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(54) Title: STARCH-IODINE USED AS A PRESERVATIVE

(57) Abstract

The treatment and preservation of blood, blood derivatives and other body tissues, fluids and cells with starch-iodine and then quenching the biocidal potential of iodine in the iodine to kill pathogenic microbes without destroying the utility of the tissues, fluids and cells is disclosed.

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Starch-Iodine used as a preservative

Cross-Reference to Related Patent Applications

This is a continuation-in-part of Serial No. 577,204, and Serial No. 577,295, both Filed September 4, 1990, and Serial No. 753,734, filed September 3, 1991; Serial No. 753,814, filed September 3, 1991; and Serial No. 754,752, filed September 4, 1991, to which priority is claimed.

Field of the Invention

This invention relates to the treatment and preservation of blood and blood derivatives, the treatment and preparation of other body tissues and cells, the treatment and preparation of tissue cultures and tissue culture products, and the preparation of laboratory reagents, standards and samples. According to the invention complexes of iodine with starch *per se*, or components of starch, e.g. amylose or amylopectin, and analogous iodine-binding polysacharoses and derivatives thereof, all of which are referred to generally and collectively as "starch" hereinafter, unless otherwise indicated by context, are used in the treatment of treating biological materials. Thereafter a physiologically compatible reducing agent such as an ascorbate salt, as an additive or on a solid support, e.g. in a bed or filter of solid albumin, starch, povidone, etc,, may be used to remove the last traces of biocidal iodine. This invention may, thus, be used to kill or inactivate virus, bacteria, chlamydia, rickettsia, mycoplasma and other potentially pathogenic microorganisms and to remove all biocidal iodine.

The treatment and preparation of human blood, tissues, etc. and of the blood, tissues, etc. of other animals are contemplated. In general, the field of this invention lies in medicine and veterinary practice; most examples being related to the practice of medicine for the benefit of human patients, use in analogous fields of veterinary medicine to the extent applicable being within the scope of the invention.

Background of the Invention

Iodine was officially recognized by the Pharmacopeia of the United States in 1930, also as tincture iodine (tincture of iodine) and linimentum iodi (liniment of iodine). Clinicians and microbiologists described a great number of experimental data and clinical applications, which can be found in numerous

surveys. Despite the successes that have been achieved with iodine, it was ascertained early that it also possesses properties unsuitable for practical application, including, for example, the fact that iodine has an unpleasant odor. In addition, it stains the skin with an intensive yellow-brownish color, causes blue stains in the laundry in the presence of starch, and combines with iron and other metals, its solutions are not stable, it irritates animal tissue, and is a poison. The adverse side effects of iodine, its painfulness on open wounds and the possibility of allergic reactions in the past 100 years led to the production of a great many iodine compounds (and iodine preparations), with the aim of avoiding these incompatibilities without a significant loss of germicidal efficiency. In this connection, the iodophors finally succeeded as near-ideal forms of application. A new iodine complex that has all or most of the advantages of the iodophors, e.g. povidone-iodine is described in this application.

Although exact details about the killing of a living cell by the I_2 molecule (or one of the reaction products occurring in aqueous solution) are not known, it can be assumed that iodine reacts:

(1) With basic N-H functions that are parts of some amino acids (e.g., lysine, histidine, arginine) and the bases of nucleotides (adenine, cytosine, and guanine) forming the corresponding N-iododerivatives. By this reaction, important positions for hydrogen bonding are blocked, and a lethal disorder of the protein structure may occur.

(2) Oxidizing the S-H group of the amino acid cysteine, through which the connections of protein chains by disulfide (-S-S-) bridges, as an important factor in the synthesis of proteins, are lost.

(3) With the phenolic group of the amino acid tyrosine, forming mono- or diiodo-derivatives. In this case, the bulk of the iodine atom(s) in the ortho position may cause a form of steric hindrance in the hydrogen bonding of the phenolic OH group.

(4) With the carbon-carbon double bond (C=C) of the unsaturated fatty acids. This could lead to a change in the physical properties of the lipids and membrane immobilization.

Iodine is consumed by proteinaceous substrates and its efficacy as a disinfectant is reduced at certain antiseptic applications. This is due to a reducing effect of the material to be disinfected which leads to the conversion of iodine into non-bactericidal iodide. Thus, not only the reservoir of available iodine is diminished but also the equilibrium of triiodide is influenced as well. Both of these effects cause a decrease in the proportion of free molecular iodine, the actual anti-microbial agent.

Iodine is used widely in human medicine is the disinfection of skin, (e.g., the preoperative preparations of the skin, the surgical disinfection of hands, the disinfection of the perineum prior to delivery, and the disinfection of the skin prior to infections and transfusions). Iodine preparations are also used for therapeutic purposes, e.g., the treatment of infected and burned skin but is a strong irritant. Iodine has also been used for the disinfection of medical equipment, such as catgut, catheters, knife blades, ampules, plastic items, rubber goods, brushes, multiple-dose vials, and thermometers.

The use of "oxidizing iodine" including "compounds incorporating molecules of biocidal iodine" e.g. absorbed or grafted on a purified vegetable carbon, as blood-contacting reagents having bactericidal and bacteriostatic action are mentioned in passing in connection with an autotransfuser device in U.S. Patent 4,898,572, Surugue nee Lasnier, et al but without any explanation or elucidation.

