,573). The '573 patent employed dialkyl- or trialkylphosphate in an amount between about 0.01 mg/ml and about 100 mg/l. The amount of detergent employed, according to the '573 patent, could range from about 0.001% to about 10%. Similarly, U.S. Patent No. 4,314,997 employed a detergent concentration could vary from 0.25% to as high as about 10%.

Another detergent approach of viral inactivation is to subjecting plasma protein products to prolonged contact with non-denaturing amphiphile (see U.S. Patent No. 4,314,997).

Amiphiphiles can be anionic, cationic, nonionic detergents. The detergent molecule is hydrophobic at one end and hydrophilic at the other end, which makes it useful for purification of therapeutic blood proteins. Ionic detergents, either anionic or cationic tend to more active than nonionic detergents. While being effective at destroying viruses, detergents may readily destroy or damage living cells. Detergents are capable not only of destroying viruses, but also disrupting other vital lipid-based structure like biomembranes that surrounds and form a significant internal structural component of every animal and plant cell. Further, high concentrations of detergent are likely to damage or denature proteins that are present and/or desirable for isolation from the biological sample. The incubation of plasma, plasma derived therapeutic proteins, plasma cryoprecipitate, or plasma cryosupernatants with such high concentration of the detergents not only would harm the plasma components but also are known to damage biomembranes. Moreover, the high concentration of detergent is extremely harmful when injected intravenously and hence such detergent-treated plasma would not be suitable for injections. To avoid damage to living cells and proteins, a lower concentration of detergent can be employed, but at the risk of being ineffective for viral inactivation. Therefore, to ensure living cells and proteins are not damaged and at the same time viral inactivation is effective, removal of detergents is imperative.

Commonly employed methods for removal of detergent include affinity or ion-exchange chromatography. These methods are lengthy and time consuming, and involve multiple steps. As one skilled in the art would appreciate, each recovery step is often associated with the loss of proteins of interest and hence result in lower yield. Further, these methods are suitable only for the particular protein factor as an end product and would not be appropriate for the whole plasma. To make it applicable to whole plasma, whole plasma would need to be reconstituted after each of the factors is successively separated and purified. After each step, some amount of time and product would be lost, which may ultimately lead to significant overall loss.

After solvent-detergent treatment, the detergent can be removed by employing several steps chosen among diafiltration, adsorption on chromatographic or affinity chromatographic supports, precipitation and lyophilization, etc. Dialkyl- or trialkylphosphate is often removed by precipitation of the protein with glycine and sodium chloride (see U.S. Patent No. 4,540,573). The process of the '573 patent is particularly time-consuming as nonionic

detergents employed with the trialkylphosphate are removed by diafiltration using either insolubilization or lyophilization. One skilled in the art appreciates that these processes are cumbersome, expensive, time consuming, and/or can result in loss of vital components of plasma.

Removal of detergent and solvent can additionally be preformed by partitioning the protein solution against an organic liquid such as castor or soy bean oil. The detergent and solvent partition into the organic liquid and are thus eliminated. The organic liquid that is oil is then removed by chromatography. This procedure involves partitioning of plasma and regenerating or replacing the chromatographic components, which tend to be very tedious, time consuming and cost intensive.

Another method for reducing of virus-inactivating chemicals and/or detergent is by high salting out effect (see U.S. Patent No. 5,817,765). In this procedure, a concentration above 0.5M of salt with high salting out effect according to Hofmeister series is added to the aqueous plasma protein solution for forming vesicles containing the virus-inactivating chemicals and/or detergents. The vesicles are removed from the aqueous phase, for example by phase separation or filtration. The technique of phase separation, however, particularly that of vesicles, is laborious, imprecise, and difficult method, which would be very cumbersome in large scale operations because of operational issues like cleaning, sanitization, process validation, etc. Additionally, the further step of protein recovery from aqueous solution may result in the loss of final protein yield. Any trace of salt remaining in the plasma product, would also be undesirable as it would render it unsuitable for the most therapeutic applications.

Removal of solvent/detergent can alternatively be performed by using carbon either in the form of activated carbon or charcoal. For example, Chinese Patent number CN1371992 employs solid phase material containing active carbon as adsorbent for removing organic solvent used as virus inactivator and/or detergent from aqueous solution. U.S. Patent No. 5,834,420 uses precipitation of viral inactivated fraction in a solution containing an amino acid at an acidic pH and filtering. Preferably the filtration step is carried out through a filter of activated carbon of which AKS-4 and AKS-7 are particularly suited. Carbon, however, is a nonspecific adsorbent, and when employed to adsorb virus inactivator and/or detergent, may

also adsorb some of the important peptides components of interest from the plasma. The use of carbon could result in a final product devoid of these useful components.

