PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A01N 1/02, A61K 41/00
A61M 37/00, A61N 1/30

A1

(11) International Publication Number: **WO 93/17553**

(43) International Publication Date: 16 September 1993 (16.09.93)

(21) International Application Number: PCT/US93/01786

(22) International Filing Date: 26 February 1993 (26.02.93)

(30) Priority data:
07/844,790 2 March 1992 (02.03.92) US

(71)(72) Applicants and Inventors: LIN, Lily [US/US]; 80 Hill Road, Berkeley, CA 94708 (US). CORASH, Laurence [US/US]; Vincente Drive, Berkeley, CA 94708 (US). ISAACS, Steven, T. [US/US]; 79 Loma Vista Drive, Orinda, CA 94563 (US). HANSON, Carl, Veith [US/US]; 1427 Edith Street, Berkeley, CA 94703 (US). CIMINO, George, D. [US/US]; 4839 Full Moon Drive, Richmond, CA 94803 (US).

(74) Agent: CARROLL, Peter, G.; Steritech, Inc., 2525 Stanwell Drive, Suite 300, Concord, CA 94520 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: DECONTAMINATING BLOOD COMPONENTS WITH 8-METHOXYPSORALEN

(57) Abstract

Methods and compositions for treating contaminants in material intended for *in vivo* use, and in particular blood and blood products for human use. Inactivation of contaminants in blood cell preparations prior to long term storage and transfusion.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**MR**	Mauritania
AU	Australia	**GA**	Gabon	**MW**	Malawi
BB	Barbados	**GB**	United Kingdom	**NL**	Netherlands
BE	Belgium	**GN**	Guinea	**NO**	Norway
BF	Burkina Faso	**GR**	Greece	**NZ**	New Zealand
BG	Bulgaria	**HU**	Hungary	**PL**	Poland
BJ	Benin	**IE**	Ireland	**PT**	Portugal
BR	Brazil	**IT**	Italy	**RO**	Romania
CA	Canada	**JP**	Japan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SK**	Slovak Republic
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SN**	Senegal
CM	Cameroon	**LI**	Liechtenstein	**SU**	Soviet Union
CS	Czechoslovakia	**LK**	Sri Lanka	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**MC**	Monaco	**UA**	Ukraine
DK	Denmark	**MG**	Madagascar	**US**	United States of America
ES	Spain	**ML**	Mali	**VN**	Viet Nam
FI	Finland	**MN**	Mongolia		

"DECONTAMINATING BLOOD COMPONENTS WITH 8-METHOXYPSORALEN"

FIELD OF THE INVENTION

The invention generally relates to the inactivation of contaminants in material intended for in vivo use, and in particular the inactivation of microorganisms in blood preparations prior to long term storage and transfusion.

BACKGROUND

Whole blood collected from volunteer donors for transfusion recipients is typically separated into its components: red blood cells, platelets, and plasma. Each of these fractions are individually stored and used to treat a multiplicity of specific conditions and disease states. For example, the red blood cell component is used to treat anemia; the concentrated platelet component is used to control bleeding; and the plasma component is used frequently as a source of Clotting Factor VIII for the treatment of hemophilia.

Ideally, all blood cell preparations should be from freshly drawn blood and then immediately transfused to the recipient. However, the logistics of operating a blood donor center preclude this possibility in the vast majority of cases. Transfusions are needed day and night and it is difficult, if not impossible, to arrange for donor recruiting at unusual hours. Consequently, modern blood donor centers must use stored blood products.

In the United States, blood storage procedures are subject to regulation by the government. The maximum storage periods for the blood components collected in these systems are specifically prescribed. For example, whole blood components collected in an "open"

(i.e. non-sterile) system must, under governmental rules, be transfused within twenty-four hours and in most cases within six to eight hours. By contrast, when whole blood components are collected in a "closed" (i.e. sterile) system the red blood cells can be stored up to forty-two days (depending upon the type of anticoagulant and storage medium used) and plasma may be frozen and stored for even longer periods.

Murphy and Gardner, New Eng.J.Med. 280:1094 (1969), demonstrated that platelets stored as platelet-rich plasma (PRP) at 22°C possessed a better in vivo half-life than those stored at 4°C. Thus, more acceptable platelet concentrates could be transfused after storage at room temperature. Until recently, the rules allowed for platelet concentrate storage at room temperature for up to seven days (depending upon the type of storage container). However, it was recognized that the incidence of bacterial growth and subsequent transfusion reactions in the recipient increased to unacceptable levels with a seven day old platelet concentrate. Platelet concentrates may now be stored for no more than five days.

Blood bags used for platelet concentrate preparation are in themselves sterile, as are the connected satellite bags. One might believe, therefore, that it is a relatively simple matter to keep the blood preparation sterile during the manipulations needed to concentrate the platelets. However, bacteria can be introduced by at least two different means. First, if the donor is experiencing a mild bacteremia, the blood will be contaminated, regardless of the collection or storage method. Adequate donor histories and physicals will decrease but not eliminate this problem. See B.J. Grossman et

al., Transfusion 31:500 (1991). A second, more pervasive source of contamination is the venepuncture. Even when "sterile" methods of skin preparation are employed, it is extremely difficult to sterilize the crypts around the sweat glands and hair follicles. During venepuncture, this contaminated skin is often cut out in a small "core" by a sharp needle. This core can serve to "seed" the blood bag with bacteria that may grow and become a risk to the recipient.

Indeed, many patients requiring platelet transfusions lack host-defense mechanisms for normal clearing and destruction of bacteria because of either chemotherapy or basic hematologic disease. The growth of even seemingly innocuous organisms in stored platelets can, upon transfusion, result in recipient reaction and death. See e.g. B.A. Myhre JAMA 244:1333 (1980). J.M. Heal et al. Transfusion 27:2 (1987).

The reports assessing the extent of contamination in platelets have differed in their methods, sample size, and bacterial detection schemes. D.H. Buchholz, et al., Transfusion 13:268 (1973) reported an overall level of platelet contamination of 2.4% when a large (>1000 bags) sample was examined and extensive measures were taken for bacterial culturing. While some units were heavily contaminated after just 24 hours of storage, the incidence as a whole varied according to the age of the concentrate and increased with the widespread practice of pooling individual units; over 30% of pools were contaminated at 3 days. See also D.H. Buccholz, et al., New Eng. J. Med. 285:429 (1971). While other clinicians suggest lower numbers, recent studies indicate that septic platelet transfusions are significantly underreported. See e.g. J. F. Morrow et al. JAMA 266:555 (1991).

Pre-culturing platelets is not a solution to the bacterial contamination problem. The culture assay takes 48 hours to detect growth. Holding platelet units for an additional two days to await the results of the assay would create, ironically, a smaller margin of safety. See Table 2 in J. F. Morrow et al. JAMA 266:555 (1991). While heavily contaminated units would be detected at the outset, lightly contaminated units would be allowed to grow for two days. Older and potentially more contaminated units would end up being transfused.

Washing the blood cells (e.g. with saline) or filtering the bacteria are also not practical solutions. These techniques are time consuming and inefficient, as they can reduce the number of viable blood cells available for transfusion. Most importantly, they typically involve an "entry" into the storage system. Once an entry is made in a previously closed system, the system is considered "opened," and transfusion must occur quickly, regardless of the manner in which the blood was collected and processed in the first place.

Finally, antibiotics are not a reasonable solution. Contamination occurs from a wide spectrum of organisms. Antibiotics would be needed to cover this spectrum. Many recipients are allergic to antibiotics. In addition, there is an every increasing array of drug-resistant strains of bacteria that would not be inactivated.

In sum, there is a need for a means of inactivating bacteria from blood components prior to storage and transfusion storage in a way that lends itself to use in a closed system. This approach must be able to handle a variety of organisms without harm

to the blood product or the transfusion recipient.

SUMMARY OF THE INVENTION

The present invention relates to methods for treating contaminants in material intended for in vivo use, and in particular blood and blood products for human use. In accordance with the present invention, a nucleic acid binding compound is selectively employed to treat contamination by microorganisms. In one embodiment, the present invention contemplates a method of inactivation microorganisms in blood preparations prior to long term storage and transfusion, comprising: a) providing, in any order, i) 8-methoxypsoralen; ii) means for activating 8-methoxypsoralen; iii) a blood preparation intended for in vivo use suspected of being contaminated with microorganisms; b) adding 8-methoxypsoralen to the blood preparation at a final concentration of approximately 30 ug/ml or less; and c) activating 8-methoxypsoralen, so that the nucleic acid binding compounds bind covalently to the nucleic acid of a portion of the microorganisms.

The method of the present invention is particularly useful where the microorganisms comprise single cell and multicellular organisms, such as bacteria, fungi, mycoplasma and protozoa. The present invention is employed with success with platelets and plasma.

In a preferred embodiment, the activating means comprises a photoactivation device capable of emitting a given intensity of a spectrum of electromagnetic radiation comprising wavelengths between 180 nm and 400 nm. Preferrably, the intensity is less than 20 mW/cm^2 and the blood preparation is exposed to this intensity for less than ten minutes. In one embodiment, the

blood preparation is exposed to this intensity for only approximately five minutes.

In another embodiment, the present invention contemplates a method of treating material intended for in vivo use, comprising: a) providing, in any order, i) one or more photoreactive nucleic acid binding compounds; ii) means for photoactivating the nucleic acid binding compounds; and iii) material intended for in vivo use suspected of being contaminated with microorganisms; b) adding the photoreactive nucleic acid binding compound to the material; and c) photoactivating the photoreactive nucleic acid binding compounds with a spectrum of electromagnetic radiation having a peak intensity at wavelengths between 330 nm and 350, so that the nucleic acid binding compounds bind covalently to the nucleic acid of a portion of the microorganisms. Preferrably, the photoactivation means comprises filters providing wavelength cutoffs at 320 nm, below which no irradiation is transmitted, and at 360 nm, above which no irradiation is transmitted. Preferrably, the intensity is less than 20 mW/cm^2.