Iodine is an excellent, prompt, effective microbicide with a broad range of action that includes almost all of the important health-related microorganisms, such as enteric bacteria, enteric viruses, bacterial viruses, and protozoan cysts, if the sometimes severe limitations inherent in its use are overcome. Mycobacteria and the spores of bacilli and clostridia can also be killed by iodine. Furthermore, iodine also exhibits a fungicidal and trichomonacidal activity. As to be expected, varying amounts of iodine are necessary to achieve complete disinfection of the different classes or organisms. Within the same class, however, the published data on the disinfecting effect of iodine correspond only to a small extent. In particular, the published killing time os spores and viruses are widely disparate.

Various authors have tried to summarize the disinfecting properties of iodine and the other halogens by reviewing the literature and analyzing the existing data. The most important conclusions are:

(1) A standard destruction (i.e., a 99.999% kill in 10 minutes at 25o C) of enteric bacteria, amoebic cysts, and enteric viruses requires I_2 residuals of 0.2, 3.5, and 14.6 ppm, respectively.

(2) On a weight basis, iodine can inactivate viruses more completely over a wide range of water quality than other halogens.

(3) In the presence of organic and inorganic nitrogenous substances, iodine is the cysticide of choice because it does not produce side reactions that interfere with its disinfecting properties.

(4) Iodine would require the smallest mg/L dosage compared to chlorine or bromine to "break any water" to provide a free residual.

(5) I_2 is 2 to 3 times as cysticidal and 6 times as sporicidal as HOI, while HOI is at least 40 times as virucidal as I_2. This behavior is explained on the one hand by the higher diffusibility of molecular iodine through the cell walls of cysts and spores and on the other hand by the higher biocidal power of HOI.

Gottardi, W. ***Iodine and Iodine Compounds*** in **DISINFECTION, STERILIZATION, AND PRESERVATION, Third Edition**, Block, Seymour S., Ed., Lea & Febiger, Philadelphia, 1983, and the references cited therein provide more details respecting the background discussed above.

The classic blue starch-iodine complex is well-known and the reaction of iodine with starch is used as an indicator reaction in may diverse types of iodine analysis.

It is also known that starch-iodine complexes have some biocidal activity, although no comprehensive studies of this activity have been identified.

Solid beads of cross-linked beads of starch-iodine that absorb moisture from wounds, etc., and also provide a source of iodine are well-known; see, e.g., Holloway, G. Allen, Jr.; Johansen, Kaj H.; Barnes, Robert W.; Pierce, George E., Multicenter trial of cadexomer iodine to treat venous stasis ulcer, Western Journal of Medicine, v151 n1, p35(4), July, 1989. Cadexomer iodine is a starch polymer bead with iodine attached to the polymer. This type of polymer bead kills bacteria with the iodine and is used in the treatment of open wounds and venous stasis ulcers. Cadexomer iodine (IODOSORB® PERSTORP CARBOTEC®, Perstorp, Sweden]), is a starch polymer bead similar to dextranomer but with

iodide (0.9% weight per weight) bonded to the polymer. When fluid contacts the beads, substantial amounts of fluid--as much as 6 ml per gram of cadexomer iodine--are absorbed, as well as bacteria within the fluid. Bonded iodide becomes bactericidal in this milieu.

Other somewhat limited descriptions of the use of starch-iodine have also been published. For example, Glushankof S I, et al, Russian patent SU 1204575, describes compositions for purificationn and decontamination of water that contains starch, aluminium sulphate, iodine and activated charcoal to produce drinking water from open reservoirs in field conditions. A starch-based disinfectant composition prepared by treating starch suspension with potassium permanganate followed by a solution of iodine in potassium permanganate is described by Tatarov P G, et. al., Russian patent SU 979363 821207. One would infer that the product described by Glushankof, et al, is a solid bed of charcoal coated or containing the starch, iodine, and aluminum sulfate.

A protective coating for stored fruit and vegetables containing slurried starch, iodine, potassium iodide and sodium bicarbonate is described by Popova E R, et. al., Russioan Patent SU 959733, 820928.

Iodine starch for disinfection is described by Mochnacz, W. et. al., Ptitsevodstvo, 1980, 11, p. 37; Zh., Vet. 1981, Abstr. No. 38226 . (Amyloiodine as bactericides, disinfectants, antiseptics, fungicides, fungistats, virucides and virustats; (CAS REGISTRY NUMBERS: 7553-56-2).

Johansson, J. A. O., CA: 92(24)203621a, Australia patent AU 506419, 800103, describes the manufacture of dextrin oxyethylated iodophor disinfectant. See also, CA: 86(24)177326n, US 4010259

Alferov, V. V., et al, CA: 82(9)52180,: Tr. Uzb. Nauchno-Issled. Inst. Vet., 1973, V.21,, 238-40, describes an iodinol sperm disinfectant and starch iodinated sperm, and the disinfection of sperm;with starch,iodine complexes. See also, CA: 82(7)39339k, Use of iodinol during artificial insemination of animals, Aliev, N. Ya.; Rakhimov, K. K.; Alferov, V. V.; Pulatov, T., Tr. Uzb. Nauchno-Issled. Inst. Vet., 1973 V 21, 241-3.

CA: 68(10)43119s, Iodinated high polymers and their application in medicine and veterinary science, Mokhnach, V. O., Botan., Iodonol Med. Vet.,

1967 5-20, mentions iodine complexes with polyvinyl alcohols, starch, and polymers.

CA: 67(14)67490m, Bactericidal water filter , Panzer, Hans W. P.; Brown, Jerry Hugh, France patent FR 1462968, describes water purification by filtration and sterilization by iodine. The use of soluble organo-iodine compounds in water purification is, of course, well-known. One of the more popular water purifiers is tetraglycine hydroperiodide, which is widely used because of its effectiveness against giardia. Iodine has also been used to treat swimming pools, etc., but is objectionable because it irritates the eyes and stains the pool walls an unattractive yellow color.

