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(54) Title: INACTIVATION OF PATHOGENS IN BLOOD AND/OR BLOOD PRODUCTS USING FORMALDEHYDE

(57) Abstract

A method of inactivating pathogens present in blood products containing plasma protein and/or in whole blood intended for blood transfusion. In accordance with the teachings of the present invention, the method comprises treating the whole blood or blood product with formaldehyde in a concentration of about 0.05 %-0.25 %, preferably about 0.1 %-0.2 % and more preferably about 0.15 %, by weight of the whole blood or blood product so as to inactivate any pathogens present therewithin. The present invention is also directed to a method of treating a patient in need of whole blood or a blood constituent which comprises treating a sample of whole blood or a blood product comprising the blood constituent and plasma protein with formaldehyde in the manner described above and then administering the formaldehyde-treated blood or blood constituent to the patient in need thereof. Preferably, after the treating step and prior to the administering step, residual formaldehyde is removed from the blood sample.

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INACTIVATION OF PATHOGENS IN BLOOD AND/OR BLOOD PRODUCTS USING FORMALDEHYDE

FIELD OF THE INVENTION

The present invention relates generally to the inactivation of pathogens in blood and/or blood products and more particularly to the inactivation of pathogens in blood and/or blood products using formaldehyde.

BACKGROUND OF THE INVENTION

The spread of infectious disease resulting from the transfusion of contaminated blood or from the administration of contaminated blood products has been well documented and is recognized as a major public health concern. Most notably, the transmission of viral hepatitis and Acquired Immune Deficiency Syndrome (AIDS) through contaminated blood and blood products has received widespread attention. It is to be noted, however, that viral hepatitis and AIDS are only two of the many diseases that can be spread through the use of contaminated blood and blood products. Lesser known pathogens, such as T-cell lymphotropic viruses (Types I and II), cytomegalovirus, Epstein-Barr virus, the parvoviruses and *Plasmodium* (malaria-causing) protozoa, may also be spread through contaminated blood and blood products. In addition, still other microorganisms that have not yet even been identified or recognized as being pathogenic may be transmitted through contaminated blood and blood products and, therefore, similarly pose a serious public health risk. The HIV virus is illustrative of a pathogen that, until recently, was not even recognized. Today, there are over 10 million people worldwide who have contracted AIDS, many of these people having contracted the disease through the use of infected blood or blood products; however, less than two decades ago, AIDS was not even a recognized disease. Thus, it is clear that there is a great need for a method for effectively inactivating pathogens in our blood supply.

In response to the aforementioned need, a number of techniques have been devised for inactivating pathogens, particularly infectious viral agents, in blood and/or blood products. A review of many of these techniques is presented in Suomela, "Inactivation of Viruses in Blood and Plasma Products," *Transfusion*

Medicine Reviews, Vol. VII, No. 1, pp. 42-57 (January 1993), which is incorporated herein by reference.

One such technique which has been used to inactivate viruses in blood and/or blood products is pasteurization. See e.g., Burnouf-Radosevich et al., "A Pasteurized Therapeutic Plasma," *Infusionstherapie*, 19:91-94 (1992), which is incorporated herein by reference. The pasteurization of blood and/or blood products is most often effected by heating them in the liquid state for 10 hours at 60°C. A small amount of a protein stabilizer, such as caprylate or tryptophanate, is often added to the preparation. After pasteurization has been completed, the stabilizer typically must be removed from the preparation prior to its clinical use. As is the case with many of the existing viral inactivation techniques discussed herein, pasteurization is more effective in inactivating enveloped viruses (i.e., viruses having a lipid envelope surrounding the viral capsid) than in inactivating non-enveloped viruses (i.e., viruses which lack a lipid envelope surrounding the viral capsid).

Another technique which has been used to inactivate viruses in blood and/or blood products is the solvent/detergent (S/D) method. See e.g., Hellstern et al., "Manufacture and in vitro Characterization of a Solvent/Detergent-Treated Human Plasma," *Vox Sang*, 63:178-185 (1992); Horowitz et al., "Solvent/Detergent-Treated Plasma: A Virus-Inactivated Substitute for Fresh Frozen Plasma," *Blood*, 79(3):826-831 (February 1, 1992); and Piquet et al., "Virus Inactivation of Fresh Frozen Plasma by a Solvent Detergent Procedure: Biological Results, *Vox Sang*, 63:251-256 (1992), all of which are incorporated herein by reference. The S/D method, which is limited to use in inactivating enveloped viruses, involves treating a blood preparation with an organic mixture which disrupts the lipid envelope of enveloped viruses. The disruption of the lipid envelope leads either to complete structural disruption of the virus or to destruction of the cell receptor recognition site on the virus. In either case, the virus is rendered noninfectious. The solvent used in the S/D method is most often tri-(n-butyl)phosphate (TNBP), and the detergent is either Tween 80, Triton X-100 or Na-cholate. Temperature and time influence the efficacy of the S/D method, typical temperatures being in the range of 24°C to

37°C and the typical duration of treatment being at least 6 hours. As is the case with most additives used in existing viral inactivation techniques, the substances responsible for viral inactivation must be removed from the treated products prior to their clinical use.

Still another technique which has been used to inactivate viruses in blood and/or blood products is photochemical inactivation. See e.g., Mohr et al., "Virus Inactivated Single-Donor Fresh Plasma Preparations," *Infusiontherapie*, 19:79-83 (1992); Wagner et al., "Differential sensitivities of viruses in red cell suspensions to methylene blue photosensitization," *Transfusion*, 34(6):521-526 (1994); Wagner et al., "Red cell alterations associated with virucidal methylene blue phototreatment," *Transfusion*, 33:30-36 (1993); Mohr et al., "No evidence for neoantigens in human plasma after photochemical virus inactivation," *Ann. Hematol.*, 65:224-228 (1992); Lambrecht et al., "Photoinactivation of Viruses in Human Fresh Plasma by Phenothiazine Dyes in Combination with Visible Light," *Vox Sang*, 60:207-213 (1991), Goodrich et al., "Selective inactivation of viruses in the presence of human platelets: UV sensitization with psoralen derivatives, *Proc. Natl. Acad. Sci. USA*, 91:5552-5556 (June 1994); Virus Inactivation in Plasma Products, J.-J Morgenthaler, ed. Karger, NY (1989); and BioWorld Today, Vol. 4, No. 229, pages 1 and 4 (November 24, 1993), all of which are incorporated herein by reference. The photochemical inactivation of a blood preparation typically involves treating the blood preparation with a photoactivatable chemical and then irradiating the preparation with light of a sufficient wavelength to activate the photoactivatable chemical. Examples of photoactivatable chemicals used in the photochemical inactivation of viruses present in blood preparations include psoralens, hypericin, methylene blue and toluidine blue. It is believed that psoralens, which have an affinity for nucleic acids, inactivate viruses by intercalating between viral nucleic acid base pairs and, in the presence of UVA light, forming a covalent bond with the viral nucleic acid, thereby preventing its transcription and/or replication. The manner in which hypericin, methylene blue and toluidine blue inactivate viruses is not as well-defined as that for psoralens. However, it is believed that these chemicals, when photoactivated, generate the

highly reactive entity, singlet oxygen, which then attacks the cellular structure (e.g. viral envelope) of the virus.

Whereas photochemical inactivation has been largely successful in inactivating enveloped viruses, it has been largely unsuccessful in inactivating non-enveloped viruses. The failure of photochemical inactivation to inactivate non-enveloped viruses is significant since Poliovirus, Adenovirus, Hepatitis A and Parvovirus (Parvo B19) are among those non-enveloped viruses that are pathogenic to humans.

It should be noted that photochemical inactivation of the type described above has been most successful when applied to inactivating viruses in blood preparations lacking red blood cells (e.g., plasma). This is because blood preparations which include red blood cells typically absorb light at the same wavelengths used to photoactivate the chemicals.

Formaldehyde has long been used to embalm and to preserve plant and animal cells and tissues. In addition, formaldehyde has long been used to inactivate pathogenic toxins for use in vaccine preparations and to disinfect hospital rooms, laboratories and the like against vegetative bacteria, fungi, spores and viruses. However, because formaldehyde has historically been considered by those of ordinary skill in the art to be highly toxic to humans, formaldehyde has received scant consideration as an agent for inactivating pathogens present in our blood supply.

In U.S. Patent No. 4,833,165, inventor Louderback, which issued May 23, 1989, and which is incorporated herein by reference, there is disclosed a method of inactivating HTLV-III (the HIV virus) in saline-washed and saline-resuspended red blood cells collected for transfusion into a patient in need of red blood cells, the method comprising the steps of treating the saline-washed and saline-resuspended red blood cells with an effective amount of from about 0.1% to about 5% by weight of the washed and resuspended red blood cells of a chemical agent selected from the group consisting of phenol, formaldehyde or mixtures thereof at a temperature of about 2°C to about 40°C for at least about 5 minutes, and then thoroughly

washing away any residual chemical agent from the thus-treated red blood cells prior to their administration to a patient in need thereof.

