Forms 7 and 8 DMO:AL 17/1/2

AUSTRALIA

Patents Act 1952

DECLARATION IN SUPPORT OF A CONVENTION OR NON-CONVENTION APPLICATION FOR A PATENT OR PATENT OF ADDITION

Name(s) of Applicant(s)

In support of the application made by Robert H. PURCELL and Stephen M. FEINSTONE

Title

for a patent for an invention entitled UTILIZING A HALOHYDROCARBON CONTAINING DISSOLVED WATER TO INACTIVATE A LIPID VIRUS.

Name(s) and address(es) of person(s) making declaration

~~I~~/We, Robert H. Purcell, 17517 White Grounds Road, Boyd, Maryland 20720, U.S.A.; and Stephen M. Feinstone, 3021 Cathedral Avenue, Washington, D.C. 20008, U.S.A.

do solemnly and sincerely declare as follows:-

1. ~~I am~~/we are the applicant(s) for the patent, ~~or am/are authorised by the abovementioned applicant to make this declaration on its behalf~~.

2. The basic application~~(s)~~ as defined by Section 141 of the Act was/~~were~~ made in the following country or countries on the following date~~(s)~~ by the following applicant(s) namely:-

Country, filing date and name of Applicant(s) for the or each basic application

in United States of America on 18th May, 1984
by Robert H. PURCELL and Stephen M. Feinstone
in _____ on _____ 19____
by _____

3. The said basic application~~(s)~~ was/~~were~~ the first application~~(s)~~ made in a Convention country in respect of the invention the subject of the application.

Name(s) and address(es) of the or each actual inventor

4. The actual inventor(s) of the said invention ~~is~~/are Applicants

See reverse side of this form for guidance in completing this part

5. The facts upon which the applicant(s) is/are entitled to make this application are as follows:-

X DECLARED at Bethesda, Md, USA this 10th day of February 1986

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X Robert H Purcell

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UTILIZING A HALOHYDROCARBON CONTAINING DISSOLVED WATER TO INACTIVATE A LIPID VIRUS

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(56) Prior Art Documents
US 4405603
US 4481189
US 4424206

(57) Claim

1. A method of inactivating essential lipid-containing viruses in a dried protein product comprising

contacting said viruses for a period of 10 minutes to 10 hours at a treatment temperature of 4°C to 40°C with a treating composition consisting essentially of

(a) a treating agent selected from one member of the group consisting of halogenated hydrocarbons and

(b) water dissolved in said treating agent wherein said water is 75 to 100% of the amount of water required to saturate the treating agent at the treatment temperature; and

removing the treating agent from the protein ~~carrier~~ product.

7. The method of Claim 1 or Claims 2 wherein the treating agent is chloroform containing ethanol and the dissolved water content of the chloroform is adjusted to the amount necessary for 100% saturation of the chloroform-ethanol solution at the treatment temperature.

9. The method of Claim 8 wherein the said virus is selected from at least one member of the group consisting of Hepatitis B virus (HBV) and non-A and non-B hepatitis virus (NANBH).

10. The method of claim 8 wherein the said virus is selected from at least one member of the lipid-containing groups consisting of herpesviruses, the delta agent, togaviruses, bunyaviruses, retroviruses, orthomyxoviruses, paramyxoviruses, rhabdoviruses, poxviruses, hepadnaviruses, arenaviruses, and coronaviruses.

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(54) Title: UTILIZING A HALOHYDROCARBON CONTAINING DISSOLVED WATER TO INACTIVATE A LIPID VIRUS

(57) Abstract

A method of inactivating a lipid virus contained in a dry protein carrier by contacting said virus-containing protein carrier for an abbreviated period of time at from 4-40°C. with a composition including a halohydrocarbon treating agent and water dissolved in said treating agent. Preferred lipid viruses are Hepatitus B virus (HBV) and non-A, non-B Hepatitus (NANBH) virus.

UTILIZING A HALOHYDROCARBON CONTAINING DISSOLVED WATER TO INACTIVATE A LIPID VIRUS

This invention relates to a method of inactivating a lipid virus contained in a dry protein carrier by contacting said virus-containing protein carrier for an extended period of time and ambient temperature with a halohydrocarbon solvent or treating agent, preferably chloroform, which contains dissolved water in an amount between 75% and 100% of that amount required to saturate the solvent at the ambient temperature. Preferred viruses are Hepatitis B virus (HBV) and non-A, non-B Hepatitis (NANBH) virus.