Those who deal with blood and other invasively obtained body fluid samples risk infection from the samples. Those at risk include the doctor, nurse or clinical technician who takes the sample, the technicians who handle the sample and who use the sample in conducting analyses and tests, those who handle the sampling and testing equipment and apparatus, and the entire chain of individuals who attend to the disposal of sampling apparatus and the like, from the individuals who pick up the used apparatus through those who ultimately dispose of the apparatus, usually in specially designed high temperature furnaces. The risk is substantial, as evidenced by the fact that nearly all health care professionals with long experience carry the Epstein-Barr virus (EBV) and/or cytomegalovirus (CMV), the latter being probably the most ubiquitous of the pathogenic viruses. Other pathogenic viruses to which health care workers, and those who handle blood and fluid sampling and handling apparatus, are exposed include hepatitis and human immunodeficiency virus (HIV) as well as a large number of less life-threatening viruses.

Another organism which is frequently present in blood and blood products or fractions and which presents a serious risk in certain procedures is the bacteria *Yersinia enterocolitica* which is become a serious contaminant, surpassing *Salmonella* and *Campylobacter* as a cause of acute bacterial gastroenteritis. A significant increase in transfusion related infections of *Y. enterocolitica* has been reported, Tipple, et al., ***Transfusion*** 30, 3, p.207 (1990). *Y. enterocolitica* and other bacteria which propagate at relatively low temperatures, e.g. *Staphylococcus*

epidermidis and *Legionella pneumophila*, present, potentially, a serious threat in blood products.

It is generally recognized that proteinaceous materials destroy the biocidal effectiveness of iodine and iodophors such as PVP-I. This factor has been considered a major impediment to the use of iodine and iodophors in the presence of large amounts of biological materials. Albumin has been identified as having and extremely high capability of de-activating the biocidal power of iodine and iodophors. For example, Batts W N; Landolt M L; Winton J R, *Appl Environ Microbiol* 57 (5). 1991, 1379-1385, reported the results of using iodine in fishery waters to kill virus that iodine efficacy decreased when proteinaceous material was added to the water. Bovine serum albumin blocked iodine inactivation of the virus more effectively than did equal concentrations of fetal bovine serum or river sediment. Batts, et al, also noted that sodium thiosulfate effectively neutralized free iodine.

In addition to the risk of transmitting infectious disease via blood or blood products, the growth of bacteria in blood and blood products at various stages of production and processing introduces pyrogens into the blood component or product which must be removed before the product can be used in therapy. Introduction of molecular iodine, e.g. povidone-I_2, at an early stage in processing of blood products greatly reduces or eliminates the pyrogen-load of the ultimate product or fraction.

Protozoa give rise to many diseases, some of great medical and economic importance. Examples of such protozoa are the genus *Plasmodium*, e.g. *P. falciparum, P. malariae, P. ovale* and *P. vivax*, which causes malaria, *Trypanosoma*, which causes Chagas' disease, and *Leishmania*, which cause a variety of leishmaniasis. The method of this invention is effective in eliminating these causative organisms in blood and blood products.

Generally, this invention is applicable to the treatment of donated blood and products produced from blood, tissues and fluids for inactivating virus, bacteria, chlamydia, rickettsia, mycoplasma and other potentially pathogenic microorganisms.

Among the important potential pathogens to which this invention is applicable is cytomegalovirus (CMV), probably the most ubiquitous of the

pathogenic microorganisms found in animal fluids and tissues and herpesviruses generally. Herpesviruses, of which CMV is a member, represent a very large group of viruses which are responsible for, or involved in, cold sores, shingles, a venereal disease, mononucleosis, eye infections, birth defects and probably several cancers. The present invention is also useful in preventing the transmission of human immunodeficiency virus (HIV). While testing has made blood products safer than it was a decade ago, the complete elimination of HIV contaminated blood and blood products has not been possible using present knowledge and technology.

As used here, the term "blood" means whole blood and blood fractions, components, and products of blood, unless "whole blood" or a specific blood derivative, e.g. a blood fraction, component or product of blood is stated. Thus, the term "blood" may apply to whole blood at the time of collection or a blood derivative at any stage in processing, as indicated by context. Blood derivatives mean blood components such as blood cell concentrates (red blood cells, platelets, etc.), plasma, and serum and products and factors prepared from blood such as albumin and the blood factors. Body tissues and cells means any tissue(s), organ(s) or cells or fluids which contain tissue(s), organ(s) or cells of animal origin. Thus, in a broad sense, body tissues and cells include blood and the cellular components of blood; however, for the most part, simply for clarity in presentation, blood is treated as a separate application of the invention. Examples of body tissues and cells include bone marrow, kidneys, cornea, heart valves, tendons, ligaments, skin, homograft or xenograft implants and prosthesis generally. Tissue and cell cultures means cells and tissues grown or enhanced in culture media and the culture media per se, but not including nutrients intended for use in cell cultures. Examples of a cultured tissue is cultured skin tissue for use in burn victims, cells and cellular products prepared by standard biological and/or genetic engineering techniques are other examples of tissue cultures. Laboratory reagents and standards, as used in this specification and the claims, means reagents and standards produced from or comprising human or animal fluids, cells or tissues. Examples of such products are red blood cell panel utilized for typing blood, control sera and chemistry controls. Samples of tissues and fluids to be tested include samples of blood, urine, sputum, cell smears, etc. While the term "donor"

is not usually applied to the individual from whom such samples are acquired, that term, "donor" will be used here in a more general sense to include the individual from whom any blood, tissue, cells or fluid is obtained for any purpose, and such term will be used to refer even to an unwilling donor.