This embodiment of the present invention is also employed with success with blood preparations, such as platelets and plasma. Where blood preparations are inactivated, it is preferred that exposure to this intensity is less than ten minutes.

It is not intended that the present invention be limited to particular nucleic acid binding compounds. In one embodiment, the present invention contemplates photoreactive nucleic acid binding compounds selected from the group comprising furocoumarins. In a preferred embodiment, the furocoumarin is a psoralen, such as 8-methoxypsoralen.

The present invention also contemplates

compositions having anti-microbial properties. In one embodiment, the composition comprises an aqueous solution of 8-methoxypsoralen at a concentration less than 30 ug/ml and material intended for human in vivo use. Preferrably, the concentration is approximately 3 ug/ml.

The present invention also contemplates using nucleic acid binding compounds in combination with other reagents (including specific storage media), therapeutic preparations, and pharmaceuticals.

DESCRIPTION OF THE FIGURES

Figure 1 is a perspective view of one embodiment of the device of the present invention.

Figure 2 is a cross-sectional view of the device shown in Figure 1 along the lines of 2--2.

Figure 3 is a cross-sectional view of the device shown in Figure 1 along the lines of 3--3.

Figure 4 is a cross-sectional view of the device shown in Figure 1 along the lines of 4--4.

Figure 5 schematically shows the decontamination approach of the present invention applied specifically to blood products.

Figure 6 is a graph showing the photoaddition of 8-methoxypsoralen to nucleic acid.

Figure 7 is a graph showing the degradation of 8-methoxypsoralen (8-MOP) compared to that of 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT), as measured by HPLC.

DESCRIPTION OF THE INVENTION

The present invention relates to methods for treating contaminants in material intended for in vivo use, and in particular blood and blood products. In one embodiment, the method of the present invention comprises the treatment of platelet concentrates for contamination by microorganisms.

As noted previously, whole blood is collected and typically separated into red blood cells, platelets, and plasma. Each of these fractions are individually stored under specific conditions prior to in vivo use. In many cases, the extent of contamination is related to the storage time because of growth. A process that inactivated microorganisms at the time of blood collection would be expected to prevent growth during storage.

In one embodiment, the present invention contemplates inactivating blood products after separation but before storage. In this embodiment, a nucleic acid binding compound is selectively employed to treat contamination by microorganisms.

In one embodiment, the nucleic acid binding compound is selected from the group comprising furocoumarins. In a preferred embodiment, the furocoumarin is a psoralen that is activated by a photoactivation device.

Psoralens are tricyclic compounds formed by the linear fusion of a furan ring with a coumarin. Psoralens can intercalate between the base pairs of double-stranded nucleic acids, forming covalent adducts to pyrimidine bases upon absorption of longwave ultraviolet light (UVA). G. D. Cimino et al., Ann. Rev. Biochem. 54:1151 (1985). Hearst et al., Quart. Rev. Biophys. 17:1 (1984). If there is a second

pyrimidine adjacent to a psoralen-pyrimidine monoadduct and on the opposite strand, absorption of a second photon can lead to formation of a diadduct which functions as an interstrand crosslink. S.T. Isaacs et al., Biochemistry 16:1058 (1977). S.T. Isaacs et al., Trends in Photobiology (Plenum) pp. 279-294 (1982). J. Tessman et al., Biochem. 24:1669 (1985). Hearst et al., U.S. Patents Nos. 4,124,589, 4,169,204, and 4,196,281, hereby incorporated by reference.

Psoralens have been shown to inactivate viruses in some blood products. See H.J. Alter et al., The Lancet (ii:1446) (1988). L. Lin et al., Blood 74:517 (1989). G.P. Wiesehahn et al., U.S. Patents Nos. 4,727,027 and 4,748,120, hereby incorporated by reference, describe the use of a combination of 8-methoxypsoralen (8-MOP) and irradiation. They show that 300 ug/ml of 8-MOP together with one hour or more of irradiation with ultraviolet light can effectively inactivate viruses. However, these treatment conditions cause harm to the blood product because of energy transfer. Their approach is only feasible if the damage to cells is specifically suppressed by limiting the concentration of molecular oxygen, a difficult and expensive process.

The inactivation method of the present invention provides a method of inactivating single cell and multicellular organisms, and in particular, bacteria, fungi, mycoplasma and protozoa. In contrast to previous approaches, the method of the present invention does not cause harm to the blood product. There is no significant damage to cells and, therefore, no need to limit the concentration of molecular oxygen.

The present invention contemplates using much lower concentrations of nucleic acid binding compounds than previously employed. For example, the present

invention contemplates using 8-MOP at concentrations of 30 ug/ml or less. Indeed, a preferred concentration of 8-MOP for bacterial decontamination in platelet concentrates is 3 ug/ml or less, i.e. a one hundred-fold lower concentration than employed by G.P. Wiesehahn et al. supra.

The present invention, furthermore, contemplates using much lower doses of irradiation than previously described. This is accomplished with lower intensity irradiation sources, with wavelength cutoff filters (see below), and/or shorter irradiation times. In a preferred embodiment, the time of irradiation is variable and controlled from 1 second to 99 minutes, in one second increments.

In one embodiment, the device of the present invention is mounted on an agitator, giving horizontal unidirectional and sinusoidal motion of variable frequency and amplitude. In another embodiment, heat from the lamps, ballasts and other sources is blocked from the bags.

While it is not intended that the present invention be limited by the theory of inactivation, the use of lower compound concentrations and irradiation doses comes from and understanding that, where the present invention is applied to the decontamination of a single cell or multicellular organism (as opposed to a virus), a lower level of nucleic acid binding will achieve inactivation. In addition, it is recognized that it is not essential that inactivation be complete. That is to say, partial inactivation will be adequate as long as the viable portion is unable, within the storage period, to grow to levels sufficient to cause disease.

To appreciate that, in any given case, an inactivation method may or may not achieve complete inactivation, it is useful to consider a specific example. A bacterial culture is said to be sterilized if an aliquot of the culture, when transferred to a fresh culture plate and permitted to grow, is undetectable after a certain time period. The time period and the growth conditions (e.g. temperature) define an "amplification factor". This amplification factor along with the limitations of the detection method (e.g. visual inspection of the culture plate for the appearance of a bacterial colony) define the sensitivity of the inactivation method. A minimal number of viable bacteria must be applied to the plate for a signal to be detectable. With the optimum detection method, this minimal number is 1 bacterial cell. With a suboptimal detection method, the minimal number of bacterial cells applied so that a signal is observed may be much greater than 1. The detection method determines a "threshold" below which the method appears to be completely effective (and above which the method is, in fact, only partially effective).

This interplay between the amplification factor of an assay and the threshold that the detection method defines, can be illustrated. For example, bacterial cells can be applied to a plate; the detection method is arbitarily chosen to be visual inspection. Assume the growth conditions and time are such that an overall amplification of 10^4 has occurred. The detectable signal will be proportional to the number of bacterial cells actually present after amplification. For calculation purposes, the detection threshold is taken to be 10^6 cells; if fewer than 10^6 cells are present after amplification, no cell colonies are visually

detectable and the inactivation method will appear effective. Given the amplification factor of 10^4 and a detection threshold of 10^6, the sensitivity limit would be 100 bacterial cells; if less than 100 viable bacterial cells were present in the original aliquot of the bacterial culture after the sterilization method is performed, the culture would still appear to be sterilized.

Such a situation is common for bacterial growth assays. The sensitivity of the assay is such that viable bacterial cells are present but the assay is unable to detect them. This may explain, at least in part, the variability in results obtained by researchers attempted to determine the extent of bacterial contamination of blood products. See D.H. Buchholz, et al., Transfusion 13:268 (1973), wherein such variability is discussed.

It should be noted that, in many countries, contamination of blood products by cellular organisms is more pervasive and, therefore, more serious than viral contamination. For example, in South America, the most important blood-borne organism is T. cruzi, which is the etiologic agent of Chagas disease. Approximately 16-18 million people are infected in the Americas (including 11% of the population of Chile). It is contemplated that the decontamination method of the present invention is well-suited for inactivation of this protozoa.

The present invention contemplates devices and methods for photoactivation and specifically, for activation of photoreactive nucleic acid binding compounds. The present invention contemplates devices having an inexpensive source of electromagnetic radiation that is integrated into a unit. In general,

the present invention contemplates a photoactivation device for treating photoreactive compounds, comprising: a) means for providing appropriate wavelengths of electromagnetic radiation to cause activation of at least one photoreactive compound; b) means for supporting a plurality of blood products in a fixed relationship with the radiation providing means during activation; and c) means for maintaining the temperature of the blood products within a desired temperature range during activation. The present invention also contemplates methods, comprising: a) supporting a plurality of blood product containers, containing one or more photoreactive compounds, in a fixed relationononship with a fluorescent source of electromagnetic radiation; b) irradiating the plurality of blood products simultaneously with said electromagnetic radiation to cause activation of at least one photoreactive compound; and c) maintaining the temperature of the blood products within a desired temperature range during activation.

The major features of one embodiment of the device of the present invention involve: A) an inexpensive source of ultraviolet radiation in a fixed relationship with the means for supporting the sample vessels, B) rapid photoactivation, C) large sample processing, D) temperature control of the irradiated samples, and E) inherent safety.

A. Electromagnetic Radiation Source

A preferred photoactivation device of the present invention has an inexpensive source of ultraviolet radiation in a fixed relationship with the means for supporting the sample vessels. Ultraviolet radiation is a form of energy that occupies a portion of the electromagnetic radiation spectrum (the electromagnetic

radiation spectrum ranges from cosmic rays to radio waves). Ultraviolet radiation can come from many natural and artificial sources. Depending on the source of ultraviolet radiation, it may be accompanied by other (non-ultraviolet) types of electromagnetic radiation (e.g. visible light).