GENERAL BACKGROUND

A selected lipid type virus, viral hepatitis, has been recognized as an important and serious sequela of parenteral exposure to blood and blood components since the early 1940s. It was originally believed that all such blood-associated hepatitis was caused by the serum hepatitis virus (now called the Hepatitis B virus, or HBV). Subsequently, the development of sensitive assays for infection with this virus revealed that only approximately one-third of transfusion-associated hepatitis was caused by the HBV. It was thought that the remaining hepatitis was caused by the Hepatitis A virus (HAV). However, the development of sensitive assays for HAV led to the recognition of a new hepatitis virus, the non-A, non-B hepatitis virus (NANB) in 1975. The successful application of sensitive screening tests for HBV to blood donors has resulted in a decrease (but not disappearance) of HBV in transfusion-associated hepatitis; at present approximately 90% of such hepatitis is caused by non-A, non-B agents.

Similarly, hepatitis following administration of plasma protein derivatives such as antihemophilic factor was thought to be caused solely by HBV. However, in the late 1970s, the association of NANB agents with administration of antihemophilic factor to hemophiliacs was reported and confirmed. As with transfusion-associated

hepatitis, the application of serologic screening methods to plasma donors has resulted in a relative decrease in the importance of HBV in such blood product-associated hepatitis.

Non-A, non-B hepatitis is the major cause of transfusion associated hepatitis in the United States. Prensently, less than 10% of post-transfusion causes are caused by the hepatitis B virus. Of the remainder, cytomegalovirus may account for a small proportion but the vast majority are cuased by an as yet unidentified agent. There is a large amount of evidence supporting a transmissible agent as the cause of NANBH. This includes transmission studies done in both humans and non-human primates. Chimpanzees and marmoset monkeys have both been shown to be susceptible to infection by at least some NANBH agents. Though very costly and cumbersome to work with, these animals can be used to aid in the characterization of the infectious agent of NANBH.

Unfortunately, serologic tests for the detection of NANB agents are not available for detection and potentially infectious donors because the agents have not been adequately identified and characterized despite extensive efforts to do so. Therefore, blood and plasma protein derivative products remain potential sources for transmission of hepatitis agents to recipients. The resultant hepatitis can be quite serious, even life-threatening, and can result in not only acute hepatitis but also chronic hepatitis in a significant proportion of cases.

For these reasons, attempts to inactivate hepatitis agents in blood and plasma products have been pursued with vigor. Such approaches have included the use of heat, the addition of anti-HBV antibody, the use of solid immunoadsorbents or other chemical-specific adsorbents, exposure to ultraviolet radiation, the addition of certain inactivating substances, such as beta-~~propriolactone,~~ propiolactone surface-active substances, etc. None of the approaches has been

entirely successful and some have introduced an added potential risk (e.g., beta-~~propriolactone~~ propiolactone is carcinogenic). Failure of these approaches stems from relative resistance of the agents to physical or chemical inactivation, particularly when in the presence of high protein concentrations as occurs with blood products and from limited knowledge about the nature of the hepatitis agents, especially the NANB agents.

As part of a systematic characterization of NANB agents by standardized virologic methods, the present inventors first established that HBV and at least one NANB agent contain lipids essential for the integrity and viability of the viruses. This was established by exposing the viruses to a potent lipid solvent (chloroform) and demonstrating that such chloroform-extracted viruses were rendered non-infectious in a suitable susceptible host, the chimpanzee (Pan troglodytes).

It was later found that when viruses were contained in a dried protein carrier such as a commercial factor VIII concentrate preparation, the viruses were much more difficult to inactivate with the lipid solvent than when the protein carrier was reconstituted to a liquid with water and then treated with the lipid solvent. It was also found that the biologic activity of the protein carrier was difficult to maintain when it was treated as a liquid with the lipid solvent. Thus, this invention expands the parent invention by modifying the process in such a way that a lipid virus contained in a dry protein carrier, in particular a plasma derivative, can be readily and consistenly inactivated by a lipid solvent while the biologic activity of the plasma product is maintained at a high level.