Although some language in U.S. Patent No. 4,833,165 may seem to indicate that whole blood and blood products other than washed red blood cells may be treated in a similar manner with phenol, formaldehyde or mixtures thereof to inactivate HTLV-III present therewithin, the file history of the aforementioned patent clearly teaches away from extending the teachings contained therein to whole blood or to blood products containing plasma protein. More specifically, the file history explicitly teaches that formaldehyde treatment of blood samples containing plasma protein would be inappropriate since formaldehyde would react with the plasma protein, thereby making the blood sample unsuitable for transfusion to a patient. Consequently, the file history clearly contemplates, as a necessary step, the removal of plasma protein from the blood sample prior to formaldehyde treatment.

Other patents and publications of interest include an abstract entitled "Sterilization of Red Blood Cells," by A. Louderback, Transfusion, Vol. 32, p. 66S (October 1992:Supplement); and U.S. Patent No. 4,675,159, inventor Al-Sioufi, which issued June 23, 1987, both of which are incorporated herein by reference. In the Al-Sioufi patent, there is disclosed a method and device for disinfecting whole blood intended for medical evaluation, the method comprising providing a receptacle for receiving and holding a sample of whole blood, the receptacle being provided with a disinfectant prior to introduction of the sample thereinto. The disinfectant is provided in an amount sufficient to destroy viral contamination in the specimen and the receptacle without compromising the integrity of the specimen for medical evaluation. The disinfectant is preferably a mono or dialdehyde, such as either glutaraldehyde or formaldehyde or a combination thereof, with glutaraldehyde being the most preferred. The effective concentration of glutaraldehyde is about 0.1 to 2.5 weight percent, preferably 0.13 to 2.0 weight percent based upon the total quantity of biological fluid to be placed in the receptacle. In a particularly preferred embodiment, the closed sample container is evacuated and provided with an elastomeric stopper adapted to receive the

hollow needle of a syringe whereby the blood specimen is introduced into the receptacle directly from the donor.

It is to be noted that the above-mentioned patent is drawn specifically to the inactivation of viruses in whole blood specimens which are drawn for medical evaluation (e.g., lab testing) and does not contemplate or in any way relate to the inactivation of viruses in blood or blood products which are intended to be introduced back into a person's body, such as in a blood transfusion.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a new method for inactivating pathogens in blood samples containing plasma protein (e.g., whole blood, red blood cell component, red blood cell concentrate, platelet component, platelet concentrate, plasma component, etc.) which are collected and intended for use in blood transfusions to patients in need thereof.

It is another object of the present invention to provide a method as described above which overcomes at least some of the problems described above in connection with existing pathogen inactivation techniques.

It is still another object of the present invention to provide a method as described above which is effective in inactivating a wide range of pathogens including viruses (both enveloped and non-enveloped), bacteria, fungi and protozoans.

Additional objects, as well as features and advantages, of the present invention will be set forth in part in the detailed description which follows, and in part will be obvious from the detailed description or may be learned by practice of the invention. Various embodiments of the inventions will be described in sufficient detail to enable those skilled in the art to practice the invention, and it is to be understood that other embodiments may be utilized and that changes may be made without departing from the scope of the invention. The following detailed description is, therefore, not to be taken in a limiting sense, and the scope of the present invention is best defined by the appended claims.

The present invention is based on the surprising discovery that formaldehyde, when used in appropriate concentrations, can be used to inactivate pathogens present in blood samples containing plasma protein (e.g., whole blood, red blood cell component, red blood cell concentrate, platelet component, platelet concentrate, plasma component, etc.) without rendering such samples unsuitable for use in blood transfusions. This discovery is contrary to the present thinking in the art, which is that formaldehyde is highly toxic to humans and is highly reactive with plasma proteins. Accordingly, the present thinking in the art is that formaldehyde should not be used to inactivate pathogens in plasma protein-

containing blood samples intended for use in blood transfusions since the plasma proteins will compete with the pathogens for reaction with formaldehyde and since the plasma proteins, upon reacting with formaldehyde, will form neo-antigenic species which will provoke an immunogenic response in a patient transfused therewith. Without wishing to be limited by any particular theory, the present inventors have discovered that, contrary to the present thinking in the art, formaldehyde has a lesser affinity for plasma proteins than for nucleic acids and, in fact, binds reversibly with plasma proteins while binding irreversibly with nucleic acids. Furthermore, the present inventors have noted that, when used in appropriate concentrations, formaldehyde not irreversibly bound to nucleic acids can rapidly be converted to normal levels by the naturally-occurring conversion of formaldehyde into formic acid by a dehydrogenase present in red blood cells.

One application of the above-described discovery is to a method of inactivating pathogens present in a blood product, the blood product comprising plasma protein. In accordance with the teachings of the present invention, such a method comprises the step of treating the blood product with formaldehyde in a concentration of about 0.05%-0.25%, preferably about 0.1%-0.2% and more preferably about 0.15%, by weight of the blood product so as to inactivate the pathogens present within the blood product. The aforementioned method may be used to inactivate a wide range of pathogens, including enveloped and non-enveloped viruses, bacteria, fungi and protozoans.

Another application of the above-described discovery is to a method of treating a patient in need of a blood constituent. (For purposes of the present specification and claims, the term "blood constituent" is intended to indicate a single element or basic building block of blood, such as, but not limited to, red blood cells, white blood cells, platelets, stem cells or a plasma protein; notwithstanding the above, a "blood constituent" may be contaminated with and include pathogens.) In accordance with the teachings of the present invention, such a method comprises the steps of (a) treating a sample comprising the blood constituent and plasma protein with formaldehyde in a concentration of about 0.05%-0.25%, preferably about 0.1%-0.2% and more preferably about 0.15%, by

weight of said sample so as to inactivate any pathogens present within said sample; and (b) administering the formaldehyde-treated blood constituent to the patient in need thereof. The aforementioned method may be used to inactivate a wide range of pathogens, including enveloped and non-enveloped viruses, bacteria, fungi and protozoans.

Preferably, the treating step comprises treating the sample at a temperature of about 2°C to about 40°C for at least five minutes, more preferably about 30 to about 60 minutes. Although the removal of residual formaldehyde from the sample after said treating step is not essential, the method preferably further comprises, after said treating step and before said administering step, the step of removing residual formaldehyde from the formaldehyde-treated blood constituent. The administering step preferably comprises transfusing the patient with the formaldehyde-treated blood constituent. The formaldehyde-treated blood constituent may be administered to the patient, either separately from the remainder of the sample or together with at least a portion of the remainder of the sample.

According to one embodiment of the above-described method, the blood constituent is a plasma protein, such as human serum albumin (HSA), antihemophilic factor or immunoglobulin G (IgG), and the sample containing the blood constituent and plasma protein is platelet rich plasma, platelet poor plasma, source plasma, fresh frozen plasma or whole blood. According to a second embodiment of the method, the blood constituent is red blood cells, and the sample is red blood cell component, red blood cell concentrate or whole blood. According to a third embodiment of the method, the blood constituent is platelets, and the sample is platelet component, platelet concentrate, platelet rich plasma or whole blood. According to a fourth embodiment of the method, the blood constituent is white blood cells, and the sample is whole blood or any component or portion thereof containing white blood cells. According to a fifth embodiment of the method, the blood constituent is progenitor (i.e., stem) cells, and the sample is whole blood or any component or portion thereof containing progenitor cells.

Still another application of the above-described discovery is to a method of treating a patient in need of whole blood, said method comprising the steps of: (a) treating a sample of whole blood with formaldehyde in a concentration of about 0.05%-0.25%, preferably about 0.1%-0.2% and more preferably about 0.15%, by weight of said sample so as to inactivate any pathogens present within said sample; and (b) administering the formaldehyde-treated sample to the patient in need thereof. The aforementioned method may be used to inactivate a wide range of pathogens, including enveloped and non-enveloped viruses, bacteria, fungi and protozoans.