U.S. Patent No. 6,610,316 discloses a "sugar detergent" rendered insoluble by being bound to an inert substrate. The method described in the '316 patent requires an additional step of binding the detergent to the resin. Further, additional testing protocol could be required to check or ensure sufficient binding of the detergent in order to avoid leaching of the detergent into the blood solution. Detergent that is not bound sufficiently to the resin could contaminate the blood product and render it unsafe for the desired use. Further, the method disclosed in the '316 patent is directed to blood or aqueous liquid containing blood cells, and does not demonstrate the suitability of this method for plasma or plasma derived proteins.

From the forgoing reasons, it is evident that it is imperative to treat plasma or plasma derivatives with virus-inactivating agents to render it safe for therapeutic applications. It is also important to improve such virus-safe plasma or plasma derivatives by clearing of the virucidal agents employed for inactivating viruses to desired acceptable level for clinical use. However in view of the drawbacks associated with the methods discussed above, the need continues to exist to provide a simple, reproducible process, not compromised on time and yield, and yet easily validated for improving virus-safe biological fluids including plasma or plasma derivatives by clearance of the virucidal agents like detergent and solvent to acceptable levels by effective and simple methods without affecting the plasma composition significantly.

SUMMARY OF THE INVENTION

The present invention provides a process for improvement of virus-safe biological materials including, but not limited to plasma, plasma derived proteins, plasma cryoprecipitates, plasma cryosupernatants, blood products and any other biological fluid by clearance of the virucidal agents to the desired level and/or pharmaceutically acceptable level.

It is also an aspect of the present invention to provide a simple, yet effective process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or a pharmaceutically acceptable level, in a single step.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to desired levels, wherein the process does not substantially damage the labile components of the plasma or biological fluids.

The present invention also relates to a present invention to provide the process for improvement of virus-safe biological materials by clearance of virucidal agents to desired level and/or pharmaceutically acceptable level, applicable to laboratory scale.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level, suitable for commercial large-scale manufacturing.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level, wherein the method is easily validated and reproducible.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level as recommended in official pharmacopeia monographs, to make the biological fluid acceptable for therapeutic clinical administration.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level by methods suitable for the said purpose.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level by methods, which would not leach in the product solution and thus would reduce the risk of contamination by such agent.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level by methods reusable for different batches of biological material for said purpose.

The present invention also relates to a process for improvement of virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable

level by methods, which would not alter the composition of plasma or biological materials significantly and thereby preserve substantially the original composition.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts a flow chart of an example of processing of a 5 liter batch of plasma.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides novel processes for removal and/or inactivation of viral contaminants from biological materials.

Definitions

As used herein, "virus-safe" for the purpose of the present invention refers to any biological material, which has undergone virus inactivation treatment, such as biological fluid with solvent and/or detergent to substantially inactivate viruses. Substantial inactivation is obtained to the extent of at least "4 logs," i.e., virus is inactivated to the extent determined by infectivity studies where the virus was present in the untreated serum in such a concentration that even after dilution to 10^4 viral activity can be measured. Alternatively, substantial inactivation is obtained by a process which, when challenged with 6 logs of virus (10^6 infective units) less than two logs of virus is recovered following completion of the process.

As used herein, "virucidal agent(s)" for the purpose of present the invention refers to any agent like solvent, detergent and/or combinations thereof, which has ability to substantially inactivate viruses, particularly lipid-coated or enveloped viruses from biological material.

As used herein, "clearance" for the purpose of the present invention refers to removal or reduction of the virucidal agents.

The present invention relates to a novel process to remove viral contaminants from biological materials to create virus-safe biological materials by clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level.

The present invention relates to a process for removal of virus-inactivating agents used against lipid-coated viruses including, but not limited to HIV, hepatitis B and hepatitis C viruses, as well as other viruses including, but not limited to cytomegalovirus, Epstein Barr virus, lactic dehydrogenase-elevating viruses (e.g., arterivirus), herpes group viruses, rhabdoviruses,

leukoviruses, myxoviruses, alphaviruses, Arboviruses (group B), paramyxoviruses, arenaviruses, and coronaviruses..

In one embodiment, the biological materials that can be processed in accordance with the present invention include, but are not limited to plasma, plasma concentrate, plasma derived proteins, plasma cryoprecipitate, plasma supernatant, vaccine, blood product or any such biological fluid.

In another embodiment, the process is used in treating solid components of blood, lysates, or proteins secreted by cells. Thus, also contemplated are treatment of platelet concentrates, white cell (leuckocyte) concentrates, and leukocyte–poor packed red cells as well as platelet rich plasma, platelet concentrates, and platelet poor plasma including packed cell masses comprising the white buffy coat consisting of white blood cells above packed red cells. Also contemplated is the treatment of masses containing concentrates of granulocytes, monocytes, interferon, and transfer factor.