The present invention relates to a method of inactivating lipid viruses that frequently contaminate a plasma protein product. Said viruses are most frequently hepatitis B virus and non-A, non-B hepatitis virus but are

also defined to include members of the herpesvirus group (cytomegalovirus, Epstein-Barr virus, herpes zoster virus, herpesvirus type 1 and type 2), the delta agent (a type of non-A, non-B hepatitis virus), togaviruses (including rubella virus), bunya viruses, retroviruses (including the human T-cell leukemia viruses), orthomyxoviruses (including influenza), paramyxoviruses (measles, mumps), rhabdoviruses (rabies, Marburg agent), arenaviruses (Lassa fever, other hemorrhagic fevers), coronaviruses, hepadnaviruses, and poxvirus group (smallpox, vaccinia virus). Other viruses, known or suspected, such as the putative agent of acquired immune deficiency syndrome (AIDS) are included as viruses possibly containing essential lipids. (Evidence that the agent of AIDS is a retrovirus has recently been published.)

The plasma protein product is defined as a protein derived from blood or blood plasma that is intended for human medical uses most often to correct a deficiency of that particular blood protein, or as an aid to treating some disease that might benefit from an increased concentration of the particular blood protein. Over 100 such plasma proteins have been identified and perhaps many more will eventually be found (cf. Putnam, Plasma Proteins, pp. 36-41 and Table 1). Examples of such blood products are antihemophilic factor (factor VIII), factor IX, fibrinogen, fibronectin, albumin, complement components, plasminogen, transferrin, and haptoglobin, and many other plasma proteins that have indicated medical uses but that may not at this time be marketed. In general, lipoproteins would not survive the process of this invention in their native state. In addition, certain blood proteins that are not intended for therapeutic use but may be used, for example, in diagnostic tests, may also be treated to reduce their hazard to the person handling them. Such plasma protein products are often dried by lyophilization during the manu-

facture process in order to preserve their biologic potency, increase shelf life and for ease of handling and shipping. The residual moisture content of such dried products ranges between about .5 and 1.5%.

In the present invention the contaminating lipid containing viruses are inactivated by treating (extracting or contacting) the dry plasma product with a lipid solvent in which water has been dissolved such that the lipid solvent contains between about 75% to 100% of the amount of water required to reach its dissolved water saturation point. The preferred lipid solvent is chloroform ($CHCl_3$) or $CHCl_3$ and a lower alcohol (e.g., methanol or ethanol), or the fluorocarbons (trichlorotrifluoroethane) which include the most common agents such as CCl_3F, CH_2F_2, CCl_2F_2, CCl_2FCClF_2 and others sold under the trademark registration Freon® or Genetron®. Throughout the present invention the biologic activity of the plasma protein is retained but the infectivity of the virus is removed.

The period of time for the treatment is about 10 minutes to about 10 hours and at least about 10 minutes. The temperature of the treatment is from about 4°C. to 40°C.

The quantity of lipid solvent used to treat dried plasma product is from about equal to the weight of the dry blood product to about 1,000 times the weight of the dry blood product.

The lipid solvent or the treating agent may be removed from the plasma product by evaporation with a stream of pure, dry nitrogen gas (N_2), by vacuum evaporation or by a combination of these or other physical methods which returns the plasma product to the dry state and free of the lipid solvent.

Commercial chloroform contains 0.5% (v/v) ethanol as a stabilizer. The quantity of dissolved water required to saturate pure chloroform varies between 0.019% (wt/v) at 3°C to 0.065% at 22°C to 0.118% at 43°C. (Stephen and

Stephen (eds.), Solubilities of Inorganic and Organic Compounds, Vol. 1, MacMillan Co., New York 1963, p. 370). Therefore, the absolute quantity of water in the chloroform can be increased by increasing the temperature. In addition, the quantity of dissolved water can be increased by increasing the ethanol concentration.