Particular types of ultraviolet radiation are herein described in terms of wavelength. Wavelength is herein described in terms of nanometers ("nm"; 10^{-9} meters). For purposes herein, ultraviolet radiation extends from approximately 180 nm to 400 nm. When a radiation source, by virtue of filters or other means, does not allow radiation below a particular wavelength (e.g. 320 nm), it is said to have a low end "cutoff" at that wavelength (e.g. "a wavelength cutoff at 300 nanometers"). Similarly, when a radiation source allows only radiation below a particular wavelength (e.g. 360 nm), it is said to have a high end "cutoff" at that wavelength (e.g. "a wavelength cutoff at 360 nanometers").

For any photochemical reaction it is desired to eliminate or least minimize any deleterious side reactions. Some of these side reactions can be caused by the excitation of endogenous chromophores that may be present during the photochemical activation procedure. In a system where only nucleic acid and psoralen are present, the endogenous chromophores are the nucleic acid bases themselves. Restricting the activation process to wavelengths greater than 320 nm minimizes direct nucleic acid damage since there is very little absorption by nucleic acids above 313 nm.

In blood products, the nucleic acid is typically present together with additional biological chromophores. If the biological fluid is just protein,

the 320 nm cutoff will be adequate for minimizing side reactions (aromatic amino acids do not absorb above 320 nm). If the biological fluid includes cells and/or cellular constituents, there will be many other chromophores, including hemes and flavins.

Hemes are abundant in blood products where they arise from the lysis of red cells. Flavins, like hemes, are required for metabolic respiration. Both of these endogenous chromophores will cause damage to cells if excited by photoirradiation.

Hemes have three principle absorption bands: two are in the red region of the visible spectrum; the other is centered about 400 nm. Flavins have two principle absorption peaks: one at 450 nm and the other at 370 nm.

In view of the presence of these endogenous chromophores in blood products, it is intended that the device of the present invention be designed to allow for irradiation within a small range of specific and desirable wavelengths, and thus avoid damage to cells caused by energy transfer. The preferred range of desirable wavelengths is between 320 and 350 nm.

Some selectivity can be achieved by choice of commercial irradiation sources. For example, while typical fluorescent tubes emit wavelengths ranging from 300 nm to above 400 nm (with a broad peak centered around 360 nm), BLB type fluorescent lamps are designed to remove wavelengths above 400 nm. This, however, only provides an upper end cutoff.

In a preferred embodiment, the device of the present invention comprises an additional filtering means. In one embodiment, the filtering means comprises a glass cut-off filter, such as a piece of Cobalt glass. In another embodiment, the filtering

means comprises a liquid filter solution that transmit only a specific region of the electromagnetic spectrum, such as an aqueous solution of $Co(No_3)_2$. This salt solution yields a transmission window of 320-400 nm. In a preferred embodiment, the aqueous solution of $Co(No_3)_2$ is used in combination with $NiSO_4$ to remove the 365 nm component of the emmision spectrum of the fluorescent or arc source employed. The Co-Ni solution preserves its initial transmission remarkably well even after tens of hours of exposure to the direct light of high energy sources.

It is not intended that the present invention be limited by the particular filter employed. Several inorganic salts and glasses satisfy the necessary requirements. For example. cupric sulfate is a most useful general filter for removing the infra-red, when only the ultraviolet is to be isolated. Its stability in intense sources is quite good. Other salts are known to one skilled in the art. Aperture or reflector lamps may also be used to achieve specific wavelengths and intensities.

When ultraviolet radiation is herein described in terms of irradiance, it is expressed in terms of intensity flux (milliwatts per square centimeter or "mW cm^{2}"). "Output" is herein defined to encompass both the emission of radiation (yes or no; on or off) as well as the level of irradiance. In a preferred embodiment, intensity is monitored at 4 locations: 2 for each side of the plane of irradiation.

A preferred source of ultraviolet radiation is a fluorescent source. Fluorescence is a special case of luminescence. Luminescence involves the absorption of electromagnetic radiation by a substance and the conversion of the energy into radiation of a different

wavelength. With fluorescence, the substance that is excited by the electromagnetic radiation returns to its ground state by emitting a quantum of electromagnetic radiation. While fluorescent sources have heretofore been thought to be of too low intensity to be useful for photoactivation, in one embodiment the present invention employs fluorescent sources to achieve results thus far achievable on only expensive equipment.

As used here, fixed relationship is defined as comprising a fixed distance and geometry between the sample and the light source during the sample irradiation. Distance relates to the distance between the source and the sample as it is supported. It is known that light intensity from a point source is inversely related to the square of the distance from the point source. Thus, small changes in the distance from the source can have a drastic impact on intensity. Since changes in intensity can impact photoactivation results, changes in distance are avoided in the devices of the present invention. This provides reproducibility and repeatability.

Geometry relates to the positioning of the light source. For example, it can be imagined that light sources could be placed around the sample holder in many ways (on the sides, on the bottom, in a circle, etc.). The geometry used in a preferred embodiment of the present invention allows for uniform light exposure of appropriate intensity for rapid photoactivation. The geometry of a preferred device of the present invention involves multiple sources of linear lamps as opposed to single point sources. In addition, there are several reflective surfaces and several absorptive surfaces. Because of this complicated geometry,

changes in the location or number of the lamps relative to the position of the samples to be irradiated are to be avoided in that such changes will result in intensity changes.

B. Rapid Photoactivation

The light source of the preferred embodiment of the present invention allows for rapid photoactivation. The intensity characteristics of the irradiation device have been selected to be convenient with the anticipation that many sets of multiple samples may need to be processed. With this anticipation, a fifteen minute exposure time or less is a practical goal.

In designing the devices of the present invention, relative position of the elements of the preferred device have been optimized to allow for fifteen minutes of irradiation time, so that, when measured for the wavelengths between 320 and 350 nanometers, an intensity flux greater than approxiamtely 1 mW cm^2 is provided to the sample vessels. In a preferred embodiment, the device irradiates both sides of the bag.

C. Processing of Large Numbers of Samples

As noted, another important feature of the photoactivation devices of the present invention is that they provide for the processing of large numbers of samples. In this regard, one element of the devices of the present invention is a means for supporting a plurality of blood products, and in particular, blood bags. In the preferred embodiment of the present invention the supporting means comprises glass plates between two banks of lights with a capacity of six 50 ml bags (equivalent to Dupont Stericell bag) plus connectors and tubing, at one time. By accepting

commonly used commerically available blood bags, the device of the present invention allows for convenient processing of large numbers of samples.

D. Temperature Control

As noted, one of the important features of the photoactivation devices of the present invention is temperature control. Temperature control is important because the temperature of the sample in the sample at the time of exposure to light can dramatically impact the results. For example, conditions that promote secondary structure in nucleic acids also enhance the affinity constants of many psoralen derivatives for nucleic acids. Hyde and Hearst, Biochemistry, 17, 1251 (1978). These conditions are a mix of both solvent composition and temperature. With single stranded 5S ribosomal RNA, irradiation at low temperatures enhances the covalent addition of HMT to 5S rRNA by two fold at 4°C compared to 20°C. Thompson et al., J. Mol. Biol. 147:417 (1981). Even further temperature induced enhancements of psoralen binding have been reported with synthetic polynucleotides. Thompson et al., Biochemistry 21:1363 (1982).

With respect to bacteria, it sould be noted that repair of crosslinks occurs during irradiation. However, where a lower temperature is employed during irradiation, the bacterial repair process is supressed. Thus, a 15°C irradiation has a significant effect on the level of inactivation that is observed.

E. Inherent Safety

Ultraviolet radiation can cause severe burns. Depending on the nature of the exposure, it may also be carcinogenic. The light source of a preferred embodiment of the present invention is shielded from the user. This is in contrast to the commercial hand-

held ultraviolet sources as well as the large, high intensity sources. In a preferred embodiment, the irradiation source is contained within a housing made of material that obstructs the transmission of radiant energy (i.e. an opaque housing). No irradiation is allowed to pass to the user. This allows for inherent safety for the user.

EXPERIMENTAL

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); gm (grams); mg (milligrams); μg (micrograms); L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); °C (degrees Centigrade); HPLC (High Pressure Liquid Chromatography).

EXAMPLE 1

As noted above, the present invention contemplates devices and methods for the activation of photoreactive nucleic acid binding compounds. In this example, a photoactivation device is described for decontaminating blood products according to the method of the present invention. This device comprises: a) means for providing appropriate wavelengths of electromagnetic radiation to cause activation of at least one photoreactive compound; b) means for supporting a plurality of blood products in a fixed relationship

with the radiation providing means during activation; and c) means for maintaining the temperature of the blood products within a desired temperature range during activation.

Figure 1 is a perspective view of one embodiment of the device integrating the above-named features. The figure shows an opaque housing (100) with a portion of it removed, containing an array of bulbs (101) above and below a plurality of representative blood product containing means (102) placed between plate assemblies (103, 104). The plate assemblies (103, 104) are described more fully, subsequently.

The bulbs (101), which are connectable to a power source (not shown), serve as a source of electromagnetic radiation. While not limited to the particular bulb type, the embodiment is configured to accept an industry standard, dual bipin lamp.

The housing (101) can be opened via a latch (105) so that the blood product can be placed appropriately. As shown in Figure 1, the housing (100), when closed, completely contains the irradiation from the bulbs (101). During irradiation, the user can confirm that the device is operating by looking through a safety viewport (106) which does not allow transmission of ultraviolet light to the user.