In another embodiment, the process is used in inactivating virus present in products of normal or cancerous cells. For instance, by the same treatment, one can inactivate virus present in products produced using normal or cancer cells, the exudates from normal or cancerous cells, hybridomas and products produced by gene splicing. Cells used for production of desired product can be mammalian as well as non-mammalian.

It is an embodiment of the present invention to provide a process for making plasma substantially free from solvent-detergent and not substantially alter vital components of the plasma. Vital components include, but are not limited to fibrinogen, factor VIII, properdin, IgG, IgM, IgA, beta-lipoprotein, prothrombin, plasminogen, plasmin inhibitor , thrombin, isoagglutinins, factor V, factor VII, factor IX, factor X, cerutoplasmin, alpha- and beta-globulins, albumin, alpha-1-proteinase inhibitor, vWF, alpha-1-lipoprotein, transferring, and thyroxine binding globulin.

Pooled plasma either to be used for patients in the need of coagulation factor therapy or to serve as a source for therapeutic protein factors therefrom is commonly subjected to treatment with virucidal agents like solvent detergent to inactivate viruses and render the plasma virus-safe for clinical applications or improve the safety profile of the coagulation factors derived

therefrom. However in view of the deleterious effect of the solvent detergent they are required to be eliminated from the virus-safe biological fluids to the acceptable levels. A pharmaceutically acceptable amount in the final product is less than 2 mcg/ml for tri–n-butylphosphate and less than 5 mcg/ml for Triton®-X 100.

One embodiment of the invention provides a process for clearance of virucidal agents to a desired level and/or pharmaceutically acceptable level as laid down in official pharmacopoeia and thereby improving its clinical profile. Guidelines promulgated by the UD Food and Drug Administration can be accessed over the internet at www.fda.gov/cber/guidelines.htm. The present invention novel process for clearance of virucidal agents is a single step, simple and rapid process unlike the hitherto disclosed methods for the same intent. Other advantageous features of the present invention process are that can be conveniently validated and is reproducible.

The present invention can be employed to prepare virus-safe plasma, plasma concentrate, plasma derived proteins, plasma cryoprecipitate, plasma supernatant, blood product or any such biological fluid already treated with virucidal agents or it can be employed after subjecting the said biological fluid to virus inactivating treatment to render them virus-safe.

For inactivation of virus, biological fluid is subjected to treatment with virucidal agents like solvent and/or detergent.

The solvents that may be employed as virucidal agent can be selected from dialkyl- or trialkylphosphates having branched or unbranched, substituted or unsubstituted alkyl groups, suitably with 1 to 10 carbon atoms or combination thereof. Mixtures of various dialkylphosphates can also be used, as well as mixtures of various trialkylphosphates. Mixtures of dialkyl- and trialkylphosphates are also possible within the scope of the present invention. The tri-alkyl phosphates that may be employed can be selected from those where the alkyl group is n-butyl, t-butyl, n-hexyl, 2-ethylhexyl and n-decyl or combinations thereof. The preferable virus inactivating solvent is tri-n-butyl phosphate (TNBP).

Detergents that can be employed as virucidal agents include a non-ionic detergent, such as a polyoxyethylene ether, e.g. a TRITON®, or a polyoxyethylene sorbitan fatty acid ester, such as polyoxyethylene-(20)-sorbitan monolaurate or polyoxyethylene-(20)-sorbitan monooleate,

sodium deoxycholate, synthetic zwitterionic detergent known as "sulfobetaines" such as N-dodecyl-N,N-methyl-2-ammonio-1 ethane sulphonate and its congeners or nonionic detergent such as octyl-beta-D-glucopyranoside. One suitable detergent is TRITON® X-100.

The virus inactivation treatment involves addition of virucidal agents that are solvents and detergents to the aqueous solution of interest. The amount of solvent and detergent would vary depending upon the volume of solution to be treated. Solvent can be employed up to the concentration of about 2 percent weight by weight. The detergent can be added up to the concentration of about 2 percent weight by weight. The virus inactivation treatment can be carried out for about 1 hour to about 16 hours, at temperature ranging from about 4° C to about 50° C.

In accordance with the present invention the virus-safe biological fluid, which has undergone virus inactivation treatment by virucidal agents, is subjected to a single step of clearance of the virucidal agents. Surprisingly it was discovered by the inventors that it was possible to remove both the detergent as well as solvent by employing a single step of clearance as disclosed the present invention.

In an embodiment of the present invention, material such as organic matrix is employed to clear virucidal agents. The biological fluid is contacted with organic matrix with a sufficient surface area for a limited period of time. The virucidal agent gets physically adsorbed on the surface of organic matrix and the separation of supernatant gives biological fluid with virucidal agents eliminated to the significant level.

The process of clearance of virucidal agents can be successfully employed at laboratory as well as commercial production levels. Figure 1 illustrates one embodiment for the manufacturing process of clearance of virucidal agents. Further the process can be carried out either in a batch mode or a column mode.