It has been found that dry (water-free) chloroform is inconsistently effective in inactivating vaccinia virus. It is believed that increases in the dissolved water content of the treating agent tend to increase the effectiveness of the inactivation treatment, with superior results achieved as the water dissolved in the treating agent approaches 100% saturation of the treating agent at the temperature of treatment. It is believed that inactivation treatments should be conducted at saturation levels of from about 75 to about 100%. A two-phase system including water and treating agent, while relatively effective in inactivating virus, may destroy activity of blood products, such as Factor VIII.

MATERIAL INFORMATION DISCLOSURE

A review of the prior art of patents is as follows:

US 4,113,712 Funakoshi - Utilization of a surfactant such as the Tritons or Tweens for hepatitis B surface antigen particles.

US 4,139,630 Asculai et al. - Utilization of non-ionic surfactants as inactivating agents for herpes simplex virus.

US 4,314,997 Shanbrom - a non-denaturing amphiphile used to inactivate hepatitis viruses B and non-A, non-B in amount of 0.25-10% by weight and citing non-anionic, anionic, and cationic surfactants.

US 3,847,737 Kanarek - A method of inactivating myxoviruses by utilizing a treating composition consisting of Tween 20, 40, 60 or 80, e.g., a polyoxyethylene ester of partial oleic acid together with an organic solvent

consisting of a chlorinated lower hydrocarbon having 2-5 carbon atoms.

US 4,315,919 Shanbrom - Similar disclosure to 4,314,997 above.

US 4,031,204 Davis - This patent notes at column 4 that a 50% chloroform concentration will inactivate totally the feline viral rhinotracheitis (FVR_m) virus.

US 4,302,444 Baxendale - This patent indicates that for a vaccine for protecting against egg drop disease, the inactivation can be carried out with formaldehyde, with organic solvents, particularly halogenated hydrocarbons in the presence of a surface active agent, such as a polyoxy ethylene sorbitan mono-oleate or with beta-propriolactone.

Non-patent literature is as follows:

Purcell, "The Hepatitis Viruses: An Overview and Historical Perspective," Viral Hepatitis, 1981, International Symposium, Szmuness, Alter and Maynard (eds.), Franklin Institute Press, Philadelphia, pp. 3-12.

Philipson, "Water-Organic Solvent Phase Systems." Methods in Virology, Maramorosch et al. (eds.), Vol. II, 1967, pp. 235-244.

Feinstone et al., "Inactivation of Hepatitis B virus and Non-A, Non-B Hepatitis by Chloroform," Infection and Immunity, Vol. 41, August 1983, pp. 816-821.

EXAMPLE 1

Vaccinia virus, a member of the pox virus group was used as an example of lipid-containing viruses. The pox viruses are known to be the most difficult group of lipid-containing viruses to inactivate by lipid solvents.

A sealed ampoule of lyophilized vaccinia virus, Elstree Strain ATCC #VR-862, was obtained from the American Type Culture Collection. The dry contents of the single ampoule were removed, divided into four approximately equal parts and placed into pre-weighed vials. The vials were then re-weighed to determine the weight of the dry powder containing the vaccinia virus. The vials were numbered 1, 2, 3 and 4 and they were then treated as follows:

Vial #1 - 1 ml of chloroform from a freshly opened bottle (J.T. Baker Chemical #9180-1).

Vial #2 - 1 ml of chloroform (from an identical bottle) that had been saturated with water by shaking it for four hours with water; then allowing the phases to separate upon standing overnight.

Vial #3 - 0.34 ml H_2O (0.236 ml H_2O/mg of vaccinia powder) was added to dissolve the vaccinia powder; then 0.66 ml fresh chloroform was added.

Vial #4 - 0.446 ml H_2O (0.236 ml/mg vaccinia powder) was added to dissolve the vaccinia powder.

All vials were then placed on a rotary shaker and were shaken vigorously for four hours at room temperature (approximately 20°C).

The chloroform in vials #1 and #2 was evaporated off by a stream of purified, dry N_2 gas. The aqueous (upper) phase was pipetted off the chloroform in vial #3. Vial #4 was not further treated. All vials were stored at -70°C until infectivity testing was performed.

The amount of infectious vaccinia virus remaining after the various treatments was assayed by tissue culture infectivity using BSC-1 cells. Quadruplicate wells of 24-well tissue culture plates containing monolayers of BSC-1 cells were inoculated with serial 10-fold dilutions of the vaccinia virus suspensions after they were treated. The BSC-1 cells were examined microscopically for the typical cytopathic effect produced by vaccinia virus.