The housing (100) also serves as a mount for several electronic components on a control board (107), including, by way of example, a main power switch, a count down timer, and an hour meter. For convenience, the power switch can be wired to the count down timer which in turn is wired in parallel to an hour meter and to the source of the electromagnetic radiation. The count down timer permits a user to prest the irradiation time to a desired level of exposure. The

hour meter maintains a record of the total number of radiation hours that are provided by the source of electromagnetic radiation. This feature permits the bulbs (101) to be monitored and changed before their output diminishes below a minimum level necessary for rapid photoactivation.

Figure 2 is a cross-sectional view of the device shown in Figure 1 along the lines of 2--2. Figure 2 shows the arrangment of the bulbs (101) with the housing (100) opened. A reflector (108A, 108B) completely surrounds each array of bulbs (101). Blood product containing means (102) are placed between upper (103) and lower (104) plate assemblies. Each plate assembly is comprised of an upper (103A, 104A) and lower (103B, 104B) plates. The plate assemblies (103, 104) are connected via a hinge (109) which is designed to accomodate the space created by the blood product containing means (102). The upper plate assembly (103) is brought to rest gently on top of the blood product containing means (102) supported by the lower plate (104B) of the lower plate assembly (104).

Detectors (110A, 110B, 110C, 110D) may be conviently placed between the plates (103A, 103B, 104A, 104B) of the plate assemblies (103, 104). They can be wired to a printed circuit board (111) which in turn is wired to the control board (107).

Figure 3 is a cross-sectional view of the device shown in Figure 1 along the lines of 3--3. Six blood product containing means (102) (e.g. teflon platelet unit bags) are placed in a fix relationship above an array of bulbs (101). The temperature of the blood product can be controlled via a fan (112) alone or, more preferrably, by employing a heat exchanger (113) having cooling inlet (114) and outlet (115) ports

connected to a cooling source (not shown).

Figure 4 is a cross-sectional view of the device shown in Figure 1 along the lines of 4--4. Figure 4 more clearly shows the temperature control approach of a preferred embodiment of the device. Upper plate assembly plates (103A, 103B) and lower plate asembly plates (104A, 104B) each create a temperature control chamber (103C, 104C), respectively. The fan (112) can circulate air within and between the chambers (103C, 104C). When the heat exchanger (113) is employed, the circulating air is cooled and passed between the plates (103A, 103B, 104A, 104B).

EXAMPLE 2

Figure 5 shows an embodiment wherein platelets are treated by the method of the present invention. Following fractionation, platelets are transferred to a bag containing a nucleic acid binding compound (shown in Figure 1 as a shaded bag). This bag, which has transmission properties and other characteristics suited for the present invention, is then placed in an irradiation device (such as that described in Example 1, above) and is irradiated. The free compound may be collected or "captured" as desired by a capture device. In such a case, the bag would contain only compound that is contained in cells; the bag would have no free compound (this bag is indicated in Figure 1 as unshaded).

EXAMPLE 3

In this example, the decontamination methods of the present invention are applied to inactivate Yersinia enterocolitica, wild type, serotype 3, biotype 4. This organism is found in blood products.

See generally R.Y. Dodd, In: Transfusion Medicine in the 1990's (American Assoc. Blood Banks 1990) (S.J. Nance, ed.). See also B.J. Grossman et al., Transfusion 31:500 (1991).

An overnight culture of the organism was made by inoculating 10 ml of brain-heart infusion (BHI) broth from a motility stab. This was maintained at 35°C and 0.1 ml of it was used to inoculate 20 ml of BHI broth for use in the experiment. After overnight incubation at 35°C, the stationary culture was pelleted for 15 minutes at 1900 g, the supernatant was discarded, and the bacterial pellet was resuspended in 1 ml of heat-inactivated normal serum pool. This was infused into a freshly expired unit of human platelets obtained from the Blood Bank of Alameda-Contra Costa Medical Association. 5 ml aliquots of bacteria containing platelet concentrate were drawn from the bag and received specified amounts of 8-MOP and UVA irradiation, except for the controls, which were irradiated without psoralen, or received no treatment (see Table 1). Temperature was maintained at 25°C during irradiation by placing the platelet concentrate in stoppered glass water-jacketed chambers attached to a circulating water bath. The irradiation device (Derma Control, Dolton, Ill.; Model No. 1224-Special) employed two arrays (six lamps/array spaced at 2.5 inches), one array above the sample and one bank below the sample (the sample is thus approximately 3 inches from the lamps). Each array is separated from the other by approximately six inches, has a polished metal reflector behind it, and is covered by a UVA-transmitting acrylic plastic sheet. The sample to be processed (e.g. platelet bag) sits on the lower sheet.

TABLE 1

	drug	8-MOP/ml	irr. time (min)	log/ml	-titer
1	no drug		0	9.1	
2	no drug		10	9.3	0.2
3	8-MOP	30 ug	10	<0	>-9.1
4	8-MOP	10	10	<0	>-9.1
5	8-MOP	3	10	3.4	-5.7
6	8-MOP	.2	10	6.8	-2.3
7	8-MOP	.06	10	9.0	-0.1

Derma Control F587T12-BL-HO type bulbs were used. These are "black light" tubes (engineered to emit specific wavelengths by means of an internal phosphor coating) 24 inches in length. The peak wavelength is below 360 nm, unlike simple mercury lamps or common "BLB" fluorescent bulbs. Total intensity is less than 20 mW/cm^2.

Bacteria was quantified by plating 0.1 ml of serial 10-fold dilutions in BHI broth onto 100 mm petri dishes containing BHI agar. After 24 hr incubation at 35°C, colonies were counted and bacterial concentration was calculated on a per ml basis. The results (Table 1) show that as little as 3 ug/ml of 8-MOP is able to inactivate almost six logs of bacteria. With 10 ug/ml, ten minutes provides more than enough irradiation. Indeed, with 10 ug/ml, five minutes of irradiation should be adequate.

EXAMPLE 4

Artuc and co-workers examined the solubility of 8-MOP in human and bovine serum proteins, and showed that at 8-MOP concentrations ranging form 100 to 1000 ng/ml. concentrations similar to those observed in patients undergoing psoralen ultraviolet A (PUVA) therapy for psoriasis, 75% to 80% of the 8-MOP was bound to albumin. M. Artuc et al., Brit. J. Derm. 101:669 (1979).

In this example, the binding of 8-MOP to Calf Thymus DNA is compared using plasma and a protein free media in order to validate the efficiency of psoralen-nucleic interactions under the decontamination methods of the present invention. Although this measurement used eukaryotic nucleic acid rather than bacterial nucleic acid, it is a useful indicator of the degree of adduct formation for bacteria.

^{3}H-8-MOP was prepared to a concentration of 115 ug/ml in ethanol at a specific activity of 4.7 x 10^{6} CPM/microgram (hereinafter "8-MOP stock"). Thereafter 130.5 or 22 ul of 8-MOP stock (2 each) for samples containing DNA ("+ DNA") and 52.2 or 8.7 ul for samples not containing DNA ("- DNA") were dried down. To + DNA samples, 40 ul of DNA stock (7.7 mg/ml) was added as well as either 460 ul plasma (day old frozen) or 450 ul Tris-EDTA ("TE") buffer. To the latter was also added 10 ul 5M NaCl. For - DNA samples (i.e. the controls), 184 ul plasma and 16 ul water was added.

The samples were mildly vortexed for approximately one hour and the counts were checked to confirm that the 8-MOP dissolved.

Each sample (100 ul) was irradiated on an HRI-100 (HRI Research Inc., Concord, CA) at 25°C for 0, 2, 4, 8, and 16 minutes. Samples were kept at 4°C overnight

after irradiation. Thereafter, the samples were extracted. First, a phenol solution was prepared at pH 8 by equilibrating with 0.1 M Tris pH 8. Each sample was then extracted with 100 ul phenol. Each sample was cetrifuged for 5 minutes to remove the aqueous phase to a new tube. A second extraction was performed with 100 ul 1:1 phenol:chloroform. A final extraction was performed with 100 ul chloroform.

The final aqueous phase was precipitated by adding 50 ul NaCl adjusted to give a final concentration of NaCl of 0.2 M and then adding 250 ul ethanol. The samples were again centrifuged (10 minutes). The supernatant was removed and the pellets were dried. The pellets were resuspended in 100 ul TE and reprecipitated. This was repeated for a total of 3 precipitations. The final pellets were brought up in 600 ul water and 100 ul was counted. Each sample was assayed for DNA by measuring absorbance (260 nm). 8-MOP levels were plotted as adducts per 1000 base pairs ("8-MOP:kBP"). The results (Figure 6) show that plasma does not significantly change the addition kinetics of 8-MOP to DNA.

The frequency of 8-MOP-DNA adduct formation predicts a high multiplicity of modification of the bacterial genome. Furthermore, this type of biochemical measurement has the potential to provide a means to monitor the efficiency of the photochemical inactivation method.

EXAMPLE 5

Photoactivation of psoralens and isopsoralens may result in a variety of photoproducts. "Photoproduct" is best understood by considering the possible reactions of photoreactive compound when exposed to activating wavelengths of electromagnetic radiation. While not limited to any precise mechanism, it is believed that the reaction of photoreactive compound in its ground state ("C") with activating wavelengths of electromagnetic radiation creates a short-lived excited species ("C*"):

$$C \rightarrow C^*$$

What happens next is largely a function of what potential reactants are available to the excited species. Since it is short-lived, a reaction of this species with nucleic acid ("NA") is believed to only be possible if nucleic acid is present at the time the excited species is generated. Thus, the reaction must, in operational terms, be in the presence of activating wavelengths of electromagnetic radiation, i.e. it is "photobinding"; it is not dark binding. The reaction can be depicted as follows:

$$C^* + NA \rightarrow NA{:}C$$

The product of this reaction is hereinafter referred to as "Photoaddition Product" and is to be distinguished from "Photoproduct."