In one embodiment of the present invention process clearance of virucidal agents in a batch mode is carried out in a vessel wherein the virus-safe biological fluid, with virucidal agents remaining therein after virus inactivation treatment, is contacted with organic matrix in a predefined ratio, under stirring, at a temperature of about 10 – 40 °C, preferably 18 – 30°C for a period of about 0.1 hour to 4 hours, preferably between about 0.15 hr. to about 2 hrs. The

supernatant is separated from the organic matrix by removal of organic matrix by employing by any suitable technique like simple filtration, filtration by applying vacuum, centrifugation or any like technique. The virucidal agents, which get physically adsorbed onto the organic matrix, are eliminated to a significant level from the biological fluid.

In another embodiment of the present invention process the clearance of virucidal agents in a column mode, is carried out in a column prepacked with organic matrix, through which the biological fluid contaminated with the virucidal agents is passed. The contact time between the biological fluid of interest and organic resin is adjusted so as to range from about 0.1 hr – 4 hrs, preferably from about 0.15 hr. to 2 hrs. A column, which can be employed for that purpose, is 1-25 cm, preferably 4 - 16 cm, in height and the diameter of which can be adjusted according to the volume of the biological fluid to be treated.

The organic matrix to be employed for the purpose of the present invention for the learance of virucidal agents is selected from synthetic polymers, which are polyaromatic or methacrylate based resins, which generally do not have tendency to leach in the solution undergoing treatment. The contemplated polyaromatic resin for the present invention purpose is selected from class of polystyrene-divinylbenzene copolymer resins with grades preferably having higher surface area. The polystyrene-divinylbenzene copolymer resin can be selected from HP 20, HP20 SS, SP 285, or the like. The methacrylate-based resins can be selected from HP-2MG, SP 70, SP 207, or the like. Alternatively any other adsorbent matrix can be employed for the purpose of present invention having the ability to sufficiently adsorb the virus inactivating agents.

The organic matrix can be reused for different batches, after regenerating and sanitizing action.

The ratio of resin to the virus-safe biological fluid to be treated can vary between 1:1 to 1:40, preferably 1:2 to 1:25, and more preferably between 1:3 to 1:10.

The process for improvement of virus-safe biological fluids by clearance of virucidal agents can be effectively employed both for single component protein derived from the plasma or to the whole plasma it self either a single unit fresh frozen plasma unit or pooled plasma useful for patients with deficiency of coagulation factors, deficiency of acquired multiple coagulation factors, also in patients needing reversal of warfarin effects, thromocytopenia, long

prothrombin time, head injuries, joint bleeds, dental bleeds, subdural hematoma, hematuria, gastro intestinal bleeding, hemoperitoneum, or any such like disorder.

The process when employed for virus-safe plasma does not alter the vital components of the plasma. Thus, the composition of the plasma processed in accordance with the present invention is almost like the original plasma. Accordingly the improved viral safe plasma obtained as a result of the present invention process may have lower levels of immunogenicity, as highly conserved proteins tend to have rather low immunogenicity and hence therapeutically more useful.

It is to be understood by those skilled in the art that the foregoing specification is to indicate the nature of the invention which is non limiting and the present invention may be embodied in the specific forms without departing from the spirit or attributes thereof and various modifications and changes may be made without departing from the scope of the present invention.

The removal of solvent–detergent from viral inactivated pooled plasma is illustrated in the examples set forth and the compiled tabulated data reflect the procedure for selecting the most beneficial and preferred compounds.

The following details of the studies conducted exemplify the process of invention without limiting the scope thereof.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all and only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric.

Example 1: Experimental details:

Step 1: VIRUS INACTIVATION BY SOLVENT AND DETERGENT:

The plasma of donors of specific group are tested, taken from –20°C freezer and thawed at 30°C in water bath. The plasma is then pooled after thawing and filtered through AP 25 millipore.

The pooled plasma is then treated with solvent tri(n-butyl)phosphate (TNBP) and the detergent Triton X-100 for 4 hours at 30°C. This type of detergent and solvent is known to inactivate enveloped virus (including HIV, HBV, HCV).

This type of virus inactivation by above mentioned solvent and detergent is a well-known process (see U.S. Patent No. 4,540,573).

Step 2: REMOVAL OF SOLVENT AND DETERGENT:

Unlike pre-existing technologies, which employ expensive, time consuming processes for removal of solvent and detergent from virus-inactivated plasma of step 1, the process of this invention is carried out by hydrophobic interaction chromatography using a synthetic polymer.

The synthetic polymers are selected from the class of polystyrene based divinyl copolymers. These polymers which are highly porous and hydrophobic in nature are specifically available from the Mitsubishi Chemical Corporation (Japan) under the name of SEPABEADS® with different grades available, such as SP70, HS20 SS, SP 825, SP850, SP207 and the like. (Itochu Chemicals America, Inc., White Plains, New York)

Solvent detergent removal by synthetic polymer resin:

The resin, for example SP 825, is activated and equilibrated by standard procedures.