Factor VIII concentrate (Koate, Cutter Laboratories) was treated in a way similar to the vaccinia virus in order to test for retention of Factor VIII activity following extraction by chloroform. Factor VIII activity was determined by a commercial laboratory using standard techniques.

Results

The results are summarized in the following table.

Sample No.	Treatment	Vaccinia Titer ($TCID_{50}$)	Factor VIII Retention * (% of control)
1	Dry chloroform	10^{5}	75.6
2	H_2O Sat $CHCl_3$	$10^{0.5}$	75.6
3	H_2O + $CHCl_3$ (2 phase)	$10^{1.25}$	15.0
4	H_2O only (control)	$10^{5.25}$	100.0

* Compared to non-chloroform treated control (sample #4)

Interpretation

The dry chloroform was not effective in inactivating the dry vaccinia virus. The two-phase system and the water saturated $CHCl_3$ were effective inactivators of vaccinia. However, the two-phase (aqueous and $CHCl_3$) system also destroyed approximately 85% of the Factor VIII activity. Treatment of dry factor VIII with either the dry $CHCl_3$ or the water saturated $CHCl_3$ resulted in a retention of 75% of the Factor VIII activity.

Thus, it was shown that vaccinia virus in the lyophilized state could be readily inactivated by chloroform if the chloroform was first saturated with water. The pox virus group, of which vaccinia virus is a member, is known to be the group of viruses containing essential lipids that is the most difficult to inactivate by lipid solvents such as chloroform. Therefore, it can be reasoned that other viruses that contain essential lipids will also be readily inactivated in the dry state by the process of extracting them with chloroform that has been saturated in dissolved water.

EXAMPLE 2

The purpose of this experiment was to determine the degree of H_2O saturation of the chloroform required to effectively kill viruses contained in a dry powder. Vaccinia virus and Factor VIII concentrate were treated in

various ways similar to the methodology of Example 1. The treatments were as follows:

1) $CHCl_3$ 100% saturated by water
2) $CHCl_3$ 75% saturated by water
3) $CHCl_3$ 50% saturated by water
4) $CHCl_3$ 25% saturated by water
5) $CHCl_3$ fresh, dry from a newly opened bottle of the same lot # as in 1-4
6) Fresh dry $CHCl_3$ + water in a two-phase system
7) Water only

The $CHCl_3$ that contained water at less than the saturation level was prepared by mixing appropriate volumes of 100% water-saturated $CHCl_3$ with dry $CHCl_3$ from a freshly opened bottle. In this way the desired saturation percent was easily achieved. Vials containing the dry vaccinia powder or the Factor VIII concentrate powder and the appropriate treating agent were shaken vigorously on a rotary shaker at room temperature (approximately 20°C) for 4 hours. The $CHCl_3$ was evaporated off by a stream of nitrogen gas or the aqueous phase was separated from the $CHCl_3$ by centrifuging at 1000 RPM for 10 minutes and the aqueous phase (top layer) was carefully pipetted off. The redried powders were reconstituted with a measured amount of water so that 0.2 ml of water was added per mg of vaccinia powder. The samples were then stored forzen at -70°C until testing.

Vaccinia and Factor VIII were tested as described in Example 1.

Results Treatment	Vaccinia Titer ($TCID_{50}$)	Factor VIII Level (% of Control)
100% H_2O saturated $CHCl_3$	$10^{2.25}$	105%
75% " " "	$10^{4.75}$	100
50% " " "	$10^{4.25}$	100
25% " " "	$10^{4.75}$	110

Results	Vaccinia Titer ($TCID_{50}$)	Factor III Level (% of Control)
Dry fresh $CHCl_3$	10^4	110
H_2O+$CHCl_3$ (2 phase)	0	14
H_2O only	$10^{4.5}$	100

Interpretation

Essentially 100% of the vaccinia virus was killed in the two-phase system but 80% of the Factor VIII was destroyed. With 100% H_2O saturated $CHCl_3$ greater than 99% of the vaccinia was killed and there was complete retention of all Factor VIII activity compared to the control. However, when the content of the dissolved water was reduced to 75% or less of the saturation level, there was essentially no virus killing although the Factor VIII activity was maintained. It must be concluded that greater than 75% of the water saturation level of $CHCl_3$ must be achieved in order to effectively kill the virus in the dry state.