Preferably, the treating step comprises treating the sample at a temperature of about 2°C to about 40°C for at least five minutes, more preferably about 30 to about 60 minutes. Although the removal of residual formaldehyde from the sample after said treating step is not essential, the method preferably further comprises, after said treating step and before said administering step, the step of removing residual formaldehyde from the formaldehyde-treated sample. The administering step preferably comprises transfusing the patient with the formaldehyde-treated sample.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are hereby incorporated into and constitute a part of this specification, illustrate various aspects of the invention and, together with the description, serve to explain the principles of the invention. In the drawings wherein like reference numerals represent like parts:

Fig. 1 is a photograph of a bacterial host plate onto which T4 viral suspensions containing various concentrations of sodium formaldehyde bisulfite were spotted and allowed to incubate overnight in accordance with Example 7;

Figs. 2(a) and 2(b) are photographs of bacterial host plates onto which T4 viral suspensions containing various concentrations of formaldehyde and sodium formaldehyde bisulfite, respectively, were spotted and allowed to incubate overnight in accordance with Example 8;

Figs. 3(a) through 3(c) are photographs of bacterial host plates onto which T4 viral suspensions containing various concentrations of formaldehyde, dimethyltartaric acid cyclic sulfate and ethidium bromide, respectively, were spotted and allowed to incubate overnight in accordance with Example 9;

Figs. 4(a) and 4(b) are photographs of bacterial host plates onto which T4 viral suspensions containing various concentrations of formaldehyde and formaldehyde and sodium metabisulfite, respectively, were spotted and allowed to incubate overnight in accordance with Example 10;

Fig. 5 is a graphic representation of the decrease in formaldehyde concentration over time in a sample of red blood cell concentrate;

Fig. 6 is a graphic representation of the decrease in formaldehyde concentration over time in a sample of plasma and in a sample of serum; and

Fig. 7 is a graphic representation of the decrease in formaldehyde concentration over time in buffy coat samples.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

As indicated above, the present invention is directed, in one aspect, to a method of inactivating a wide variety of pathogens (e.g., viruses, bacteria, fungi, protozoans) present in blood products containing plasma protein and/or in whole blood intended for blood transfusions. This is achieved, in accordance with the teachings of the present invention, by adding formaldehyde, in a suitable concentration, to a sample of whole blood or of a blood product containing plasma protein, whereby any pathogens present therewithin are inactivated. For purposes of the present invention, a suitable concentration of formaldehyde is about 0.05%-0.25%, preferably about 0.1%-0.2% and more preferably about 0.15%, by weight of the sample. Preferably, following the addition of formaldehyde to the sample, the sample is maintained at a temperature of about 2°C to about 40°C for at least five minutes, preferably about 30 to about 60 minutes.

Examples of blood products which contain plasma protein include, but are not limited to, red blood cell component, red blood cell concentrate, platelet component, platelet concentrate, platelet rich plasma, platelet poor plasma, source plasma and fresh frozen plasma.

According to another aspect of the invention, once a sample of whole blood or of a blood product containing plasma protein has been treated in the manner described above to inactivate any pathogens present therewithin, the sample may be administered to a patient in need of one or more blood constituents contained within the sample. Alternatively, only th(os)e blood constituent(s) of which a patient is in need may be administered to the patient. In either case, a preferred mode of administration is by transfusion.

In order to lower the concentration of formaldehyde in the sample to a level closer to that endogenously produced in the human body and metabolized in blood by the naturally-occurring conversion of formaldehyde to formic acid and other innocuous products, it may be desirable, although not necessary, to remove residual formaldehyde from the sample by physical or chemical means after the treatment step and prior to the administration step. (At the concentrations contemplated by the present invention, even without removing residual

formaldehyde from the sample, the level of formaldehyde quickly decreases to normal levels by naturally-occurring processes.)

One way in which residual formaldehyde may be removed from the sample is by physical sorption of the formaldehyde on a suitable solid, i.e., by running the sample through a solid matrix of activated carbon or a similarly appropriate material for formaldehyde. It should be understood, however, that surface adsorption capacity or equilibrium partitioning between the sorbent and the fluid phase may tend to limit the extent of physical sorption.

An alternative method is to run the sample through a solid matrix of a sorbent derivatized with a formaldehyde-reactive moiety. As can readily be appreciated, the selection of a chemical moiety should be based on several considerations: (1) the specific reactivity of the chemical moiety with formaldehyde under conditions prevailing in the sample; (2) the lack of chemical reactivity and biochemical affinity of the chemical moiety towards species other than formaldehyde present in the sample; (3) the ease with which the chemical moiety can be incorporated into a solid phase medium and the stability of the chemical moiety once in the immobilized state; and (4) the cost of preparing a functionalized medium containing the chemical moiety. Based on the foregoing considerations, examples of suitable formaldehyde-reactive moieties include polymerizable nucleic acid bases of the pyrimidine series (uracil, thymine, cytosine) and of the purine series (adenine and guanine). Such nucleic acid bases contain vinyl groups in addition to free amino groups and are thus polymerizable *in-situ* to form highly aminated surfaces, which are considerably more stable than their monomeric counterparts. Hydrazine derivatives and ion exchangers in the sulfite or bisulfite form represent other suitable formaldehyde-reactive moieties.

Still another way of reducing the concentration of formaldehyde in the sample is to run the sample through a solid matrix to which is immobilized an enzyme (such as the dehydrogenase present in red blood cells) capable of converting formaldehyde into formic acid or another similarly innocuous product.

The following examples are illustrative only and should in no way limit the scope of the present invention.

EXAMPLE 1

19 μl of T4 virus stock was added to each of nine tubes. 1 μl of a formaldehyde:phosphate buffered saline stock solution was added to eight of the nine tubes to give the below-indicated concentrations. 1 μl of phosphate buffered saline was added to the ninth tube. The samples were incubated for 45 minutes at room temperature, and the PFU's were determined. The results are summarized below in TABLE I.

TABLE I

SAMPLE	FORMALDEHYDE CONCENTRATION	PFU's
1	0.53%	$<10^3$
2	0.40%	$<10^3$
3	0.30%	$<10^3$
4	0.23%	$<10^3$
5	0.12%	$<10^3$
6	0.06%	$<10^3$
7	0.03%	$150x10^4$
8	0.01%	$61x10^6$
9	0%	$161x10^8$

EXAMPLE 2

300 μl of red blood cell concentrate and 80 μl of T4 stock were added to each of eight tubes. Stock solutions of formaldehyde were prepared in phosphate buffered saline, pH 7.4. 20 μl of an appropriate formaldehyde stock solution was added to each of seven of the eight tubes to give the below-indicated

concentrations. 20 μl of phosphate buffered saline was added to the eighth tube. The samples were incubated for 60 minutes at room temperature. The samples were then diluted, and the PFU's determined. The results are summarized below in TABLE II.

TABLE II

SAMPLE	FORMALDEHYDE CONCENTRATION	PFU's
A	0.185%	$136x10^3$
B	0.111%	$119x10^5$
C	0.074%	$91x10^6$
D	0.037%	$46x10^7$
E	0.014%	$85x10^7$
F	0.021%	$92x10^7$
G	0.007%	$75x10^7$
H	0%	$52x10^7$

EXAMPLE 3

300 μl of red blood cell concentrate and 80 μl of T4 stock were added to each of seven tubes. Stock solutions of formaldehyde were prepared in phosphate buffered saline, pH 7.4. 20 μl of an appropriate formaldehyde stock solution was added to each of six of the seven tubes to give the below-indicated concentrations. 20 μl of phosphate buffered saline was added to the seventh tube. The samples were incubated for 60 minutes at room temperature. The samples were then diluted, and the PFU's determined. The results are summarized below in TABLE III.

TABLE III

SAMPLE	FORMALDEHYDE CONCENTRATION	PFU's
A	0.370%	$<10^2$
B	0.222%	$3x10^2$
C	0.148%	$109x10^3$
D	0.088%	$157x10^4$
E	0.074%	$50x10^5$
F	0.044%	$29x10^6$
G	0%	$34x10^7$

EXAMPLE 4

4 incubation mixtures were prepared, each incubation mixture containing 165 μl of blood plasma, 25 μl of T4 virus and 10 μl of a formaldehyde stock solution resulting in the below-indicated final concentration. At the times indicated below, samples were taken, diluted, mixed with host cells and overlaid onto solid medium. The plates were then incubated overnight at 37°C, and plaques were counted the next day. The results are summarized below in TABLE IV.

TABLE IV

INCUBATION PERIOD	FORMALDEHYDE CONCENTRATION	PFU's
0 minutes	0%	$8x10^8$
0 minutes	0.0349%	$4x10^8$
0 minutes	0.0712%	$4x10^8$

0 minutes	0.1028%	$5x10^8$
15 minutes	0%	Not determined
15 minutes	0.0349%	$13x10^7$
15 minutes	0.0712%	$7x10^7$
15 minutes	0.1028%	$32x10^6$
30 minutes	0%	$12x10^8$
30 minutes	0.0349%	$7x10^7$
30 minutes	0.0712%	$3x10^6$
30 minutes	0.1028%	$4x10^6$
45 minutes	0%	Not determined
45 minutes	0.0349%	$19x10^6$
45 minutes	0.0712%	$31x10^5$
45 minutes	0.1028%	$9x10^5$
60 minutes	0%	$6x10^8$
60 minutes	0.0349%	$9x10^6$
60 minutes	0.0712%	$3x10^5$
60 minutes	0.1028%	$11x10^4$

EXAMPLE 5

4 reaction mixtures were prepared, each reaction mixture consisting of 16.5 μl of red blood cell component, 2.5 μl of T4 stock and 1 μl of a formaldehyde stock solution resulting in the below-indicated final concentration. At the times indicated below, samples were taken, diluted, mixed with host cells and overlaid onto solid

medium. The 1 μl of phosphate buffered saline was added to the ninth tube. The samples were incubated for 45 minutes at room temperature. The plates were then incubated overnight at 37°C, and plaques were counted the next day. The results are summarized below in TABLE V.