With this reaction described, one can now consider the situation where nucleic acid is not available for binding at the time the compound is exposed to activating wavelengths of electromagnetic radiation. Since the excited species is short-lived and has no nucleic acid to react with, the excited species may

simply return to its ground state:

$$C^* \rightarrow C$$

On the other hand, the excited species may react with itself (i.e. a ground state or excited species) to create a ground state complex ("C:C"). The product of these self-reactions where two compounds react is referred to as "photodimer" or simply "dimer." The self-reactions, however, are not limited to two compounds; a variety of multimers may be formed (trimers, etc.).

The excited species is not limited to reacting with itself. It may react with its environment, such as elements of the solvent ("E") (e.g. ions, gases, etc.) to produce other products:

$$C^* + E \rightarrow E:C$$

It is this type of reaction that is believed to cause cellular damage (e.g., reaction with oxygen to create singlet oxygen species). Furthermore, it may simply internally rearrange ("isomerize") to a ground state derivative ("["):

$$C^* \rightarrow [$$

Finally, the excited species may undergo other reactions than described here.

The present invention and the understanding of "photoproduct" does not depend on which one (if any) of these reactions actually occurs. "Photoproduct" - whatever its nature - is deemed to exist if, following the reaction of a compound and activating wavelengths of electromagnetic radiation, there is a resultant product formed that can interact with other components of the reaction environment.

With psoralens such as 4'-hydroxymethyl-4,5',8-trimethylpsoralen (HMT), there are a number of resultant products produced when the HMT is exposed to

activating wavelngths of electromagnetic radiation. The major resultant products of HMT are two cyclobutyl photodimers. In one of the dimers, the two pyrone rings are linked in a cis-syn configuration, while in the other dimer, the linkage occurs between the furan end of one molecule and the pyrone end of the other, again with cis-syn configuration. A third resultant product of HMT is a monomeric HMT photoisomer. In this isomer, the central ring oxygens assume a 1, 4 instead of the normal 1, 3 orientation. While the two photodimers would not be expected to have an intercalating activity due to geometrical considerations, the photoisomer remains planer, and accordingly, it is contemplated that it has a positive intercalative association with double stranded nucleic acid and, thus, could be a mutagen.

In this example, the photochemical breakdown of 8-MOP is compared with AMT. The samples were analyzed by reverse phase HPLC using a Rainen Dynamax 300A column. Gradient elution was performed with 0.1 M ammonium acetate / acetonitrile (0 - 70% acetonitrile over 42 minutes). AMT elutes as a single peak at approximately 24 minutes under these conditions. Detection was by absorption at either 260 or 330 nm. The latter wavelength was used for the plasma containing samples.

Standard solutions of each compound were prepared at various concentrations. These solutions were then diluted 1:10 into water, then 300 ul injected for analysis. All samples were monitored at 300 nm. Peaks were analyzed by measuring either peak height or peak area, then converted to a gh/ml value using the standard plot. Peak area was determining by photocopying the trace, cutting out the copy of the peak, then weighing the resultant trace. The two

methods gave essentially the same result.

The results are shown in Figure 7. Clearly, AMT degrades more quickly than 8-MOP. It would, therefore, be expected to generate more photoproducts - which eventually would end up in the transfusion recipient. By contrast, it is not expected that 8-MOP generates a significant amount of photoproducts. This is important when one considers that the weight of authority has concluded that unactivated 8-MOP is nonmutagenic.

EXAMPLE 6

When platelets become activated, an alpha granule membrane glycoprotein called GMP140 becomes exposed on the platelet surface. Less than (5%) of fresh, normal unstimulated platelets express detectable GMP 140 levels by flow cytometry. See generally M.J. Metzelaar, Studies on the Expression of Activation-Markers on Human Platelets (Thesis 1991).

To measure GMP140, a small aliquot of platelet rich plasma is placed in HEPES buffer containing a GMP140-binding antibody or control mouse IgG. CD62 is a commerically available monoclonal antibody which binds to GMP 140 (available from Sanbio, Uden, the Netherlands; Caltag Labs, So. San Francisco, CA, and Becton Dickinson, Mountian View, CA). After a fifteen minute incubation, Goat Anti-Mouse IgG conjugated to FITC is added to the tube in saturating amounts. Finally, the cells are diluted in isotonic saline, fixed with paraformaldehyde and analyzed on a FACSCAN (Becton Dickinson, Mountian View, CA). The positive control is made by adding Phorbol Myristate Acetate (PMA) to the test system at a final concentration of 10^{-7} M.

In this example, CD62 was employed to measure the

impact, if any, of irradiation alone on platelet activation. The antibody was stored in small aliquots (0.01 mg/ml) at -40°C prior to use. A mouse IgG control (0.05 mg/ml) (Becton Dickinson, Mountain View, CA #9040) 5X concentrated was employed. At time of use, this was diluted 1:5 in HEPES buffer. The secondary antibody was goat Anti-Mouse IgG conjugated to FITC (TAGO, Burlingame, CA #3506). This was stored in small aliquots at -20°C. Phorbol Myristate Acetate (PMA) (Sigma, St. Louis, MO) was stored at -40°C. At time of use, this was dissolved in DMSO (working concentration was 1.62 X 10^{-5} M).

16% Paraformaldehyde (PFA) (Sigma, St. Louis, MO) was prepared by adding 16 grams paraformaldehyde to 100 ml deionized water. This was heated to 70°C, whereupon 3 M NaOH was added dropwise until the solution was clear. The solution was cooled and the pH was adjusted to 7.4 with 1 N HCl. This was filtered and stored. A commercially available isotonic buffer was used: Hematall Isotonic Diluent (Fisher # CS 606-20).

For measuring platelet activation of platelet contentrates, a unit of human platelets was obtained from the Blood Bank of Alameda-Contra Costa Medical Association. 5 ml aliquots were drawn from the bag and received specified amounts of UVA irradiation, except for the control, which received no treatment other than being placed in a chamber for irradiation. Temperature was maintained at 25°C during irradiation by placing platelet concentrate in stoppered glass water-jacketed chambers attached to a circulating water bath. The irradiation device (Derma Control, Dolton, Ill.; Model No. 1224-Special) was as described in Example 3, above. Following irradiation, the platelets were stored for 5 days. At specific time points, aliquots were taken and

processed.

Processing involved adding an aliquot (e.g. 5 microliters) of platelet concentrate to each microcentrifuge tube containing the antibody and appropriate reagents and this was mixed very gently by vortex. The samples were incubated for 15 minutes at room temperature.

The Goat anti-Mouse IgG-FITC (diluted 1:10 in HEPES buffer) was added (5 microliters) to each tube and the solution was mixed by gentle vortex. The samples were incubated for an additional 15 minutes at room temperature.

Isoton II was added (1 ml) to each tube and mixed gently with a polypropylene disposable pipet. 8% PFA in HEPES (150 microliters) was added to each diluted sample to final 1%. The platelets were analyzed on the FACSCAN. The results are shown in Table 2.

TABLE 2

	Day 3		Day 5	
Conditions	UNACTIVATED	PMA ACTIVATED	UNACTIVATED	PMA ACTIVATED
Control	17	85	25	89
UV 5'	17	87	24	86
UV 10'	51	84	77	79

Activation is expressed as a percent. Clearly, irradiation for ten minutes (UV 10') resulted in a significant negative impact on stored platelets; the platelets were highly activated. By contrast, irradiation for five minutes (UV 5') resulted in no

significant activation above the control which received no irradiation.

EXAMPLE 7

Given the results of Example 6, it is clear that either a shorter irradiation time or the use of filters is needed to avoid damage to cells by UV irradiation. In this example, CD62 is employed to measure the impact of irradiation in the presence of psoralen on platelet activation. Shorter irradiation times and wavelength filters are separately employed.

Shorter Irradiation Times. A unit of human platelets is again obtained from the Blood Bank of Alameda-Contra Costa Medical Association. 5 ml aliquots are drawn from the bag to receive five minutes (5') of UVA irradiation in the presence of 10 ug/ml 8-MOP, except for the control, which receives no treatment other than being placed in a chamber for irradiation. Temperature is maintained at 25°C during irradiation by placing platelet concentrate in stoppered glass water-jacketed chambers attached to a circulating water bath. The irradiation device (Derma Control, Dolton, Ill.; Model No. 1224-Special) is as described in Example 3, above.

Following irradiation, the platelets are again stored for 5 days as in Example 6. At specific time points, aliquots are taken and assayed with the CD62 antibody and analyzed on the FACSCAN to show that, under these conditions, platelets can be inactivated without damage to the cells and stored for five days prior to transfusion.

Wavelength Filters. An aqueous solution of $Co(No_3)_2$ is used in combination with $NiSO_4$ to substantially remove the 365 nm component of the emmision spectrum of the light source employed. The Co-Ni solution can be

conveniently used in place of water as a coolant during the irradiation.

Following a ten minute irradiation with the filter, the platelets are stored an assayed with the CD62 antibody on the FACSCAN to show that, under these conditions, platelets can be inactivated without damage to the cells and stored for five days prior to transfusion.

CLAIMS

1. A method of inactivation microorganisms in blood preparations prior to long term storage and transfusion, comprising:

a) providing, in any order, i) 8-methoxypsoralen; ii) means for activating said 8-methoxypsoralen; iii) a blood preparation intended for in vivo use suspected of being contaminated with microorganisms;

b) adding said 8-methoxypsoralen to said blood preparation at a final concentration of approximately 30 ug/ml or less; and

c) activating said 8-methoxypsoralen, so that said nucleic acid binding compounds bind covalently to the nucleic acid of a portion of said microorganisms.

2. The method of Claim 1, wherein said microorganisms comprise single cell and multicellular organisms.

3. The method of Claim 2, wherein said organisms comprise bacteria, fungi, mycoplasma and protozoa.

4. The method of Claim 1, wherein said blood preparation comprises platelets.

5. The method of Claim 1, wherein said blood preparation comprises plasma.

6. The method of Claim 1, wherein said activating means comprises a photoactivation device capable of emitting a given intensity of a spectrum of

electromagnetic radiation comprising wavelengths between 180 nm and 400 nm.