1g of resin is added for 4ml of solvent detergent treated plasma i.e. 1:4 (w/v) and stirred gently for 30 minutes at 30°C. The resin is separated using muslin cloth and the plasma is collected separately.

The plasma, which is now substantially free of solvent and detergent, is then passed through Depth filter (Microfilt, India) and then through a 0.22 μ filter.

The final product obtained thus complies with the specifications as laid down in the official British Pharmacoepia for Plasma pooled Virus Inactivated product wherein the maximum accepted limits of Triton X-100 is 5 ppm and tri(n-butyl)phosphate is 2 ppm. (*see* The British Pharmacopeia, H.M. Printing Office, London)

Example 2: Analysis of Effect of Resin on Detergent and Factor VIII Levels

The study on the various proportions of the resins and the resultant Factor VIII content and residual detergent in ppm is tabulated below:

Table 1:

Resin	**Ratio**	**Time**	**Temp.**	**Triton (ppm)**	**Factor VIII (IU/ml)**
Plasma					0.9
HP20	1:4	30 min	4°C	44	0.82
SP207	1:6	30 min	4°C	25	0.65
HP2MG	1:8	30 min	4°C	15	0.51
SP 70	1:4	30 min	4°C	5	0.72
SP825	1:4	30 min	4°C	6.16	0.82

It was found that Triton is more than acceptable limits in HP 20, SP 207 and HP2 MG. SP 70 and SP 825 removed Triton more effectively to below acceptable limits of 5ppm. Further SP 825 resin was effective in removing detergent with good recovery of Factor VIII and without any loss / inactivation of coagulation factors.

Example 3: Optimisation of temperature for SolventDetergent Removal and Factor VIII levels by SP 825 resin

Extensive studies were done on SP 825 for optimizing the process parameters like temperature and proportion of the resin with respect to plasma.

Table 2:

Resin:plasma (gm:ml)	Time (min.)	Temp. (°C)	Factor VIII (IU/ml)	Triton (ppm)
Plasma			0.83	0
1:4	30	30	0.83	3.7
1:4	30	4	0.74	6.1
1:6	60	30	0.83	4.2
1:6	60	4	0.73	13.7

The ratio of resin to plasma in the proportion of 1:4 at 30°C gave acceptable results for Factor VIII and Triton. However at 4°C it was found that residual detergent was more than 5 ppm and the recovery of Factor VIII was less.

Hence, it was inferred that the most preferred temperature was room temperature in the range of 20-30°C for effective removal of solvent and detergent.

Example 4: Optimization of contact time of sample to resin for effective removal of solvent and Detergent

The different interval of time of contact of plasma with SP 825 resin was studied for effective removal of detergent.

Table 3:

Resin:plasma (gm:ml)	Temp. (°C)	Resin Contact Time (min.)	Residual Triton (ppm)
1:4	30	10	8
1:4	30	20	6
1:4	30	30	0.45
1:4	30	40	0.38

A proportion of 1:4 of resin to plasma at 30°C for 30-40 minutes was optimum for removal of solvent detergent.

Example 5: Analysis of Effect of Resin on pH

There is no significant alteration of pH after resin treatment, as given by experimental evidence below:

Table 4:

Sample	PH before Resin treatment	pH after resin treatment
S-D Plasma	6.7	6.8
S-D Plasma	7.0	7.05
S-D Plasma	7.2	7.15

Example 6: Removal of S-D by column mode

The performance of the resin in column and in batch mode was studied by the following procedure:

1. 10 gm of resin was weighed and packed in XK 16 (Amersham Biosciences, GE Healthcare, UK) column.
2. 10 gm of resin was weighed and taken in glass beaker.
3. 40 ml of solvent detergent (S-D) treated plasma was added to the resin in beaker for batch mode.
4. 40 ml of S-D treated plasma passed through the column at room temperature.
5. Flow rate checked with the help of measuring cylinder. Typically, 2.5 ml/ min
6. Linear flow rate 75 cm/hr.
7. S-D treated plasma passed five times through the column and sample collected at every pass for analysis.

Table 5:

Sample	Mode	Triton ppm
1st pass	Column	3.89

Solvent and detergent can be removed by column mode with a flow rate of 45-75 cm/hr, preferably 60cm/hr of linear flow rate and temperature in the range of 20-30°C.

Example 7: Regeneration of the Resin.

The resin can be regenerated after every run by the following procedure as directed by the manufacturer Mitsubishi in their data sheet of Labion TM.

The resin is washed twice with water for injection to remove any remaining plasma.

Then the resin is treated with three volumes of 3% sodium hydroxide solution for 15 minutes with slow stirring at 25-30°C, then decanted and washed with three volumes of water.