TABLE V

INCUBATION PERIOD	FORMALDEHYDE CONCENTRATION	PFU's
0 minutes	0%	$14x10^8$
0 minutes	0.0694%	$18x10^8$
0 minutes	0.1388%	$7x10^8$
0 minutes	0.1850%	$11x10^8$
15 minutes	0%	Not determined
15 minutes	0.0694%	$11x10^7$
15 minutes	0.1388%	$20x10^6$
15 minutes	0.1850%	$10x10^6$
30 minutes	0%	$11x10^8$
30 minutes	0.0694%	$41x10^6$
30 minutes	0.1388%	$7x10^5$
30 minutes	0.1850%	$9x10^4$
45 minutes	0%	Not determined
45 minutes	0.0694%	$52x10^5$
45 minutes	0.1388%	$6x10^4$
45 minutes	0.1850%	$11x10^3$
60 minutes	0%	$10x10^8$

60 minutes	0.0694%	$14x10^5$
60 minutes	0.1388%	$6x10^3$
60 minutes	0.1850%	$1x10^3$

EXAMPLE 6

19 μl of T4 virus stock was added to each of seventeen tubes. 1 μl of an additive was added to fifteen of the tubes to give the below-indicated concentrations. The samples were exposed to room light or to UV radiation for 75 minutes, and the PFU's were determined. The results are summarized below in TABLE VI.

TABLE VI

SAMPLE	ADDITIVE	EXPOSURE	PFU's
1	Formaldehyde (0.5%)	Room light	$<10^3$
2	Formaldehyde (0.1%)	Room light	$<10^3$
3	Formaldehyde (0.05%)	Room light	$55x10^3$
4	Acetaldehyde (0.235%)	Room light	$24x10^6$
5	Acetaldehyde (0.047%)	Room light	$70x10^7$
6	Acetaldehyde (0.0235%)	Room light	$20x10^7$

7	Dextran sulfate (50 μg/ml)	Room light	$76x10^6$
8	Dextran sulfate (10 μg/ml)	Room light	$72x10^7$
9	Dextran sulfate (5 μg/ml)	Room light	$39x10^7$
10	Coumarin 339 (71 μg/ml)	Room light	$174x10^7$
11	Coumarin 339 (7.1 μg/ml)	Room light	$76x10^7$
12	Coumarin 339 (0.71 μg/ml)	Room light	$179x10^7$
13	None	Room light	$165x10^7$
14	Coumarin 339 (71 μg/ml)	UV light	$87x10^7$
15	Coumarin 339 (7.1 μg/ml)	UV light	$20x10^7$
16	Coumarin 339 (0.71 μg/ml)	UV light	$118x10^7$
17	None	UV light	$196x10^7$

As can be seen from the results above, formaldehyde was the most effective at killing the virus.

EXAMPLE 7

A stock solution of sodium formaldehyde bisulfite (500 mg/ml) in phosphate buffered saline, pH 7.4, (PBS) was diluted in PBS to yield additional stock solutions. 1 μl of each of the newly made stock solutions was added to 19 μl of T4 stock to give final sodium formaldehyde bisulfite concentrations of 25 mg/ml (F^0), 5 mg/ml ($F^{1:5}$), 2.5 mg/ml ($F^{1:10}$), 0.5 mg/ml ($F^{1:50}$), and 0.25 mg/ml ($F^{1:100}$). A control containing 1 μl of PBS and 19 μl of T4 was also prepared. The suspensions incubated for 1 hour at room temperature. Thereafter, 10 μl of each suspension was diluted into 1000 μl of dilution broth, and 5 μl of each suspension was spotted onto a plate overlaid with an *E. coli* host strain. After overnight incubation at 37°C, the plate was photographed (see Fig. 1).

EXAMPLE 8

A solution of 3.7% formaldehyde was diluted in phosphate buffered saline, pH 7.4, to form a series of formaldehyde stock solutions. A 50% solution of sodium formaldehyde bisulfite was also diluted in phosphate buffered saline to yield a series of stock solutions. 1 μl from each of the stock solutions was mixed with 19 μl of T4 virus stock to create a series of incubation mixtures containing formaldehyde at final concentrations of 0.185% (F^0), 0.037% ($F^{1:5}$), 0.0185% ($F^{1:10}$), 0.0037% ($F^{1:50}$), and 0.00185% ($F^{1:100}$) and a series of incubation mixtures containing sodium formaldehyde bisulfite at final concentrations of 2.5% ($NaFS^0$), 0.5% ($NaFS^{1:5}$), 0.25% ($NaFS^{1:10}$), 0.05% ($NaFS^{1:50}$) and 0.025% ($NaFS^{1:100}$). A control containing 1 μl of PBS and 19 μl of T4 stock was prepared for each series of incubation mixtures. The mixtures were incubated at room temperature for 60 minutes and diluted 1:100 in dilution broth. 5 μl from each mixture of the formaldehyde series was spotted onto a first plate overlaid with soft agar containing an *E. coli* host strain, and 5 μl from each mixture of the sodium formaldehyde bisulfite series was spotted onto a second such plate. After overnight incubation at 37°C, the plates were photographed (see Figs. 2(a) and 2(b), respectively).

EXAMPLE 9

Stock solutions of 3.7% formaldehyde, dimethyltartaric acid cyclic sulfate (500 mg/ml) and ethidium bromide (10 mg/ml) were used to generate respective dilution series of 1:5, 1:10, 1:50 and 1:100. Incubation mixtures containing 1 μl from each stock solution and the dilutions and 19 μl of virus were prepared. The mixtures incubated at room temperature for 2 hours before being diluted 1:100 and spotted (5 μl) onto respective plates containing an *E. coli* host. After incubation at 37°C, the plates were photographed (see Figs. 3(a) through 3(c), respectively).

EXAMPLE 10

Whole blood was allowed to separate at 5°C. The plasma was removed, and the red blood cell component was divided into a series of 0.5 ml aliquots. Each aliquot received 50 μl of T4 stock and half the aliquots also received 20 μl of a sodium metabisulfite (BS) solution (500 mg/ml). A stock solution of 37% formaldehyde (F^0) was diluted 1:5 ($F^{1:5}$), 1:10 ($F^{1:10}$), 1:50 ($F^{1:50}$) and 1:100 ($F^{1:100}$) in phosphate buffered saline. 25 μl of the formaldehyde stock solution and each of the dilutions was added to respective samples having and lacking BS. After 1 hour at room temperature, the mixtures were diluted 1:100 in dilution broth. 5 μl of each dilution lacking BS was spotted onto a first plate containing host cells, and 5 μl of each dilution including BS was spotted on a second plate containing host cells. After overnight incubation, the plates were photographed (see Figs. 4(a) and 4(b), respectively).

EXAMPLE 11

Red blood cell concentrate (57% Hct, 10 ml) was passed through a leukocyte removal filter and then contacted with 2.1 ml of 7871 ppm formaldehyde solution. Thus, the final solution concentration was 1420 ppm formaldehyde and 47% Hct. At various times, 0.1 ml samples were removed and diluted with saline

to 1 ml. 0.5 ml of the plasma was then analyzed for formaldehyde concentration. The remaining 0.5 ml was then washed twice with 0.5 ml of saline, and the washings were analyzed for formaldehyde concentration. Finally, the remaining 0.5 ml containing washed red blood cells was analyzed for formaldehyde concentration. (The cells were lysed using Triton X.) The results of the measurements discussed above are depicted in Fig. 5. (In Fig. 5, ■ represents readings taken from plasma, ● represents readings taken from the washings, ▲ represents readings taken from the red blood cells and ◆ represents the total of all readings.)

EXAMPLE 12

2.2 ml of 7871 ppm formaldehyde solution was added to a first 10 ml aliquot of plasma, resulting in a 12.2 ml solution containing 1420 ppm formaldehyde. At various times, 0.1 ml samples were removed and diluted with saline to 1 ml. These diluted samples were then analyzed for formaldehyde concentration, the results of these measurements being depicted in Fig. 6 (using the symbol ■).

1 ml of 1.0 M $CaCl_2$ was added to a second 10 ml aliquot of plasma. The precipitate was removed by centrifugation. Buffered saline was added to the supernatant liquid (i.e. serum) to a volume of 10 ml. 2.2 ml of formaldehyde was added to the solution and, as described above, 0.1 ml samples were removed at various times, diluted with saline to 1 ml and then analyzed for formaldehyde concentration. The results of these measurements are also shown in Fig. 6 (represented by the symbol ●).

EXAMPLE 13

Buffy coat samples (5 ml, 8.2×10^7 WBC/ml, 5.8×10^9 RBC/ml) were contacted with (1) 0.775 ml of 7871 ppm formaldehyde solution and 0.325 ml buffered saline; (2) 1.1 ml of 7871 ppm formaldehyde solution and 0.325 ml buffered saline; or (3) 1.55 ml of 7871 ppm formaldehyde solution and 0.325 ml buffered saline, respectively, to give final solutions containing 1000 ppm formaldehyde, 1420 ppm

formaldehyde and 2000 ppm formaldehyde, respectively. At various times, 0.3 ml aliquots of the solutions were removed from the various samples and diluted with saline to 1 ml. These samples were then analyzed for formaldehyde concentration. (RBC's were lysed using Triton X.) The results are depicted in Fig. 7 (with ■ representing those samples containing an initial formaldehyde concentration of 1000 ppm, ● representing those samples containing an initial formaldehyde concentration of 1420 ppm and ▲ representing those samples containing an initial formaldehyde concentration of 2000 ppm.)