7. The method of Claim 6, wherein said intensity is less than 20 mW/cm^2.

8. The method of Claim 7, wherein said blood preparation is exposed to said intensity for less than ten minutes.

9. The method of Claim 8, wherein said blood preparation is exposed to said intensity for approximately five minutes.

10. A method of treating material intended for in vivo use, comprising:

a) providing, in any order, i) one or more photoreactive nucleic acid binding compounds; ii) means for photoactivating said nucleic acid binding compounds; iii) material intended for in vivo use suspected of being contaminated with microorganisms;

b) adding said photoreactive nucleic acid binding compound to said material; and

c) photoactivating said photoreactive nucleic acid binding compounds with a spectrum of electromagnetic radiation having a peak intensity at wavelengths between 330 nm and 350, so that said nucleic acid binding compounds bind covalently to the nucleic acid of a portion of said microorganisms.

11. The method of Claim 10, wherein said photoactivation means comprises filters providing wavelength cutoffs at 320 nm, below which no irradiation is transmitted, and at 360 nm, above which no irradiation is transmitted.

12. The method of Claim 10, wherein said intensity is less than 20 mW/cm^2.

13. The method of Claim 10, wherein said material comprises a blood preparation

14. The method of Claim 8, wherein said blood preparation is exposed to said intensity for 10 minutes.

15. The method of Claim 10, wherein said photoreactive nucleic acid binding compounds are selected from the group comprising furocoumarins.

16. The method of Claim 15, wherein said furocoumarin is selected from the group comprising psoralens.

17. The method of Claim 16, wherein said psoralen is 8-methoxypsoralen.

18. A composition having anti-microbial properties, comprising an aqueous solution of 8-methoxypsoralen at a concentration less than 30 ug/ml and material intended for in vivo use.

19. The composition of Claim 18, wherein said material is intended for human use.

20. The composition of Claim 18, wherein said concentration is approximately 3 ug/ml.

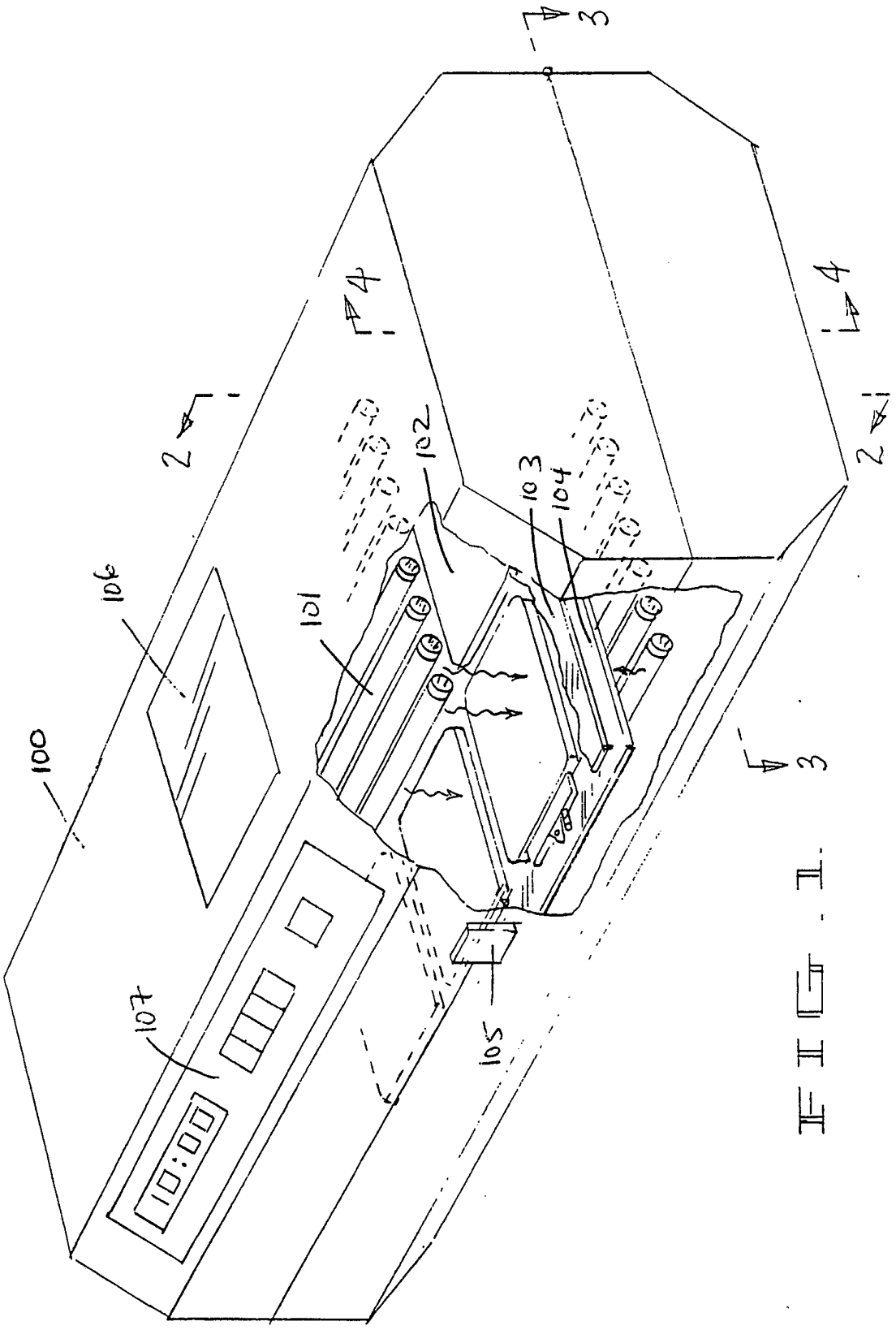


FIG. 1.

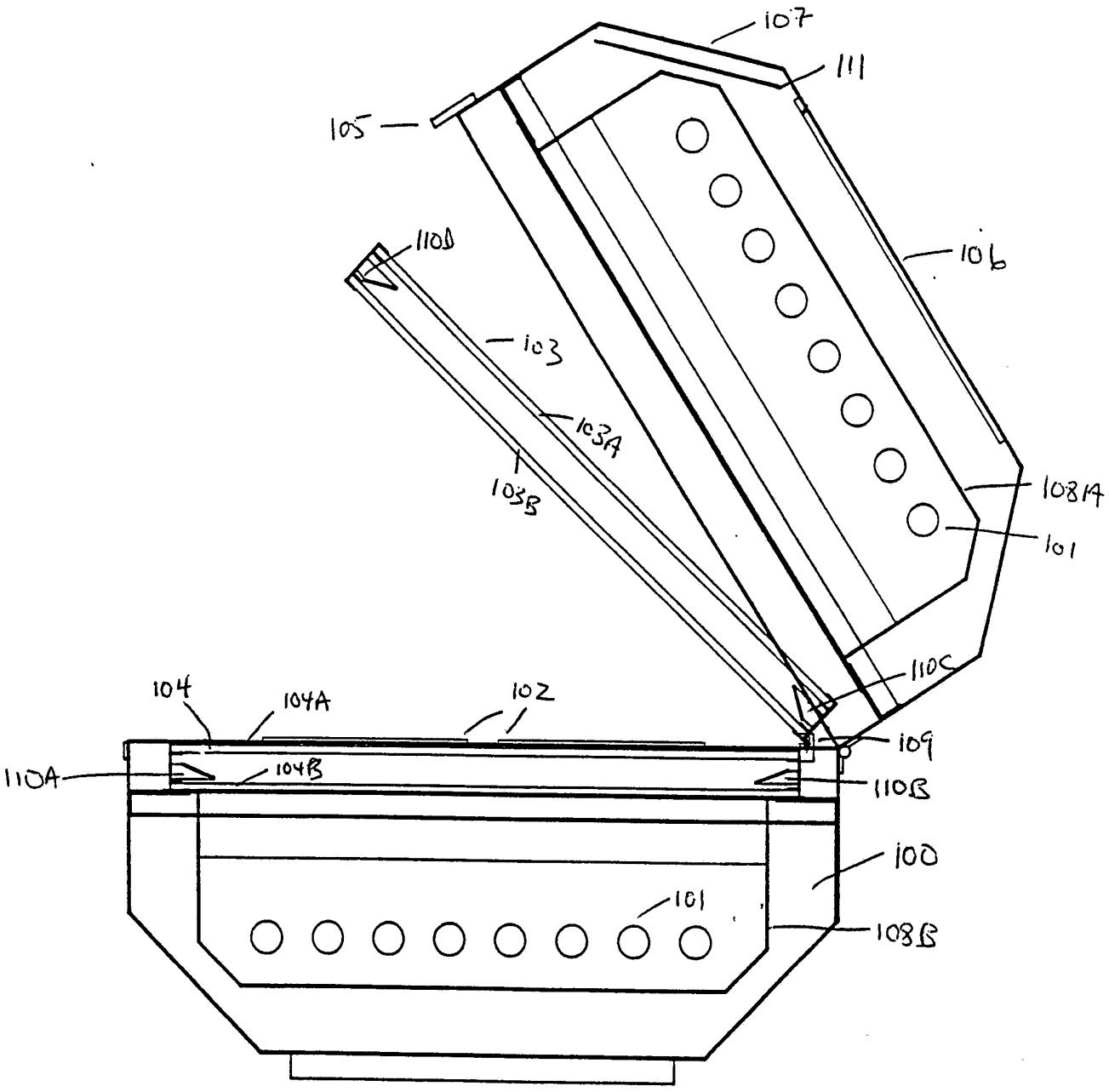

107
111
105
110D
106
103
103A
103B
108A
101
110C
104
104A
102
109
110A
104B
110B
100
101
108B

FIG. 2.