EXAMPLE 3

The purpose of this experiment was to test if increasing the H_2O content of the chloroform treating agent would improve virus killing. This was accomplished by saturating the chloroform with water at a higher temperature (37°) which increases the amount of water that can be dissolved in the chloroform before saturation is achieved. The treatment was also performed at 37°.

The second method used to increase the water content was to increase the ethanol content of the chloroform. Commercial chloroform contains about 0.5% (v/v) ethanol as a preservative. The water content of the chloroform can be increased by increasing the ethanol content of the chloroform. In this experiment the ethanol content was increased from 0.5% to 2% and 5% and then the chloroform/ethanol was saturated with water. The treatment protocol was similar to those in Examples 1 and 2 in which

weighed, lyophylized vaccinia powder and weighed, lyophylized Factor VIII concentrate were treated with the various chloroform preparations or water only as a control. Treatment was for 4 hours at the temperature stated in the protocol. Chloroform was removed by evaporation with a stream of pure, dry nitrogen gas or in the case of water/chloroform two-phase systems by contrifuging at 1000 RPM for 10 minutes and then pipetting off the aqueous phase. Dried powder was reconstituted with H_2O at 0.2 ml/mg of powdered vaccinia and 0.05 ml/mg powdered Factor VIII.

Protocol

			Post Treatment	
			Vaccinia Titer	Factor VIII Levels
Vial #	Treatment	Temp.	$TCID_{50}$	% of Control
1	Dry $CHCl_3$	20°C	10^5	75
2	Water saturated $CHCl_3$	20°C	0	94
3	Dry $CHCl_3$	37°C	$10^{4.75}$	119
4	H_2O saturated $CHCl_3$	37°C	0	75
5	Dry $CHCl_3$ - 2% ethanol	20°	10^5	100
6	H_2O saturated $CHCl_3$ - 2% ethanol	20°	0	100
7	Dry $CHCl_3$ - 5% ethanol	20°	$10^{4.75}$	119
8	H_2O saturated $CHCl_3$ - 5% ethanol	20%	0	96
9	$CHCl_3+H_2O$ 2-phase	20°	0	0.3
10	$CHCl_3+H_2O$ 2-phase	37°	0	0.3
11	H_2O only (control)	20°	$10^{5.5}$	100
12	H_2O only	37°	$10^{5.5}$	15

Interpretation

Chloroform saturated with water killed all of the $10^{5.5}$ infectious vaccinia viruses in the lyophylized dry powder. Chloroform without dissolved water at or near the saturation point killed less than 10% of the infectious vaccinia virus and was therefore poorly effective in killing the vaccinia virus. Increasing the temperature or ethanol content of the chloroform had little effect on virus killing if the chloroform or the chloroform alcohol were not also saturated with water. While water and chloroform together in a two-phase system was effective in virus killing, it also destroyed most of the Factor VIII activity. Factor VIII activity was maintained when the chloroform treatment was performed in a one-phase system whether or not the chloroform or chloroform/ethanol was saturated with water.

It is concluded from Examples 1, 2, and 3 that lipid containing viruses contained in a dry protein powder can be killed by the lipid solvent, chloroform, if the chloroform is saturated with water. The water saturation point of chloroform can be increased by increasing the temperature or by adding ethanol. However, virus killing is effective as long as the dissolved water content approaches the saturation point at the temperature or ethanol level used. It is further concluded that a blood product such as Factor VIII that is commonly prepared as a dry powder can be treated as a dry powder with water saturated chloroform without a significant loss of activity.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of inactivating essential lipid-containing viruses in a dried protein product comprising

contacting said viruses for a period of 10 minutes to 10 hours at a treatment temperature of 4°C to 40°C with a treating composition consisting essentially of

(a) a treating agent selected from one member of the group consisting of halogenated hydrocarbons and

(b) water dissolved in said treating agent wherein said water is 75 to 100% of the amount of water required to saturate the treating agent at the treatment temperature; and

removing the treating agent from the protein ~~product carrier~~.