If a tissue is explanted into the culture media for the purpose of propagating its cells, the procedure is called tissue culture whereas the explanting of individual cells into culture media would be called cell culture; however, both procedures are often referred to by the term "tissue culture" procedures without differentiation, unless the distinction is critical for some ancillary reason. This general usage of the term is employed here.

Tissue cultured cells are extremely fragile in many ways, having exacting requirements not only as to nutrients but also to the amount and type of resident organisms which can be tolerated, and culture media are highly susceptible to bacterial and/or viral infection.

It is, generally, impossible to define with precision the exact materials required to propagate a given cell line and, therefore, it is common practice to use media based upon or containing serum and to add nutrient serum as needed during the cell propagation. Bovine serum from adult animals may be suitable in some instances, but fetal bovine serum (FBS) (sometimes referred to as fetal calf serum (FCS)) is required for the safe propagation of many cell lines, and where high purity is critical. Even the use of FBS is not, however, a guarantee of freedom from infective agents. Indecd, every lot of commercially produced FBS is contaminated with infectious bovine viral diarrhea (BVD) virus and infections with infectious bovine rhinotracheitis (IBR), parainfluenza 3 (Pl 3) are extremely common. At best, pools of raw serum probably contain at least 10^4 infectious BVD virus particles per milliliter. Serum filtration is a common step in reducing the load of infectious organisms in serum, but serum quality can be damaged by filtration if significant amounts of serum components are adsorbed to the filters or if macromolecules are sheared. Shearing of macromolecules during filtration occurs generally when tangential flow filtration is used and turbulence develops. It is currently very difficult to obtain reliable results on the removal of BVD viruses from serum using filtration.

The presence of adventitious viruses in cell cultures is well recognized, and when the cultures are of primate origin there are serious hazards for the production of human viral vaccines. This is one reason for the increasing use of bovine cell cultures. These cultures, however, are not free from viral contamination. Calf kidney (CK) and calf testis (CT) cells were often infected by non cytopathic mucosal disease virus (MDV): the cells seemed morphologically healthy, but nearly all showed fluorescence with BVD antiserum and rabbit anti-bovine conjugate.

The risks of infection from whole blood are well-known. One of the great tragedies of modern medicine is the infection of many patients, most frequently hemophiliacs who require frequent blood transfusions, with HIV. The purification of the nation's and the world's whole blood for transfusion would constitute a monumental step forward in the history of medicine. The risks of infection from red blood cell concentrates is similar to comparable risks associated with whole blood.

The teachings of the prior art suggest that neither elemental (diatomic) iodine nor complexed iodine would be an effective and reliable biocide in a fluid or in a body, e.g. blood, packed or concentrated cells, organs, etc. in which massive amounts of protein are be available to react with the iodine.

Various medical and blood handling procedures are referred to hereinafter. These are all well-known procedures and steps in these procedures are fully described in the literature. The following references are provided for general background and as sources for detailed reference to the literature as to specific procedures: TECHNICAL MANUAL of the American Association of Blood Bankers, 9th Ed. (1985); **HLA TECHNIQUES FOR BLOOD BANKERS**, American Association of Blood Bankers (1984); **Developments in Biological Standardization**, Vols. 1-57, S. Karger, Basel; **CLINICAL IMMUNOCHEMISTRY**, The American Association for Clinical Chemistry; **MEDICINE**, Vols. 1 - 2, Scientific American, New York; **Care of the SURGICAL PATIENT**, Vols 1 - 2, Scientific American, New York; **CURRENT PROTOCOLS IN MOLECULAR BIOLOGY**, Greene Publishing Associates and Wiley-Interscience, John Wiley & Sons, New York.

Summary of the Invention

The present invention encompases processes of treating a liquid composition that contains bacteria, virus, or other pathogenic organisms to sterilized the same by reaction with starch-iodine in the liquid phase or contacting the liquid with starch-iodine such as passing the liquid through solid starch-iodine filters or columns. Starch-iodine may be iodine complexed with essentially the entire starch molecule, of any of the great variety of starches available from different sources, or with a polysacharide component, or derivative thereof, of starch, or an equivalent polysacharide, that binds to starch, amylose an amylopectin being the most widely available of these components.

Thereafter, if it is desired to assure total iodine removal, the solution can be passed into contact with solid starch, e.g. filtered through a bed or column of solid starch particles that are less than saturated with iodine, preferably having no more than a trace of iodine or another iodine absorbing material, such as cross-linked povidone to remove the excess iodine. Likewise a reducing agent such as a reducing sugar, ascorbate, sodium sulfite, etc., may be added to eliminate the last traces of biocidal iodine. Reducing sugars, ascorbic acid (Vitamin C) and its salts, and sodium sulfite are well-known, readily available reducing agents that are physiologically acceptable. However, any physiologically acceptable reducing agents may be used.

The invention is embodied in a method of disinfecting biological materials. The steps of the method include treating biological material before separation of the components thereof with starch-iodine to provide from a concentration of 0.1^{w}/o to 5^{w}/o starch-iodine in said material before separation of the components thereof. A derivative of the material resulting from the preceding step is prepared and, optionally, also treated with starch-iodine to provide from 0.1^{w}/o to 5^{w}/o starch-iodine in the derivative. Also optionally the derivative may be treated by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual iodine. These methods are applicable, for example, to whole blood, plasma, tissue,

culture nutrient, packed red blood cells and cell-bearing liquids or non-cell-bearing biological liquids.