EXAMPLE 14

A 500 ml stock solution containing approximately 0.4 g human IgG and 1.2 g HSA/100 ml was made by mixing 40 ml of 50 mg IgG/ml solution with 24 ml of 25% HSA and bringing the total weight of solutions to 500 g. (IgG was obtained from Armour Pharmaceutical Co., Kankakee, IL, and 25% HSA (human serum albumin) was obtained from New York Blood Center, New York, NY.) Phosphate Buffered Saline (PBS, obtained from Sigma Chemical Co., St. Louis, MO) was added to ten 50 ml polypropylene centrifuge tubes in the amounts indicated below in TABLE VII. Appropriate quantities of formaldehyde (37% solution, U.S.P./N.F., Spectrum Chemical Mfg. Corp., CA) were then added to the ten tubes to yield the concentrations indicated in TABLE VII. 25 g of the IgG/HSA stock solution was then added to each of the ten tubes. The contents of each tube were mixed well for 2 minutes and were allowed to react for the period indicated in TABLE VII.

A 5% sodium bisulfite solution was made by dissolving 1.0 g of sodium bisulfite (a mixture of sodium bisulfite and sodium metabisulfite, Aldrich Chemical Co., St. Louis, MO) in 20 ml PBS, pH 7.4. At the end of 1 hour, 8.0 ml aliquots from samples 1 through 5 of TABLE VII were removed and added to 2.0 ml aliquots of 5% sodium bisulfite solution in separate 15 ml polypropylene centrifuge tubes. The samples were added to sodium bisulfite because it is known that sodium bisulfite forms an adduct with formaldehyde and, therefore, would be removed from solution. The contents of each tube were mixed well, and the solutions were then

analyzed for human IgG concentration using a rate nephelometer (a Beckmann Immunochemistry Analyzer II operated according to the human IgG protocol). Rate nephelometry measures the rate of increase in intensity of light scattered from particles suspended in solution as a results of larger complexes formed during an antigen-antibody reaction. The antigen in these measurements is IgG treated with formaldehyde; the antibody is an immunoglobulin G antiserum developed by Beckmann for human IgG which recognizes particular epitopes of human IgG. The results of the above-described measurements are shown in TABLE VII.

Sephadex® G-25M (PD-10) gel filtration columns (8 ml gel bed volume, Pharmacia LKBBiotech, Uppsala, Sweden) were prepared and exchanged with PBS by passing 10 ml through the columns. At the end of six hours, 8.0 ml aliquots of the reaction mixtures of samples 6 through 10 were added to 2.0 ml of 5% sodium bisulfite solution in separate 15 ml polypropylene centrifuge tubes and were mixed well. In addition, 2.0 ml aliquots of the reaction mixtures of samples 6 through 10 were added to the top of the gel columns, and 2 ml fractions were collected by eluting with PBS. Most of the proteins, as measured by A 280, were found in the second fraction. The fractions were saved, and the IgG concentration in the fractions was measured by nephelometry. The results are also given in TABLE VII.

TABLE VII

SAMPLE	HCHO (ppm) / HCHO (µl 37% Soln)	Reaction Time (hrs)	PBS (g)	IgG Conc. (g/l)	IgG Conc. (g/l) 2d fraction
1	0 / 0	1	25	26.2,2.42 2.57	
2	500 / 67.5	1	24.93	2.42,2.25	

3	750 101.5	1	24.9	2.47,2.42	
4	1000 135	1	24.86	2.53,2.50	
5	1250 169	1	24.83	2.28,2.35	
6	500 67.5	6	24.93	2.42,2.42	1.44
7	750 101.5	6	24.9	2.38,2.42	1.54
8	1000 135	6	24.86	2.4,2.37	1.62
9	1250 169	6	24.83	2.55,2.5	1.57
10	0 0	6	25	2.48,2.47 2.45	1.52,1.54

As can be seen from TABLE VII, within experimental error, no difference could be recognized between samples treated with formaldehyde and the controls, both for the 1-hour treatment and the 6-hour treatment. TABLE VII also shows that when the samples were passed through a gel column to remove unreacted formaldehyde, no difference, within experimental error, could be seen between the formaldehyde-treated samples and the controls.

Additional aliquots from samples 1, 2 and 5 were also analyzed by two-dimensional electrophoresis. Examination of the gel showed no noticeable

difference between the control (sample 1) and the formaldehyde-treated samples (samples 2 and 5).

The remaining reaction mixtures of samples 1 through 5, after being stored for 6 hours at room temperature, were stored at 4°C for 22 hours. Rate nephelometry was then performed on the samples. The results are given below in TABLE VIII.

TABLE VIII

SAMPLE	HCHO Conc. (ppm)	REACTION TIME (room temp.)	REACTION TIME (4°C)	IgG Conc. (g/l)
1	0	6	22	2.58, 2.78
2	500	6	22	2.57,2.48
3	750	6	22	2.75,2.75
4	1000	6	22	2.67,2.88
5	1250	6	22	2.8,2.77

As can be seen from TABLE VIII, there was no difference, within experimental error, between the formaldehyde-treated samples (samples 2 through 5) and the control (sample 1).

EXAMPLE 15

Packed red blood cells (55% Hct) were exposed to various levels of formaldehyde for 1 hour. The cells were then spun 10 minutes at 1500 rpm. The hemoglobin level of the plasma was then measured using a commercially available hemoglobin assay from Sigma Chemical so that cell lysis could be correlated with formaldehyde concentration. The results are given below in TABLE IX.

TABLE IX

FORMALDEHYDE CONCENTRATION	Hb ASSAY (600 nm)	Hb CONCENTRATION (mg/dl)
blank (TMB)	0.036	-
standard	0.184	-
0 ppm	0.178	28.7
1000 ppm	0.194	32.0
1500 ppm	0.335	60.6
2000 ppm	0.103	13.5
3000 ppm	0.179	28.9
4000 ppm	0.218	36.8

As can be seen from the results above, with the exception of the high of 60.6 mg/dl and the low of 13.5 mg/dl, all of the hemoglobin levels appear to be comparable to one another. Therefore, it appears that the concentrations of formaldehyde used above do not cause unusual levels of hemolysis.

EXAMPLE 16

Various levels of formaldehyde were added to samples of red blood cells in saline solution and to samples containing red blood cells and certain plasma proteins. The samples were incubated by mixing in a low speed mixer for 1 hour at room temperature and were then centrifuged at 3000 rpm for 10 minutes. The supernatant from each sample was then assayed for hemoglobin using the above-mentioned Sigma hemoglobin assay. The results are given below in TABLE X.

TABLE X

SAMPLE	HCHO CONC.	Hb ASSAY (600 nm)	ASSAY DILUTION	mg% Hb
RBC in 0.95% NaCl	0	0.179	1:20	301.3
RBC in 0.95% NaCl	500	0.137	1:20	230.6
RBC in 0.95% NaCl	750	0.116	1:20	195.2
RBC in 0.95% NaCl	1000	0.148	1:20	249.1
RBC in 0.95% NaCl	1250	0.164	1:20	276.0
RBC in 0.2g IgG+0.6g HSA/100 ml	500	0.159	No dilution	13.4
RBC in 0.2g IgG+0.6g HSA/100 ml	750	0.183	No dilution	15.4
RBC in 0.2g IgG+0.6g HSA/100 ml	1000	0.176	No dilution	14.8
RBC in 0.2g IgG+0.6g HSA/100 ml	1250	0.29	No dilution	24.4

RBC in 0.2g IgG+0.6g HSA/100 ml	0	0.202	No dilution	17.0
30 mg% Hb standard	0	0.364	No dilution	30.6
30 mg% Hb standard	0	0.349	No dilution	29.4

As can be seen from the results above, no change in hemoglobin release can be attributed to the presence of formaldehyde in the concentrations used above. Also, it appears from the results above that hemoglobin release was 10 to 20 times higher in the absence of the plasma proteins than in the presence of the plasma proteins. Although not indicated in TABLE X, it should also be noted that the 95% NaCl samples, including the controls, all showed red color in the supernatant whereas the samples with IgG and HSA did not show red color in the supernatant.

The embodiments of the present invention recited herein are intended to be merely exemplary and those skilled in the art will be able to make numerous variations and modifications to it without departing from the spirit of the present invention. All such variations and modifications are intended to be within the scope of the present invention as defined by the claims appended hereto.

WHAT IS CLAIMED IS:

1. A method of inactivating a pathogen present in a blood product, the blood product comprising plasma protein, said method comprising the step of treating the blood product with formaldehyde in a concentration of about 0.05%-0.25% by weight of the blood product so as to inactivate the pathogen present within the blood product.

2. The method as claimed in claim 1 wherein said treating step comprises treating the blood product with formaldehyde in a concentration of about 0.1%-0.2% by weight of the blood product.

3. The method as claimed in claim 2 wherein said treating step comprises treating the blood product with formaldehyde in a concentration of about 0.15% by weight of the blood product.