After complete removal of water, the resin is treated with three volumes of 80% isopropanol and then finally stored at room temperature.

The samples of the resin are then analyzed for isopropanol and protein by optical density at 280nm.

This regenerated resin can be reused only after extensive cleaning, validation and regulatory approval.

Example 8: Manufacturing Process on a Pilot Scale of 5 Liters

The manufacturing process involves the following steps exemplified in the flow diagram shown in Figure 1:

Plasma frozen within 15 hours of collection from volunteer donors was debagged, pooled and thawed at a temperature not to exceed 35°C. The product underwent 1.0 μm filtration and was transferred to a process tank. The adjusted pooled plasma was virus-inactivated by the addition of 0.3% TNBP and 1% Triton X-100. The mixture was incubated at 30 ± 2°C for four hours.

TNBP and Triton X-100 were removed by hydrophobic interaction chromatography at 20-30°C for 30 min. followed by depth filtration and 0.22μ filtration. The product was then filled in a designated bag, labeled and stored at –20°C.

The results of the scale up of laboratory scale to pilot scale by using the above resins for solvent detergent removal is given below indicating the industrial applicability to this process.

Table 6:

Batch	**Blood Group**	**Vol (ml)**	**Triton (ppm)**
Pool	**B** +ve	100	
1	**B** +ve	100	0.48
2	**B** +ve	100	0.55
3	**B** +ve	100	0.53

Batch	**Blood Group**	**Volume in ml**	**Triton In ppm**	**TnBP in ppm**
Pool 1	**B** +ve	1000		
1	**B** +ve	1000	1.24	below detectable limits
Pool 2	**O** +ve	1000		
2	**O** +ve	1000	0.59	0.44
Pool 3	**A** +ve	1000		
3	**A** +ve	1000	0.81	Below detectable limits

Example 9: Analytical Procedures for Triton and Tri-(N-Butyl) Phosphate

(a) Sample preparation for Residual Triton analysis:

S-D treated plasma after HIC resin treatment was extracted on C-18 cartridge with 75% isopropanol (v/v). Extraction procedure was checked by spiking known amount of Triton in the sample. Almost 99 % amount of Triton was recovered.

(b) Method of HPLC analysis:

Extracted sample was loaded on C-8 column and UV detector 280 nm was used to detect the residual triton.

(c) Sample preparation for tri(n- butyl)phosphate:

Final sample was then extracted with hexane. Ethanol was added to get a clear supernatant. 1 µl of sample of supernatant is used for Gas chromatography analysis. Extraction procedure was monitored by spiking known amount TNBP and recovering almost 99 % of the amount.

HP-5 column with FID detector was used for analysis. Detector temperature was at 250°C.

The resultant product has been characterized, and was found to be biochemically similar to the starting material, human frozen plasma.

This method of analysis is described by Kaliappanadar *et al.* in "Validation of a simple and sensitive gas chromatographic method of analysis of Tri-n-butyl phosphate from virally inactivated human Immunoglobulin" J. Chromatography B, 757:181-189 (1993).

PRODUCT SPECIFICATIONS:

The product obtained for solvent detergent treated pooled plasma which employed the process of the present invention complies with the limits or specifications set forth in British Pharmacoepia.

Table 7:

Tests	Limits	Batch1	Batch2
Antibodies to HIV 1&2	Absent	Absent	Absent
HCV	Absent	Absent	Absent
HbsAg	Absent	Absent	Absent
Ouchterlony test	Human origin	Complies	Complies
Osmolality	240mosmol/kg	Complies	Complies
Citrate	25mmol/L	13.075	11.10
Calcium	5mmol/L	1.35	1.27
Potassium	5mmol/L	0.09	0.115
Sodium	200mmol/L	163	123.9
Pyrogen	Rabbit test	Complies	Complies
Sterility	Should pass	Passes	-Passes
TNBP	2 ppm	0	0.44
Triton	5 ppm	1.2	0.59
SDS-Page	Same bands	Complies	Complies
Haemaglutinin A&B	Group specific	B	O
PH	6.5 to 7.6	6.9	7.3
Total protein by Kjeldahl	Minimum 45 gm / Liter	56	57.75
Activated coagulation factors	≥ 150 sec	153s	155s
Hepatitis A virus Ab	Minimum 2 IU / ml	Complies	Complies
Irregular erythrocytes	Absent	Complies	Complies
Factor VIII	≥ 0.5 IU/ml	0.623	0.712
Factor V	≥ 0.5 IU/ml	0.98	1.02

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicate to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

1. A process of preparing a virus inactivated plasma comprising:
providing plasma suspected to contain a virus;
contacting said plasma with solvent detergent in an amount and under conditions sufficient for substantially inactivating the virus; and
removing solvent-detergent from plasma by treatment with a synthetic polymer.