Also included in the invention is the improved method of treating patients with plasma comprising the steps of collecting plasma from a donor, and thereafter infusing the plasma into the patient to be treated, of mixing the plasma with starch-iodine sufficient to resulting a starch-iodine a concentration of from about 0.1^{w}/o to about 5^{w}/o, and allowing contact of said plasma with said starch-iodine for at least about one-half minute sufficient to inactivate or destroy infective pathogenic microbes in the plasma and optionally thereafter removing biocidal iodine from the resulting mixture by passing said mixture into intimate contact with cross-linked PVP or albumin or adding a physiologically acceptable reducing agent.

An apparatus for treatment of liquid to kill microbes is also provided. The apparatus is in the form of a liquid container having, in use an upper reservoir portion for holding said liquid and a lower elutriation portion for recovering liquid and structure defining first and second beds of particulate matter, the first bed comprising substantially insoluble starch-iodine and the second bed consisting essentially of substantially insoluble PVP, starch or albumin; the beds being so formed and configured as to permit the passage of the liquid therethrough in intimate contact with the surfaces of the particles forming the respective beds. The first bed may be cross-linked PVP. The apparatus may comprising a third layer between the first and second layers, the third layer comprising substantially insoluble PVP hydrogen peroxide particulate matter. The apparatus may contain a layer of particulate matter comprising an iodine reducing agent. A layer of soluble starch-iodine may be provided on the first layer in the liquid reservoir. All or only part of the layers, after the first and second layers, may be provided.

One method of sterilizing an implantable tissue in accordance with this invention comprises placing tissue that is physiologically acceptable for implantation into a human patient into a vacuum chamber, evacuating the chamber and maintaining a vacuum on the chamber for a period long enough to extract at least about one-half of the unbound water originally present in said

tissue, and introducing into the vacuum chamber a solution of starch-iodine for thereby reconstituting into the tissue said solution in place of the water that was vacuum extracted. Optionally, iodine may be removed by washing or reconstituting the tissue with a reducing agent such as ascorbic acid or a salt thereof or sodium sulfite, for example.

Brief Description of the Drawings

Figure 1 depicts an apparatus for contacting a liquid material with starch-iodine and with either or both of (a) an iodine absorbing material and/or (b) an iodine reducing material, and for providing other materials for processing biological liquids, in particular, according to this invention.

Figure 2 depicts, largely schematically, an apparatus for treating solid tissue samples.

Description of the Preferred Embodiments

Starch-iodine may be prepared in any of a large number of methods. Substantially pure starch, that may have traces of other biological materials or be essentially free of contaminants is available commercially. Amylose and amylopectin, components of starch that are encompassed in the term "starch" as used in the general sense indicated above are also available in high purity. The chemistry of starch and polysacharides generally is well-developed and a large number of high purity starch and polysaccharide compositions are available commercially.

The term "pure," and its derivatives are used in the sense commonly used in reference to biologically isolates that inherently contain some biologicals other than the principal constituent. Trace amounts of other materials is not *per se* detrimental to the present invention, and can be tolerated unless they interfere with the iodine-amylose reaction or reaction of iodine with microbes.

The use of starch-iodine, optionally followed by treatment with a physiologically acceptable reducing agent for the manufacture of a medicament is contemplated by this invention. Such a medicament may, for example, consist essentially of blood cells in plasma or another carrier liquid. Such medicaments may be used for the treatment of disorders wherein the patient requires the transfusion of blood cells. Starch-iodine is added in an amount in

excess of that required to kill or inactivate all microbes is added. Starch-iodine may comprise, for example, from about 0.01 to 10 weight percent, preferably from 0.1^{w}/o to 5^{w}/o of the medicament. The starch-iodine is allowed to remain in contact with the blood cells or plasma, or other biological material being prepared to be a medicament, for a period of at least about a half a minute sufficient to kill the microbes, but not long enough to denature or otherwise injure the biological material. Usually, contact of under an hour is preferred. Accordingly, the contact times will be referred to as from one-half minute to one hour with the caveat that longer contact is not necessary or beneficial and may result in injury to the biological, but would, nevertheless, be within the scope of the invention. The reducing agent is then added in an amount to reduce substantially all iodine. The maximum amount of reducing agent required is easily calculated. The actual amount normally required, to which a safety margin amount will be added, is determined by an iodine assay on typical batches using known, routine procedures. A second treatment as described may be performed to assure total sterilization, if desired. Likewise, a second similar treatment may be performed on a product or fraction of the initial biological material treated as described above.

The use of starch-iodine and a physiologically acceptable iodine absorbent material, e.g.,solid albumin, solid starch, or cross-linked povidone, for the manufacture of a medicament is contemplated by this invention. Such a medicament may, for example, consist essentially of blood cells in plasma or another carrier liquid. Such medicaments may be used for the treatment of disorders wherein the patient requires the transfusion of blood cells. Either simultaneously therewith, or afterward, starch-iodine in an amount in excess of that required to kill or inactivate all microbes is added. Starch-iodine may comprise, for example, from about 0.01 to 10 weight percent, preferably from 0.1^{w}/o to 5^{w}/o of the medicament. The starch-iodine is allowed to remain in contact with the blood cells or plasma, or other biological material being prepared to be a medicament, for a period of at least about a half a minute sufficient to kill the microbes, but not long enough to denature or otherwise injure the biological material. Usually, contact of under an hour is preferred.

Accordingly, the contact times will be referred to as from one-half minute to one hour with the caveat that longer contact is not necessary or beneficial and may result in injury to the biological, but would, nevertheless, be within the scope of the invention. The mixture resulting from the above is then contacted with an iodine absorbing reagent such as cross-linked PVP, or coagulated albumin or solid starch, to remove the iodine. If desired, a reducing agent may thereafter be added in an amount to reduce any iodine that may not have been absorbed. The contact with the iodine absorbing material is preferably accomplished by passing the material undergoing treatment through a layer, i.e. a bed or filter, of solid, substantially insoluble albumin or starch. A second treatment as described may be performed to assure total sterilization, if desired. Likewise, a second similar treatment may be performed on a product or fraction of the initial biological material treated as described above.