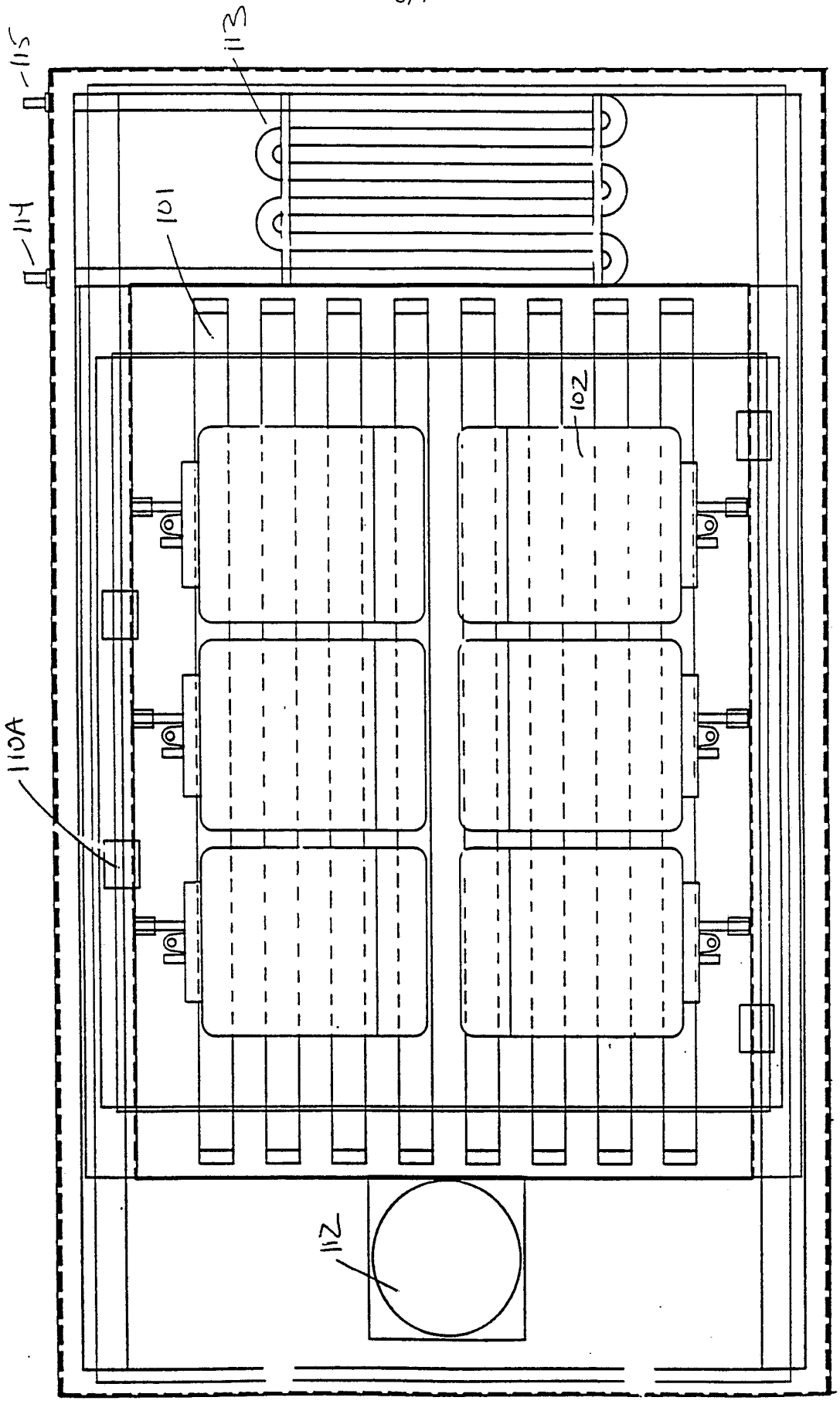


FIG. 3.