4. The method as claimed in claim 1 wherein the pathogen is selected from the group consisting of viruses, bacteria, fungi and protozoans.

5. The method as claimed in claim 1 wherein the blood product is selected from the group consisting of red blood cell component, red blood cell concentrate, platelet component, platelet concentrate, platelet rich plasma, platelet poor plasma, source plasma, fresh frozen plasma and a plasma protein.

6. A method of treating a patient in need of a blood constituent, said method comprising the steps of:

(a) treating a sample comprising the blood constituent and plasma protein with formaldehyde in a concentration of about 0.05%-0.25% by weight of said sample so as to inactivate any pathogens present within said sample; and

(b) administering the formaldehyde-treated blood constituent to the patient in need thereof.

7. The method as claimed in claim 6 wherein said treating step comprises treating the sample with formaldehyde in a concentration of about 0.1%-0.2% by weight of said sample.

8. The method as claimed in claim 7 wherein said treating step comprises treating the sample with formaldehyde in a concentration of about 0.15% by weight of said sample.

9. The method as claimed in claim 6 wherein the blood constituent is a plasma protein.

10. The method as claimed in claim 9 wherein the blood constituent is a plasma protein selected from the group consisting of human serum albumin, antihemophilic factor and immunoglobulin G.

11. The method as claimed in claim 9 wherein said sample is platelet rich plasma.

12. The method as claimed in claim 9 wherein said sample is platelet poor plasma.

13. The method as claimed in claim 9 wherein said sample is source plasma.

14. The method as claimed in claim 9 wherein said sample is fresh frozen plasma.

15. The method as claimed in claim 9 wherein said sample is whole blood.

16. The method as claimed in claim 6 wherein the blood constituent is red blood cells.

17. The method as claimed in claim 16 wherein said sample is red blood cell component.

18. The method as claimed in claim 16 wherein said sample is red blood cell concentrate.

19. The method as claimed in claim 16 wherein said sample is whole blood.

20. The method as claimed in claim 6 wherein the blood constituent is platelets.

21. The method as claimed in claim 20 wherein said sample is platelet component.

22. The method as claimed in claim 20 wherein said sample is platelet concentrate.

23. The method as claimed in claim 20 wherein said sample is platelet rich plasma.

24. The method as claimed in claim 20 wherein said sample is whole blood.

25. The method as claimed in claim 6 wherein the blood constituent is white blood cells.

26. The method as claimed in claim 6 wherein the blood constituent is progenitor cells.

27. The method as claimed in claim 6 wherein said pathogens are selected from the group consisting of viruses, bacteria, fungi and protozoans.

28. The method as claimed in claim 6 wherein said administering step comprises transfusing the patient with the formaldehyde-treated blood constituent.

29. The method as claimed in claim 6 further comprising, after said treating step and before said administering step, the step of removing residual formaldehyde from the formaldehyde-treated blood constituent.

30. The method as claimed in claim 6 wherein said treating step comprises treating the sample at a temperature of about 2°C to about 40°C for at least five minutes.

31. The method as claimed in claim 30 wherein the treatment time ranges from about 30 to about 60 minutes.

32. The method as claimed in claim 6 wherein said administering step comprises administering the formaldehyde-treated sample to the patient.

33. The method as claimed in claim 6 further comprising, after said treating step and before said administering step, the step of separating the formaldehyde-treated blood constituent from the remainder of the formaldehyde-treated sample and wherein said administering step comprises administering only the formaldehyde-treated blood constituent to the patient.

34. A method of treating a patient in need of whole blood, said method comprising the steps of:

(a) treating a sample of whole blood with formaldehyde in a concentration of about 0.05%-0.25% by weight of said sample so as to inactivate any pathogens present within said sample; and

(b) administering the formaldehyde-treated sample to the patient in need thereof.

35. The method as claimed in claim 34 wherein said treating step comprises treating the sample with formaldehyde in a concentration of about 0.1%-0.2% by weight of said sample.

36. The method as claimed in claim 35 wherein said treating step comprises treating the sample with formaldehyde in a concentration of about 0.15% by weight of said sample.

37. The method as claimed in claim 34 wherein said pathogens are selected from the group consisting of viruses, bacteria, fungi and protozoans.

38. The method as claimed in claim 34 wherein said administering step comprises transfusing the patient with the formaldehyde-treated sample.

39. The method as claimed in claim 34 further comprising, after said treating step and before said administering step, the step of removing residual formaldehyde from said sample.

40. The method as claimed in claim 34 wherein said treating step comprises treating the sample at a temperature of about 2°C to about 40°C for at least five minutes.