In a similar manner, the "addition" of a reducing agent to the material undergoing treatment may be accomplished by passing the material through a layer of substantially insoluble material that has active reducing sites thereon or equilibrates with the liquid material undergoing treatment to partially dissolve into such liquid, or make readily available in said liquid (as by swelling, for example) reducing moieties. A bed of beads or fibers, for example, that expose on the surface thereof reducing sugar moieties may be used very conveniently.

Reference is made to Figure 1 of the drawing for a better understanding of the invention in one form. Figure 1 depicts an apparatus for contacting a liquid material with starch-iodine and with either or both of (a) an iodine absorbing material and/or (b) an iodine reducing material, and for providing other materials for processing biological liquids, in particular, according to this invention. The apparatus, being shown and described in a generally schematic fashion, may be in any of many configurations. The only significant structure, insofar as this invention relates is to the arrangement of the layers

The apparatus 10 may be viewed as a filter funnel or a column. As those in the art understand, the difference between a filter and a column is often insignificant in that both "filter" a liquid and both cause the liquid to

contact solid material. A filter may, indeed must, remove only part of the material. For example, either a filter or a column may let small cells or particles pass but retain larger cells, or it may permit only liquid and extremely small particles pass. The apparatus comprises cylindrical portion 12 that, in part, defines a reservoir portion. The reservoir may be large or very small as desired. The apparatus, in the configuration depicted comprises a second, smaller cylindrical tube portion 14 and a conical transition zone 16 connecting the two cylindrical portions as is conventional in funnel manufacture. It is again emphasized, however, that it is immaterial whether the apparatus defines a reservoir and or funnel portion of any particular size or configuration.

The apparatus defines a first layer 20 and a second layer 22. The first layer is made up of substantially insoluble starch-iodine. This layer is described as being made up of particulate materials in that the use or particulates in one way or another is usually involved. Particles of solid, insoluble starch-iodine, e.g. cross-linked starch-iodine, in the form of a layer or bed of particles, either supported directly by a layer below or by way of another support, e.g. being bonded to or entrapped within a layer of fibers or particles, is contemplated. The first layer may also contain some soluble starch-iodine. A frit made of particles bound together adhesively, by heat or pressure would also be within the disclosure and invention. The starch-iodine may be formed *in situ* by iodinating a layer of starch or the layer may be made up of pre-synthesized starch-iodine.

The second layer is downstream of the first layer, i.e. the liquid to be treated flows through the first layer and then the second layer. The second layer may comprise an insoluble iodine absorbent, e.g. cross-linked povidone, or insolublized albumin or starch, or an iodine reducing agent, or a mixture of both, or be a multiple sub-layer structure with a sublayer of iodine absorbent first and then a sublayer of iodine reductant. Again, the layer may be a self-supporting frit or other structure or may be supported by a support or other layer.

The essential function of the apparatus is to cause a liquid that is to be treated to pass, with or without cells or other particles therein, first through a

layer of starch-iodine and, thereafter, to contact such liquid with absorbent to remove the iodine and/or reductant to reduce the iodine. Hence, the layers may be quite deep or quite thin, adjacent each other or spaced from each other, as is necessary or desirable to provide adequate contact of the liquid with each of the layers or beds.

Such an apparatus is conveniently suited for the treatment of liquid to kill microbes in the liquid. The liquid container that is generally defined by the overall apparatus in the simplified, schematic example of Figure 1, and has an upper or liquid inflow reservoir portion for holding liquid to be treated. This may be a very small reservoir or quite large. The reservoir may displaced from the beds or layers by a very large distance, though this is not generally beneficial. The apparatus has a lower or elutriation or recovery portion for recovering liquid that has been treated. Between these portions, first and second beds of particulate matter are defined by suitable structure. The first bed or layer comprises substantially insoluble starch-iodine. The second bed consists essentially of substantially insoluble albumin or starch, or other iodine absorbent, and/or iodine reducing agent. The beds are so formed and configured as to permit the passage of the liquid therethrough in intimate contact with the surfaces of the particles forming the respective beds. The usual and most common iodine absorbent is cross-linked povidone.

The apparatus may desirably further comprise a third layer 24 between the first and second layers. The third layer comprises substantially insoluble povidone hydrogen peroxide particulate matter. The presence of the third layer entraps and regenerates iodine and significantly increases the biocidal activity of iodine.

A fourth layer 26, which may be in the form of a sublayer within the second layer, comprising particulate iodine reducing agent may be provided downstream from the second layer to provide for the reduction of any residual iodine from I_2 to iodide, or, if reduction is earlier provided, to add a safety step to assure that all biocidal iodine has been reduced.

In many applications, it may be desirable to provide a fifth layer 28 of soluble starch-iodine on the first layer in the liquid reservoir to permit the

actual dissolution into the liquid of substantial amounts of starch-iodine and thereby provide a greater reservoir of more available iodine to the liquid.

The first and second layers are essential to the full and proper functioning of the apparatus. After those layers or beds, however, any number of additional layers or additives may be provided, so long as they do not interfere with the combined function of the first and second beds or layers.

All of the layers just described may, conveniently but not necessarily, be supported by a layer 30 that may be a frit, a filter paper or a porous layer. The thickness of the beds may be the same or greatly different. It is a simple matter to calculate contact time in a column and to provide suitable beds of materials therein.