2. A process of removal of solvent and detergent from solvent detergent virus inactivated plasma comprising:
subjecting the solvent detergent virus inactivated plasma to hydrophobic interaction chromatography comprising a synthetic polymer under conditions wherein the solvent and detergent are substantially removed.

3. A process of preparing a virus inactivated plasma with solvent detergent removed, the process comprising:
providing plasma treated with solvent detergent in an amount and under conditions sufficient for substantially inactivating a virus; and
removing the solvent and detergent from plasma by a single step treatment with a synthetic polymer.

4. The process of claim 1, 2, or 3, wherein the solvent is a tri-alkylphosphate selected from an alkyl group consisting of n-butyl, t-butyl, n-hexyl, 2-ethylhexyl and n-decyl.

5. The process of claim 1, 2, or 3, wherein the detergent is selected from the group consisting of polyoxyethylene ether, polyoxyethylene sorbitan fatty acid ester, sodium deoxycholate, synthetic zwitterionic detergent, and octyl-beta-D-glucopyranoside.

6. The process of claim 1, 2, or 3, wherein the solvent is tri(n-butyl) phosphate and the detergent is TRITON® X-100.

7. The process of claim 1, 2, or 3, wherein the synthetic polymer is a synthetic aromatic resin provided as an organic matrix.

8. The process of claim 1, 2, or 3, wherein the synthetic polymer is hydrophobic

9. The process of claim 1, 2, or 3, wherein the hydrophobic synthetic polymer is selected from the group consisting of a polystyrene-based divinyl benzene copolymer and a methacrylate-based polymer.

10. The process of claim 1, 2, or 3, wherein the synthetic polymer is selected from the group consisting of SEPABEADS in the grades of HP 20, HP20 SS SP 285, HP-2MG.

11. The process of claim 1, 2 or 3, wherein the synthetic polymer is selected from the group consisting of SP 70 and SP207.

12. The process of claim 1, 2, or 3, wherein the synthetic polymer is a polystyrene based divinyl benzene copolymer resin.

13. The process of claim 10, wherein the polystyrene based divinyl benzene copolymer resin is selected from the group consisting of HP 20, HP20 SS, and SP 285.

14. The process of claim 10, wherein the polystyrene based divinyl benzene copolymer resin is SP 285.

15. The process of claim 1, 2, or 3, wherein the synthetic polymer is a methacrylate based polymer.

16. The process of claim 15, wherein the methacrylate based polymer is selected from the group consisting of HP-2MG, SP 70, and SP207.

17. The process of claim 1,2, or 3, wherein the synthetic polymer is used in the ratio of resin to solvent detergent treated plasma in the range of 1:1 to 1:8, preferably in the range to 1:4 to 1:6

18. The single step process according to claim 3 of solvent-detergent removal from solvent-detergent treated plasma, further comprising:
treating the virus-inactivated plasma with the synthetic polymer for 10 to 60 minutes.

19. The process of claim 18 wherein the virus-inactivated plasma is treated with the synthetic polymer for 30 minutes.

20. The single step process according to claim 3 of solvent-detergent removal from solvent-detergent treated plasma, further comprising:
treating the virus-inactivated plasma with the synthetic polymer for a predetermined period at 4°C to 40°C.

21. The process of claim 20 wherein the virus-inactivated plasma is treated with the synthetic polymer at 20-30°C.

22. A process of preparing virus inactivated plasma, comprising:
(a) providing plasma from donors;
(b) treating plasma with solvent detergent in an amount and conditions sufficient for inactivating virus;
(c) contacting the treated plasma with polystyrene based divinyl benzene copolymer under conditions sufficient for removing solvent detergent.

23. The process of claim 1, 2, or 3, wherein the method is employed on a pilot scale or employed on an industrial scale.

24. The process of claim 1, 2, or 3, further wherein the synthetic polymer is regenerated following use.

25. The process of claim 1, 2, or 3, wherein the plasma is substantially free of solvent-detergent.

26. The process of claim 25, wherein the plasma is used for patients with deficiency of coagulation factors and in patients needing reversal of warfarin effects, thromobcytopenia, long prothrombin time, head injuries, joint bleeds, dental bleeds, subdural hematoma, hematuria, gastrointestinal bleeding, or hemoperitoneum.

27. The process of claim 1, wherein the plasma is substantially free from solvent-detergent and that the composition of the plasma with respect to the vital components is not

substantially altered.

28. The process of claim 1, wherein the plasma complies with the acceptable pharmacoepial limits of solvent less than 2 ppm and detergent less than 5 ppm, further wherein the plasma is substantially free from solvent-detergent.

29. A virus-inactivated plasma prepared by a method according to any of claims 1, 2, and 20.

30. The virus-inactivated plasma of claim 29, wherein the plasma is pooled from plasma of a plurality of donors.

31. A process of preparing a virus safe biological material comprising:
providing biological material suspected to contain a virus;
contacting said biological material with solvent detergent in an amount and under conditions sufficient for substantially inactivating the virus; and
removing solvent-detergent from the biological material by treatment with a synthetic polymer.