41. The method as claimed in claim 40 wherein the treatment time ranges from about 30 to about 60 minutes.

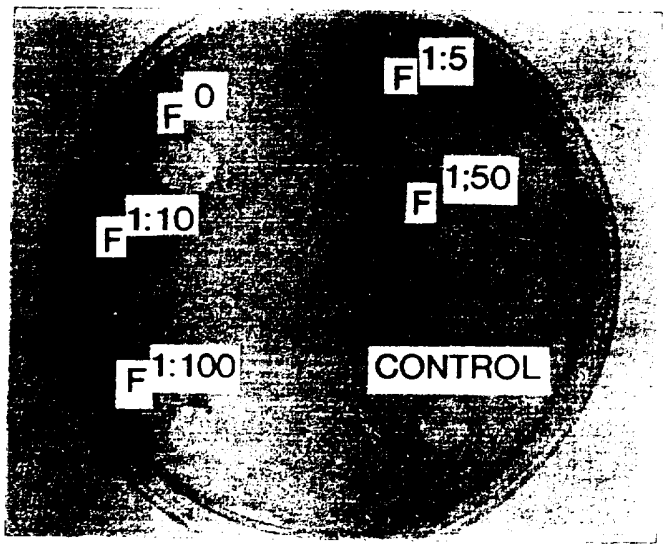


FIG.1

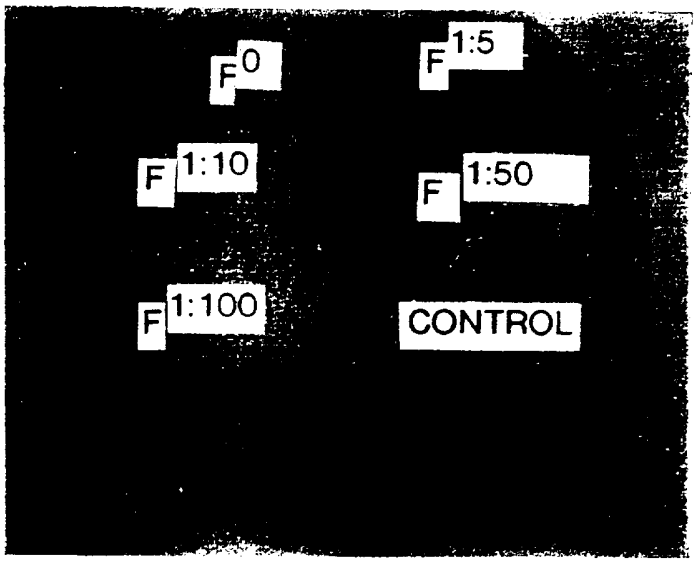


FIG.2A

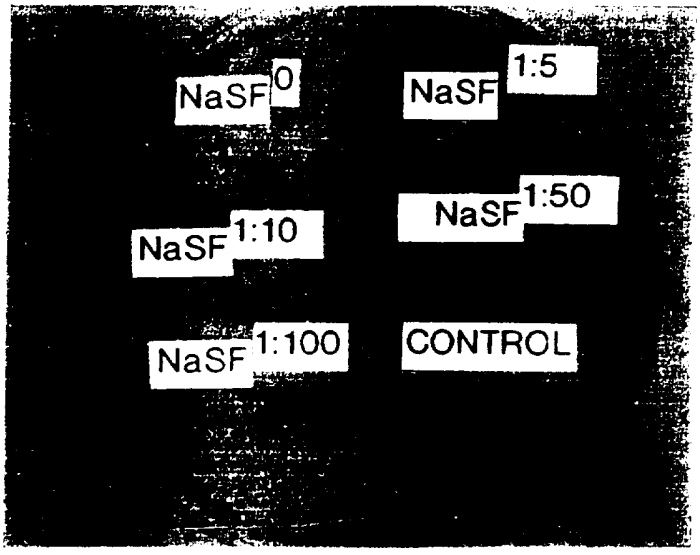


FIG.2B

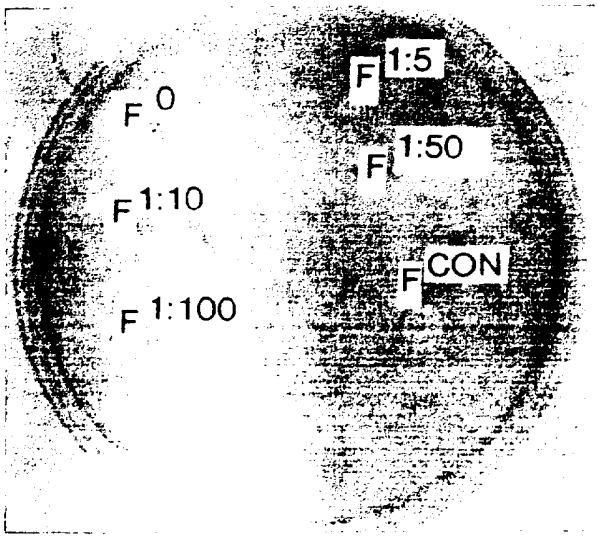


FIG.3A

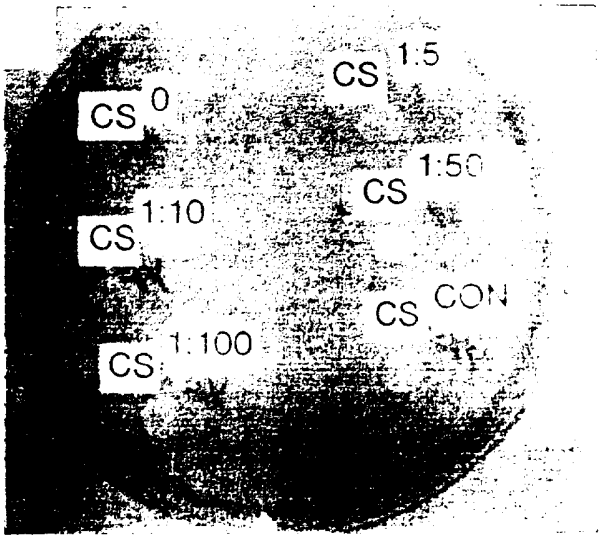


FIG.3B

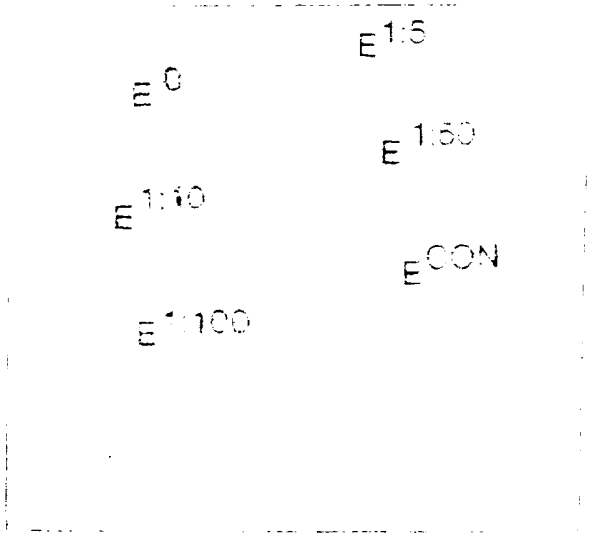
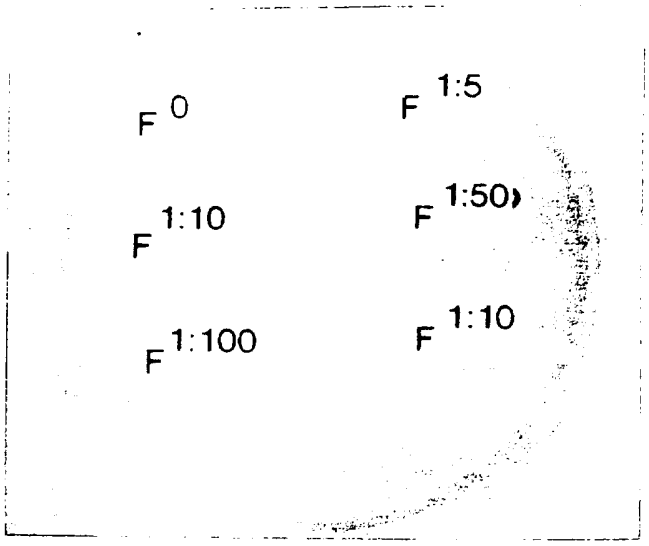


FIG.3C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F 1:10
F 1:50
F 1:100
F 1:10

FIG.4A

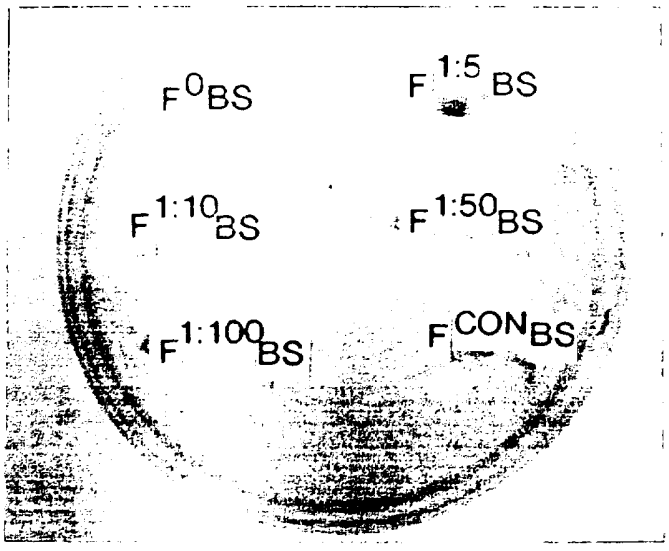
F0BS
F1:5 BS
F1:10BS
F1:50BS
F1:100BS
FCONBS

FIG.4B

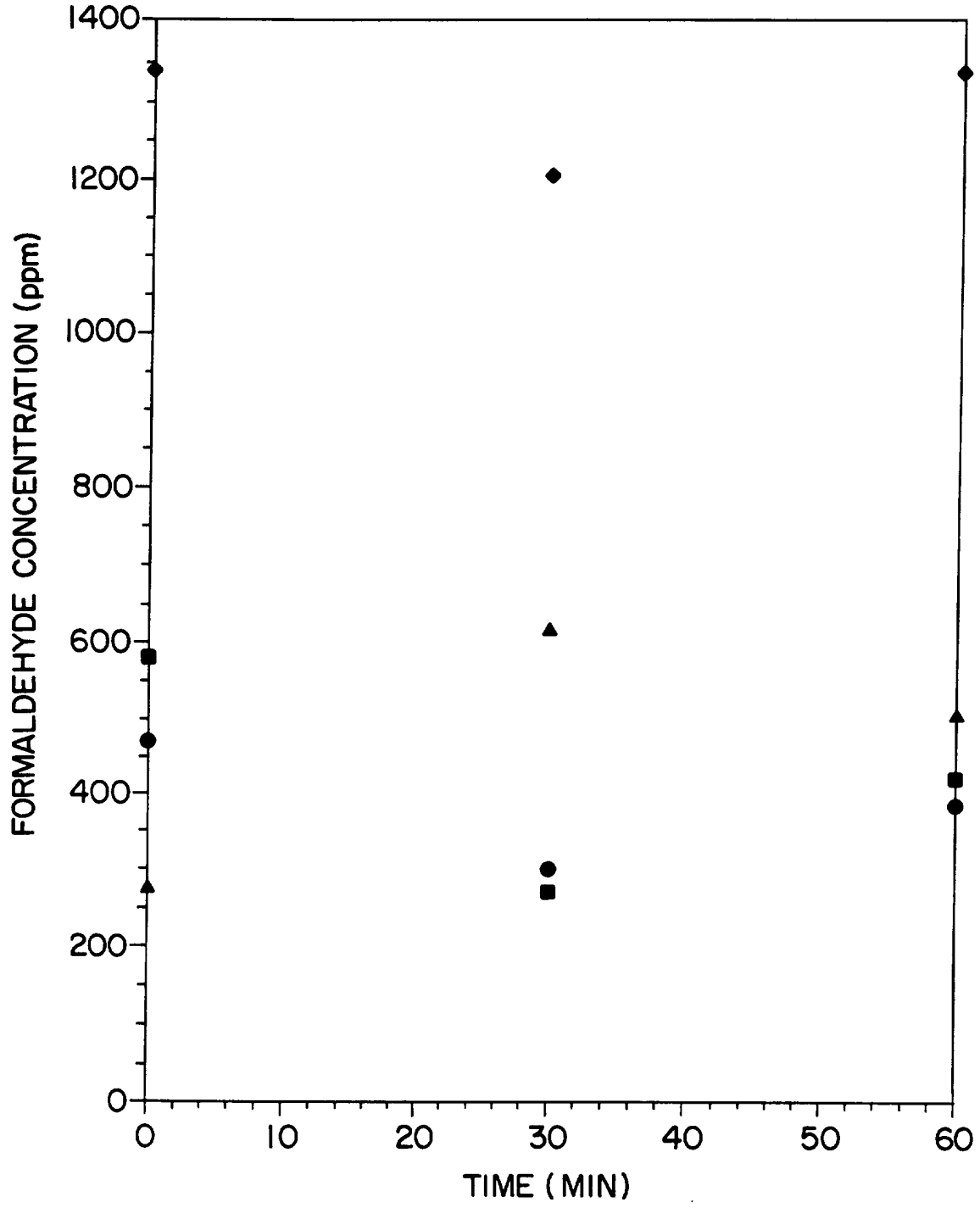
FORMALDEHYDE CONCENTRATION (ppm)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
TIME (MIN)