Any of the beds may be made up the active material, e.g. starch-iodine, reducing sugar, etc., attached to carrier particles, such as ground glass, charcoal, ion exchange resin, cellulose derivatives, etc. The particulate matter may, in a preferred form, consist essentially of particles having a diameter of from about 10 to about 100 microns, but any size that permits suitable flow rates and assures intimate contact may be used.

The use of starch-iodine and a physiologically acceptable reducing agent for the manufacture of transfusion biological material from one human or mammal for transfusion of such material to another human or mammal, or the transplant or transfusion biological material is a part of this invention. The transfusion or transplant is disinfected with a starch-iodine solution having concentration of from about 0.01 to 10 weight percent, preferably 0.1^{w}/o to 5^{w}/o, and thereafter treated with the reducing agent to reduce the residual iodine. Liquid materials may be treated in any suitable manner, such as has been described. Solid tissue samples may be treated simply be soaking, by infusing or by vacuum infusing. Figure 2 depicts, largely schematically, an apparatus for treating solid tissue samples. The apparatus comprises a chamber system 100 capable of withstanding the forces of a vacuum. In the merely exemplary form shown, a cylinder 102 is closed at the respective ends by end covers 104 and 106, the end 106 being removable to gain access to the inside of the chamber. For example, a portion 108 of the end 106 may be slipped

into the cylinder 102 and sealed using "O" rings, etc., to provide a vacuum tight seal. A vacuum line 110 through valve 112 and line 114 permits evacuation of the chamber. An input line 120, coupled to valve 122 and line 124 permits the introduction of liquid into the chamber. A platform 126, secured to the end 106, supports a tissue sample 130. The tissue sample is placed in the chamber, the chamber evacuated and then liquid is introduced, thereby substantially replacing water in the sample with the liquid introduced.

Implantable tissues may be treated to kill microbes, i.e. "sterilized" by placing tissue that is physiologically acceptable for implantation into a human patient into a vacuum chamber, evacuating the chamber and maintaining a vacuum for a period long enough to extract at least about one-half of the unbound water originally present in said tissue and then introducing into said vacuum chamber a solution of starch-iodine for thereby reconstituting into the tissue said solution in place of the water that was vacuum extracted. The thus treated tissue may then be soaked in a solution of an physiologically acceptable iodine reducing agent. Alternatively, the chamber may again be evacuated to extract the starch-iodine solution from the tissue and a solution of physiologically acceptable iodine reducing agent introduced into the vacuum chamber for saturating the tissue for reducing any residual iodine.

As a method of disinfecting blood derivatives, the invention may comprise treating blood before separation of the components thereof with starch-iodine to provide from a concentration of from about 0.01 to 10 weight percent, preferably 0.1^{w}/o to 5^{w}/o, starch-iodine in the blood, preparing a derivative of the blood from step, treating the derivative with starch-iodine to provide from about 0.01 to 10 weight percent, preferably 0.1^{w}/o to 5^{w}/o, iodine in the derivative thereafter treating the derivative by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual iodine.

Infective pathogenic microorganisms are believed to be inactivated when starch-iodine is used in solution to perfuse tissues and organs after removal from the donor and before transplantation to the recipient. The perfusion solution comprises starch-iodine in a concentration of from about 0.01 to 10

weight percent, preferably 0.1^{w}/o to about 5^{w}/o (100 to 5000 ppm I_2), preferably from about 0.25^{w}/o to about 2^{w}/o. After a period of time, most of the unreacted starch-iodine is washed away and any residual iodine is absorbed into the protein or converted to inactive iodides, e.g. using ascorbate or other reducing agent as described, and does not significantly interfere with acceptance by the recipient.

The above applications in which the material to be purified is a liquid or cells carried in a liquid can be carried out by flowing the liquid through a bed (e.g. the conventional filter structure of solid particles on a porous or foraminous support) of solid particles of starch-iodine of suitable size or by contacting the liquid and/or the cells in the liquid with particles or a membrane or surface of solid starch-iodine. Where a bed of particles is used with a cell-bearing liquid, the particles must be large enough to permit intimate contact without entrapping or binding the cells. The liquid may then be passed through a layer or in contact with solid phase starch-iodine to assure complete biocidal effect. Thereafter, the liquid is passed through or into intimate contact with cross-linked PVP to absorb the molecular iodine from the liquid. Finally, a reducing agent such as ascorbate may be added if considered necessary as a precaution.

In carrying out this facet of the invention, the liquid or cell-bearing liquid is contacted with the solid starch-iodine. This may be done most efficiently, in most cases, by passing the liquid through a settled or fluidized or packed bed of starch-iodine particles; however, such approaches will not, ordinarily, be suitable for treating cell-bearing liquids. Cell-bearingliquids may be treated by mixing the particles in a container of the liquid or passing the liquid over a surface of the starch-iodine material, e.g. over a multiple-plate array of sheets of such material. The starch-iodine may be washed and the iodine content therein regenerated between uses.

In general a solution of reducing agent , e.g. a reducing sugar (or mixtures of reducing sugars), ascorbic acid or ascorbate, a sulfite, e.g. sodium sulfite, etc. in which the agent is in a concentration of 0.001 to 1 percent is suitable and such is implicit unless otherwise noted.