32. The process of claim 31, wherein the biological material is selected from the group consisting of:
plasma, plasma concentrate, plasma derived proteins, plasma cryoprecipitate, plasma supernatant, vaccine, blood product, serum, biological fluid, solid components of blood, lysates, proteins secreted by cells, platelet concentrates, white cell (leuckocyte) concentrates, leukocyte–poor packed red cells, platelet rich plasma, platelet concentrates, platelet-poor plasma, packed cell masses comprising white buffy coat consisting of white blood cells above packed red cells, and masses containing concentrates of one or more of granulocytes, monocytes, interferon, and transfer factor.

33. A virus safe biological material prepared according to the process of claim 31.

34. The virus safe biological material of claim 33, wherein the biological material is selected from the group consisting of:
plasma, plasma concentrate, plasma derived proteins, plasma cryoprecipitate, plasma

supernatant, vaccine, blood product, serum, biological fluid, solid components of blood, lysates, proteins secreted by cells, platelet concentrates, white cell (leukocyte) concentrates, leukocyte–poor packed red cells, platelet rich plasma, platelet concentrates, platelet-poor plasma, packed cell masses comprising white buffy coat consisting of white blood cells above packed red cells, and masses containing concentrates of one or more of granulocytes, monocytes, interferon, and transfer factor.

35. The process of claim 31, wherein the virus is an enveloped virus.

36. The process of claim 35, wherein the virus is a lipid coated virus selected from the group consisting of HIV, hepatitis B and C viruses, cytomegalovirus, Epstein Barr virus, lactic dehydrogenase-elevating viruses (e.g., arterivirus), herpes group viruses, rhabdoviruses, leukoviruses, myxoviruses, alphaviruses, Arboviruses (group B), paramyxoviruses, arenaviruses, and coronaviruses

Flow Chart for Exemplar Processing of a 5 Liter Batch of Plasma

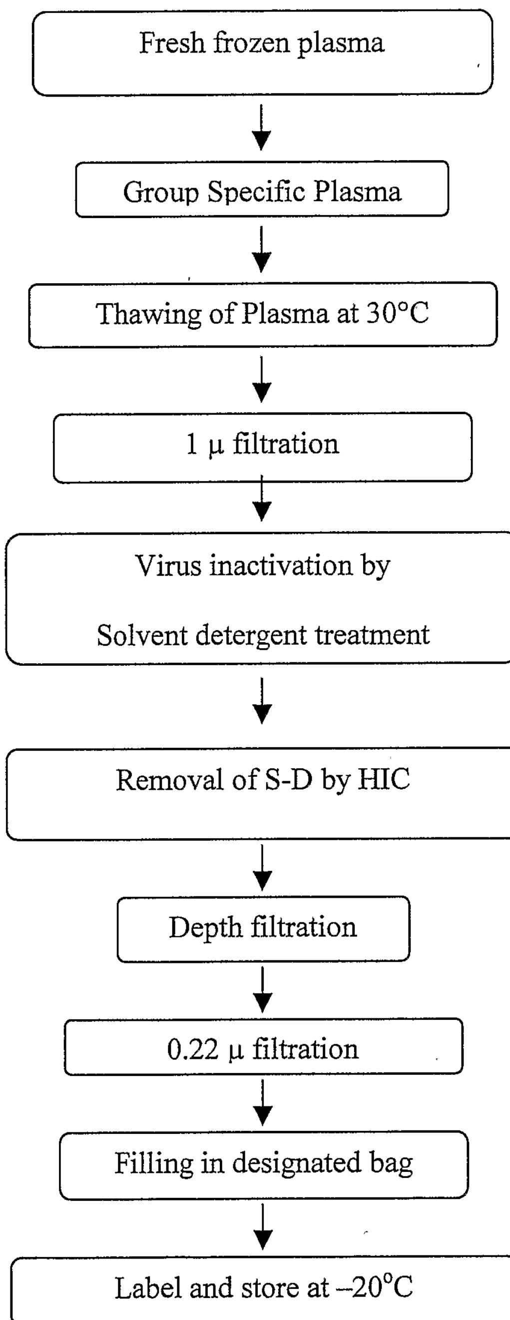

FIGURE 1

Box No. VIII (v) DECLARATION: NON-PREJUDICIAL DISCLOSURES OR EXCEPTIONS TO LACK OF NOVELTY

The declaration must conform to the standardized wording provided for in Section 215; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (v). If this Box is not used, this sheet should not be included in the request.

Declaration as to non-prejudicial disclosures or exceptions to lack of novelty (Rules 4.17(v) and 51*bis*.1(a)(v)):

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☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (v)".