FIG. 5

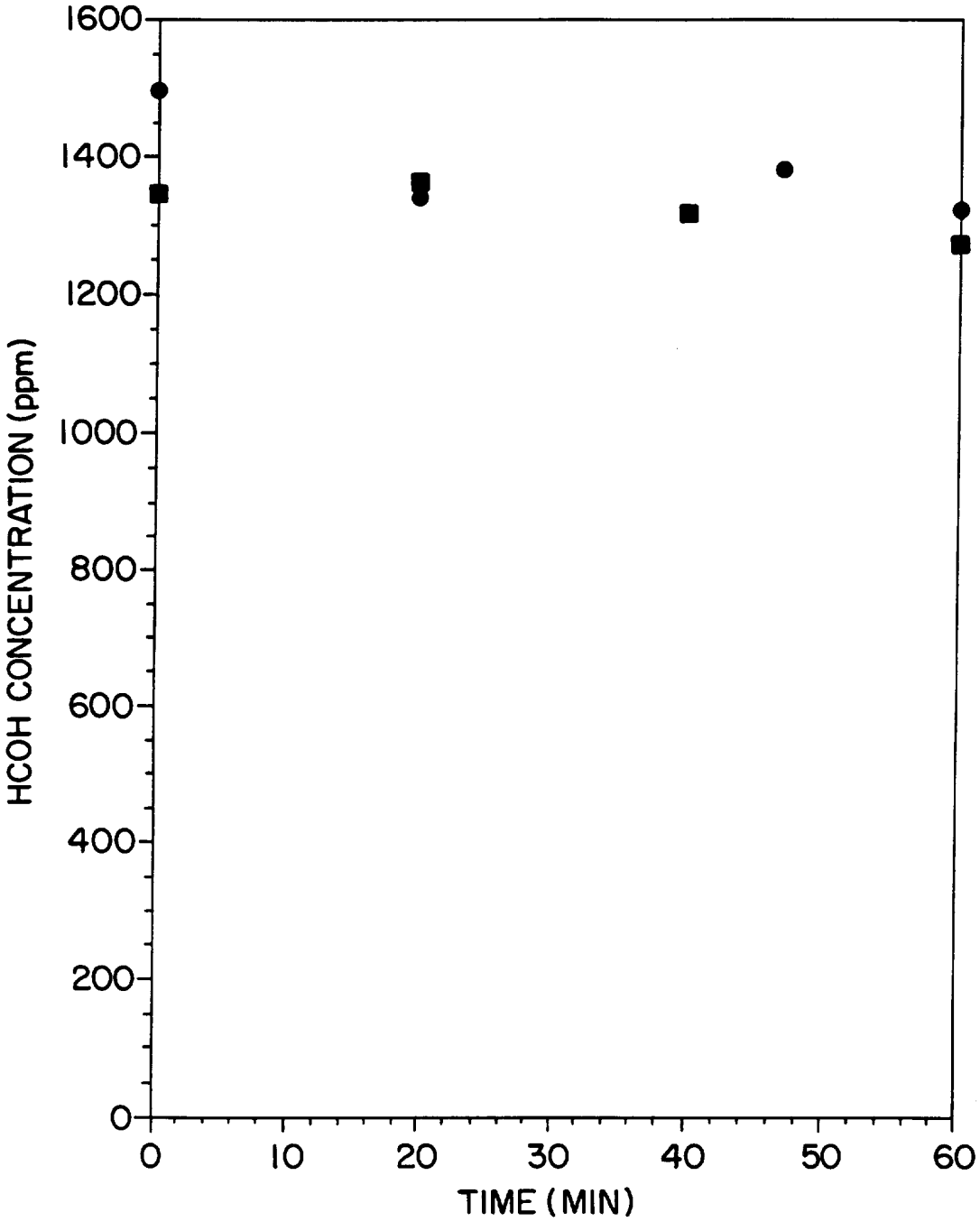
1600
1400
1200
1000
800
600
400
200
0
HCOH CONCENTRATION (ppm)
0
10
20
30
40
50
60
TIME (MIN)

FIG. 6

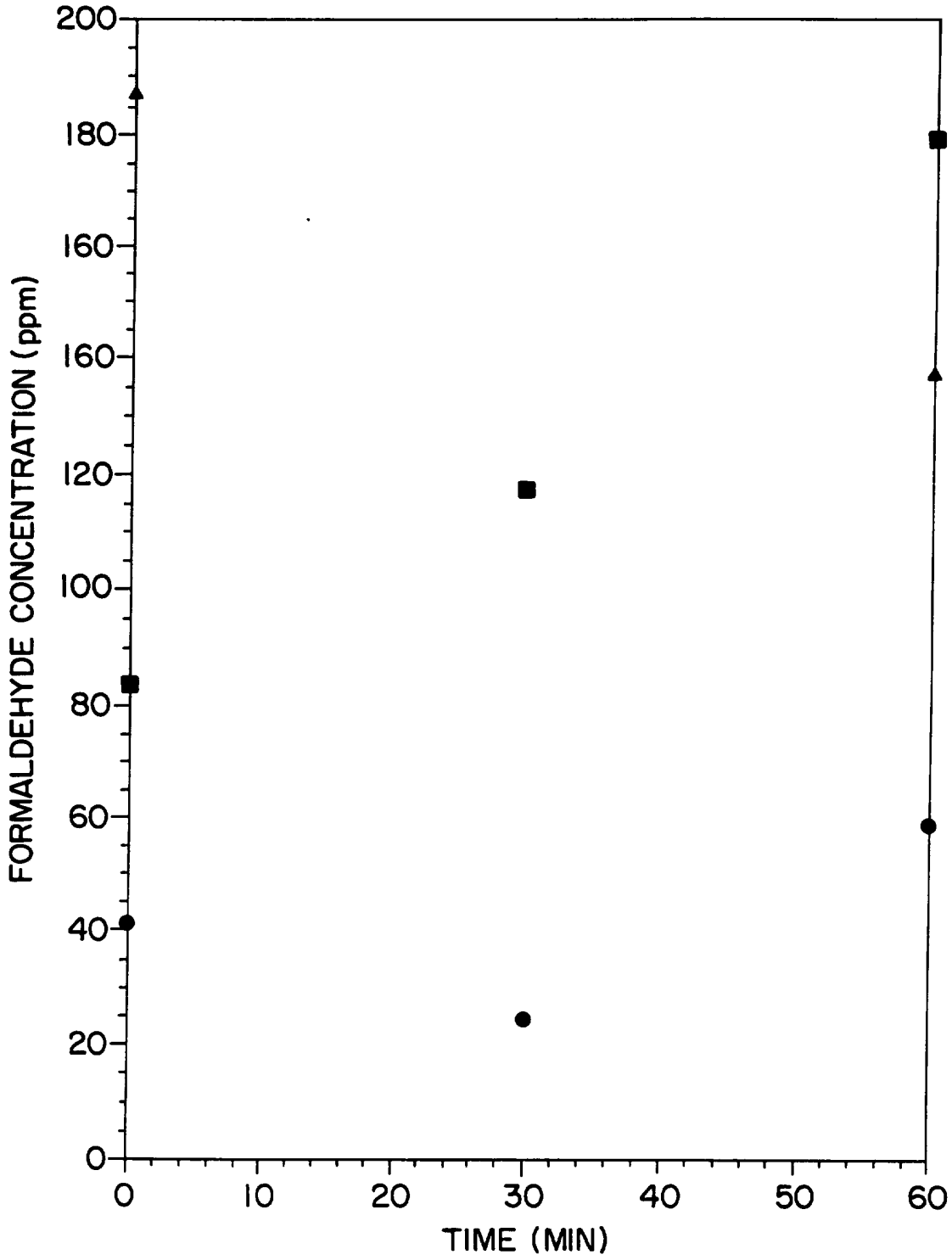
200
180
160
160
120
100
80
60
40
20
0
FORMALDEHYDE CONCENTRATION (ppm)
0
10
20
30
40
50
60
TIME (MIN)

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03213

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 1/02
US CL :422/1, 28, 36; 435/1, 2; 514/693, 695, 699
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. : 422/1, 28, 36; 435/1, 2; 514/693, 695, 699

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)

APS
search terms: blood, plasma protein, formaldehyde, transfusion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 4,833,165 (LOUDERBACK) 23 May 1989 (23.05.89), see entire document.	1-5 --- 30, 33, 39-40
X	US, A, 4,675,159 (AL-SIOUFI) 23 June 1987 (23.06.87), see entire document.	1-5
X	US, A, 4,880,602 (AL-SIOUFI) 14 November 1989 (14.11.89), see entire document.	1-5
X --- Y	Transfusion, The Journal of The American Association of Blood Banks, Vol. 32, Number 8S, issued 1992 October (Bethesda, Maryland), A. Louderbeck, "Sterilization of Red Blood Cells", page 66S.	1-28, 31-32, 34-38, 41 --- 30, 33, 39-40

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KRISANNE M. THORNTON Telephone No. (703) 308-0651

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03213

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,074,838 (KROYER) 24 December 1991 (24.12.91), see entire document.	1-41