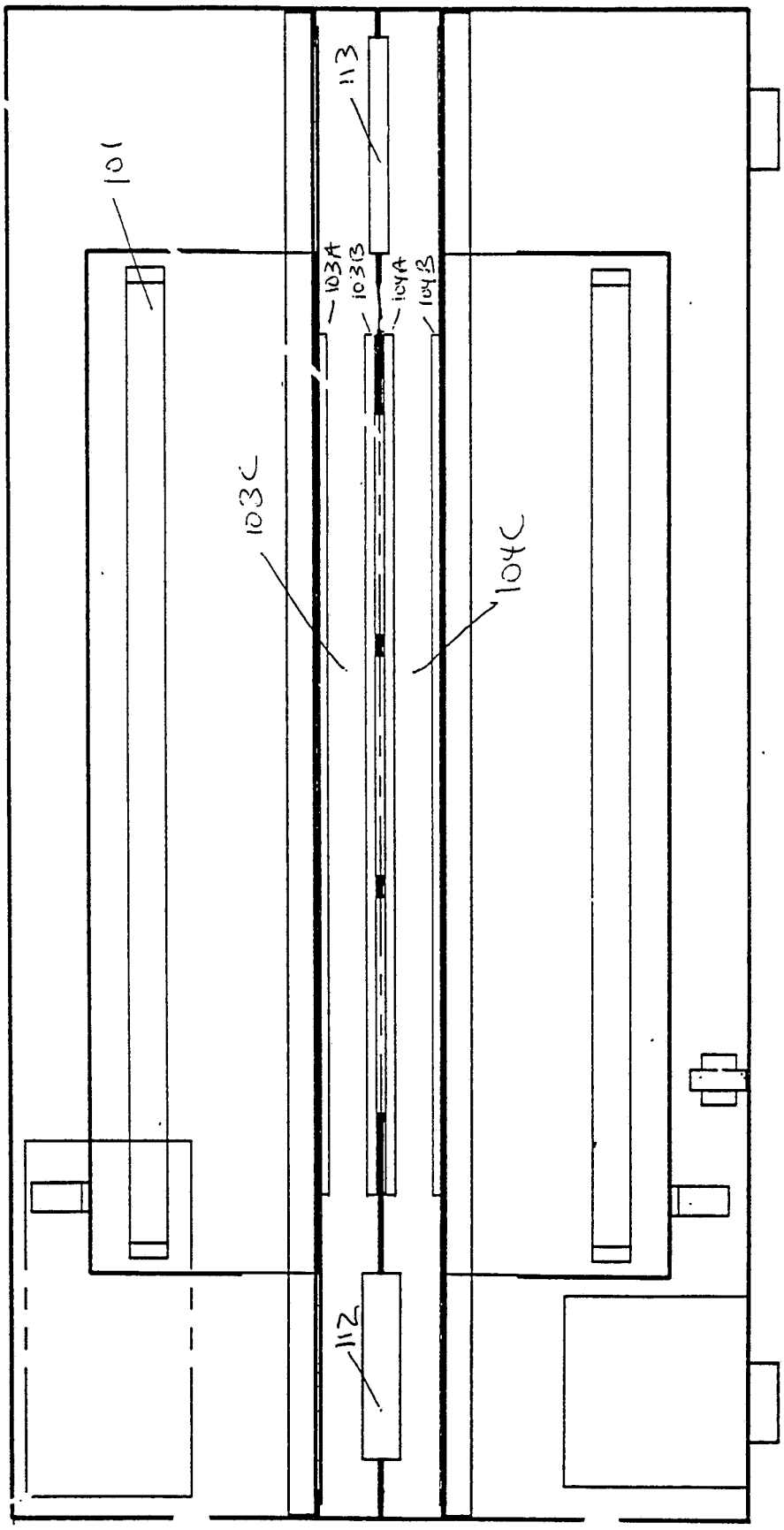


FIG. 4

FIGURE 5

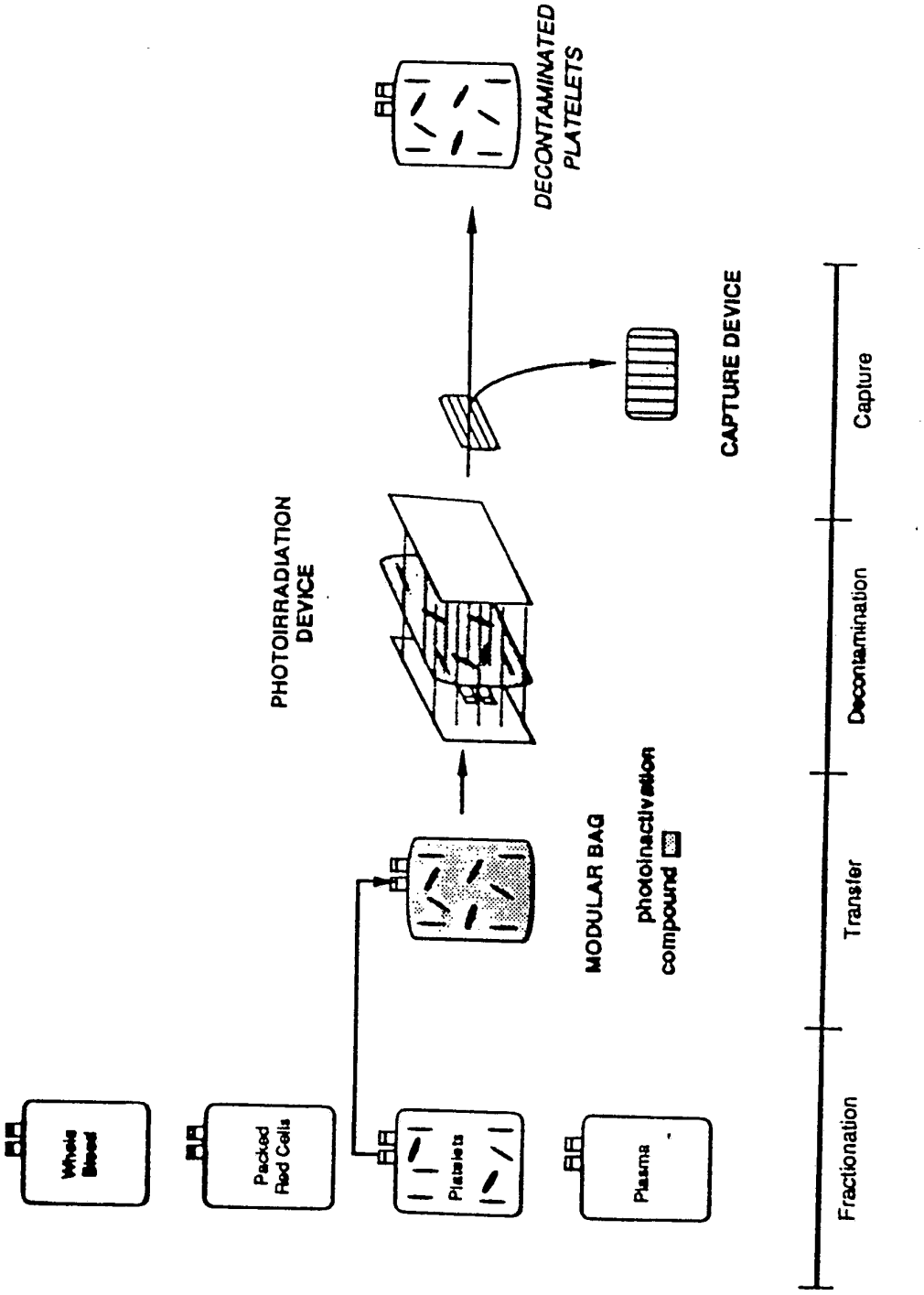
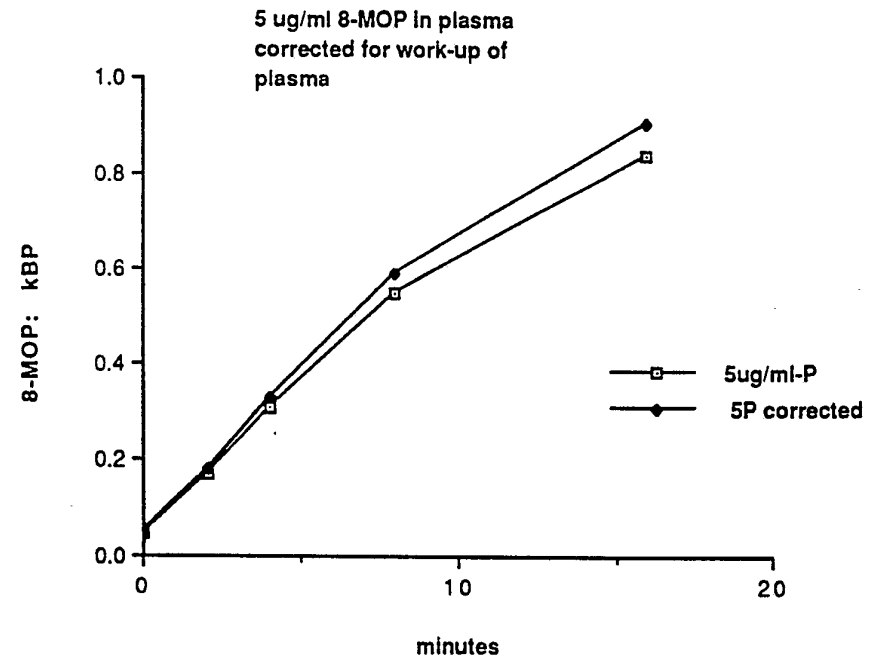

Whole Blood
Packed Red Cells
Platelets
Plasma
MODULAR BAG
photoinactivation compound
PHOTOIRRADIATION DEVICE
CAPTURE DEVICE
DECONTAMINATED PLATELETS
Fractionation
Transfer
Decontamination
Capture

FIGURE 6


5 ug/ml 8-MOP in plasma
corrected for work-up of
plasma
8-MOP: kBP
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
minutes
5ug/ml-P
5P corrected

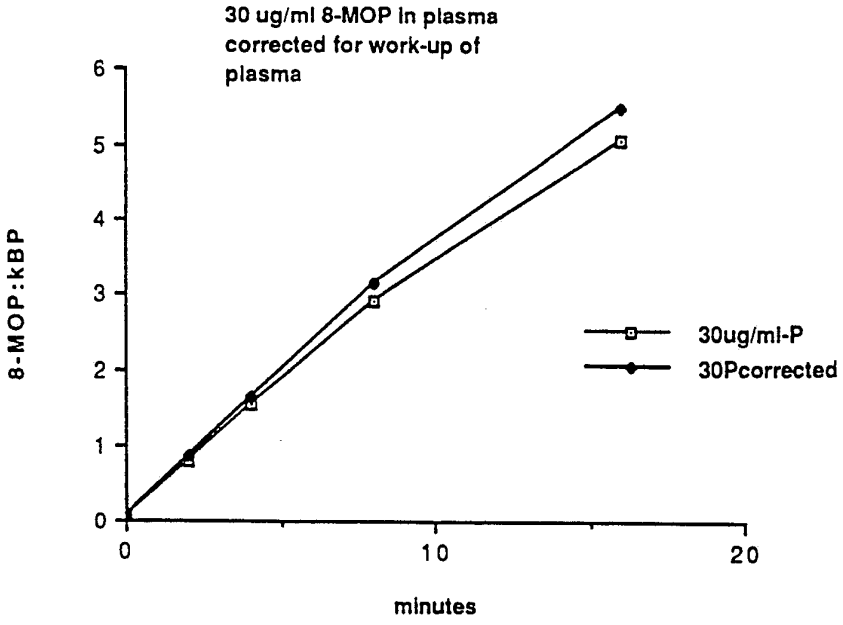
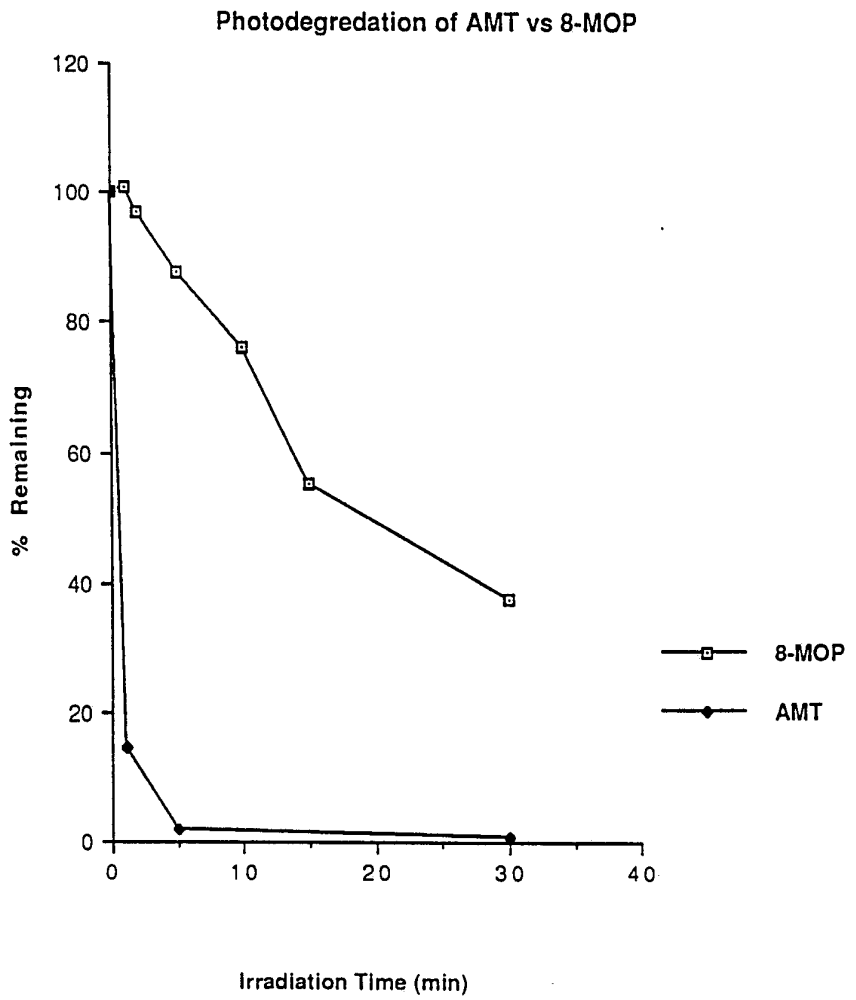

30 ug/ml 8-MOP in plasma
corrected for work-up of
plasma
8-MOP:kBP
6
5
4
3
2
1
0
0
10
20
minutes
30ug/ml-P
30Pcorrected

FIGURE 7


Photodegredation of AMT vs 8-MOP
120
100
80
60
40
20
0
% Remaining
0
10
20
30
40
Irradiation Time (min)
8-MOP
AMT

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01786

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01N 1/02; A61K 41/00; A61M 37/00; A61N 1/30
US CL :435/2; 424/90, 530; 604/4, 20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S : 435/2; 424/90, 530; 604/4, 20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online, APS, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BLOOD, Volume 74, No. 1, issued July 1989, Lin, Lily et al., "Use of 8-Methoxypsoralen and Long-Wavelength Ultraviolet Radiation for Decontamination of Platelet Concentrates," pages 517-525, see page 518.	1-20
Y	US, A, 4,878,891 (Judy et al.) 07 November 1989, see column 4 and claim 1.	1-20
Y	US, A, 4,960,408 (Klainer et al.) 02 October 1990, see columns 3 and 4, claim 2.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

"E" earlier document 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 when the document is taken alone

"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

Date of the actual completion of the international search	Date of mailing of the international search report
07 April 1993	20 APR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer RALPH GITOMER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★