2. The method of Claim 1 wherein the dry protein product is a plasma protein for therapeutic or diagnostic use.

3. The method of Claim 1 or Claim 2 wherein the treating agent is chloroform.

4. The method of any one of the preceding claims wherein the treating agent is removed from the protein product by evaporation.

5. The method of any one of the preceding claims wherein the water content of the treating agent is adjusted by controlling the temperature of treatment.

6. The method of Claim 1 or Claim 2 wherein the treating agent is a solution of chloroform and an alcohol formed by dissolving alcohol in the chloroform and then dissolving water in the chloroform-alcohol solution.

7. The method of Claim 1 or Claims 2 wherein the treating agent is chloroform containing ethanol and the dissolved water content of the chloroform is adjusted to the amount necessary for 100% saturation of the chloroform-ethanol solution at the treatment temperature.

8. A method for inactivating a lipid virus in a dry blood- or plasma-derived protein product comprising

adjusting the dissolved water content of chloroform in a treating composition consisting essentially of chloroform to about 100% of the amount required to saturate chloroform at a given treatment temperature;

contacting the virus for a period of 10 minutes to 10 hours with said water-saturated chloroform treating composition at a treatment temperature of from 4°C to 40°C; and

removing the chloroform from the product.

9. The method of Claim 8 wherein the said virus is selected from at least one member of the group consisting of Hepatitis B virus (HBV) and non-A and non-B hepatitis virus (NANBH).

10. The method of claim 8 wherein the said virus is selected from at least one member of the lipid-containing groups consisting of herpesviruses, the delta agent, togaviruses, bunyaviruses, retroviruses, orthomyxoviruses, paramyxoviruses, rhabdoviruses, poxviruses, hepadnaviruses, arenaviruses, and coronaviruses.

11. The method of Claim 8 wherein the said virus is selected from at least one member of the lipid-containing group consisting of retroviruses

12. The method of Claim 8 wherein the biological activity of the blood- or plasma-derived protein product is substantially retained by the treatment.

13. A novel product from the process of any one of claims 8 to 12 comprising a blood or plasma protein derivative rendered noninfectious for lipid viruses wherein the inactivation was made by treatment with chloroform saturated 100% with water.

DATED this 3rd day of August 1989

ROBERT H PURCELL and STEPHEN M FEINSTONE
By Their Patent Attorneys:

GRIFFITH HACK & CO
Fellows Institute of Patent
Attorneys of Australia

INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/00909

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. 424/101,89; 435/238; 514/2; 260/112B
INT.CL. A61K 35/14 ; C12N 7/06

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/101,89; 435/238; 514/2; 260/112B
I.P.C.	A61K 35/14, C12N 7/06

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X,P	US., A, 4,481,189, Published 06 November 1984, Prince	1-4,7,15 and 16
X	US., A, 4,405,603, Published 20 September 1983, Schwinn et al	15 & 16
X	US., A, 4,424,206, Published 03 January 1984, Ohmura et al	15 & 16
X	US., A, 4,446,134, Published 01 May 1984, Naito et al	15 & 16
X,P	US., A, 4,456,590, Published 26 June 1984, Rubinstein	15 & 16
X	US., A, 4,031,204, Published 21 June 1977, Davis, See column 4, lines 38-46.	1,2,5,7
T	US., A, 4,511,556, Published 16 April 1985, Purcell et al	

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
01 August 1985	14 AUG 1985
International Searching Authority [1]	**Signature of Authorized Officer [20]**
RO/US	Shawn P. Foley

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	US., A, 4,113,712, Published 12 September 1978, Funakoshi	
A	US., A, 4,302,444, Published 24 November 1981, Baxendale, See column 3, lines 48-53.	
X	US., A, 4,314,997, Published 09 February 1982, Shanbrom	15 & 16
A	US., A, 3,847,737, Published 12 November 1974, Kanarek	
X	N, Infection and Immunity, Vol. 41, No. 2, issued August, 1983, Feinstone et al.	1-5,7,15,16

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	N, Methods In Virology, Published 1967, Maramorosch et. al., eds., Academic Press, New York, Vol. 2. See chapter 7.	