Solvent extraction of iodine with a solvent for iodine that is substantially inert to blood and biological materials generally may also be used to remove iodine. N-heptane is a good solvent for iodine and has minimal effect, on short exposure, to blood and blood cells. Close n-alkane analogs and vegetable oils may also be used. Cotton seed oil, corn oil, etc. are generally biologically inert as to blood and blood cells and are also suitable solvents for iodine. The solvent extraction may be carried out in any suitable vessel that will permit intimate mixing of the blood and solvent and decantation of the hydrophobic phase from the top or withdrawal of the blood or biological liquid from the bottom. The process results in transfusion quality whole blood that is safe, being free of pathogenic microbes, and which is also free of any added chemicals except for iodide

Industrial Application

This invention finds application in medicine and veterinary science.

WHAT IS CLAIMED IS:

1. The use of starch-iodine for the manufacture of a medicament consisting essentially of blood cells in plasma or another carrier liquid for the treatment of disorders wherein the patient requires the transfusion of blood cells, the starch-iodine being added in an amount in excess of that required to kill or inactivate all microbes therein comprising from 0.01^{w}/o to 10^{w}/o of the medicament.

2. The use of starch-iodine and a physiologically acceptable reducing agent for the manufacture of transfusion biological material from one human or mammal for transfusion of such material to another human or mammal, the transplant or transfusion biological material being disinfected with a starch-iodine solution having concentration of from 0.01^{w}/o to 10^{w}/o and optionally thereafter adding said reducing agent to reduce the residual iodine.

3. A method of disinfecting biological materials comprising the steps of:

(a) treating biological material before separation of the components thereof with starch-iodine to provide from a concentration of 0.01^{w}/o to 10^{w}/o starch-iodine in said material before separation of the components thereof;

(b) preparing a derivative of the material resulting from step (a);

(c) treating said derivative with starch-iodine to provide from 0.01^{w}/o to 10^{w}/o starch-iodine in the derivative; and optionally

(d) removing residual iodine by reduction, absorption or solvent extraction.

4. The method of Claim 3 wherein the biological material is whole blood.

5. The method of Claim 3 wherein the biological material is blood plasma.

6. The method of Claim 3 wherein the biological material is body tissue.

7. The method of Claim 3 wherein the biological material is tissue culture nutrient.

8. The method of Claim 3 wherein the biological material is packed red blood cells.

9. The method of Claim 3 wherein the biological material is a cell bearing liquid.

10. In the method of treating patients with plasma comprising the steps of collecting plasma from a donor, and thereafter infusing the plasma into the patient to be treated, the improvement comprising the additional steps of: mixing the plasma with starch-iodine sufficient to resulting a starch-iodine a concentration of from about 0.01^{w}/o to about 10^{w}/o, and allowing contact of said plasma with said starch-iodine for at least about one-half minute sufficient to inactivate or destroy infective pathogenic microbes in the plasma and optionally thereafter removing biocidal iodine from the resulting mixture by passing said mixture into intimate contact with cross-linked PVP, solid starch or albumin or adding a physiologically acceptable reducing agent.

11. An apparatus for treatment of liquid to kill microbes therein comprising a liquid container having, in use an upper reservoir portion for holding said liquid and a lower elutriation portion for recovering liquid and structure defining first and second beds of particulate matter, the first bed comprising substantially insoluble starch-iodine and the second bed consisting essentially of substantially insoluble iodine absorbent material; the beds being so formed and configured as to permit the passage of the liquid therethrough in intimate contact with the surfaces of the particles forming the respective beds.

12. The apparatus of Claim 11 wherein the substantially insoluble iodine absorbent material is cross-linked PVP, albumin or starch.

13. The apparatus of Claim 11 further comprising an additional layer between the first and second layers, said additional layer comprising substantially insoluble PVP hydrogen peroxide particulate matter.

14. The apparatus of Claim 11 further comprising an additional layer of particulate matter below the second layer, said additional layer comprising an iodine reducing agent.

15. The apparatus of Claim 11 further comprising an additional layer of soluble starch-iodine on the first layer in the liquid reservoir.

16. The apparatus of Claim 11 wherein the insoluble starch-iodine particles of the first layer are physically supported by a layer of fibrous material.

17. A method of sterilizing an implantable tissue comprising:

(a) placing tissue that is physiologically acceptable for implantation into a human patient into a vacuum chamber;

(b) evacuating said chamber and maintaining a vacuum on said chamber for a period long enough to extract at least about one-half of the unbound water originally present in said tissue; and

(c) introducing into said vacuum chamber a solution of starch-iodine for thereby reconstituting into the tissue said solution in place of the water that was vacuum extracted;

18. The method of Claim 17 further comprising the step of soaking the thus treated tissue in a solution of an physiologically acceptable iodine reducing agent.

19. The method of Claim 17 further comprising the following steps after said step (c):

(d) evacuating the chamber to extract the starch-iodine solution; and

(e) introducing a solution of physiologically acceptable iodine reducing agent into the vacuum chamber for saturating the tissue with said solution for reducing any residual iodine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/05519

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61M 37/00
US CL :604/4
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. : 422/28,29,37,30,31; 435/269,2; 424/78.25,530,529,667-669,672;604/4-6,48,49

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)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ---- A	US,A, 4,971,760 (RUBINSTEIN), 20 NOVEMBER 1990, SEE ENTIRE DOCUMENT	10 ---- 1-9,11-19
Y --- A	US,A, 4,320,114 (DENZINGER ET AL), 16 MARCH 1982, SEE ENTIRE DOCUMENT.	10 ---- 1-9,11-19
Y --- A	US,A, 4,849,215 (GOTTARDI), 18 JULY 1989, SEE ENTIRE DOCUMENT.	10 ------ 1-9,11-19
A,P	US,A, 5,185,371 (RUBINSTEIN), 09 FEBRUARY 1993, SEE ENTIRE DOCUMENT.	1-19

☐ 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 part 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		

Date of the actual completion of the international search
03 AUGUST 1993

Date of mailing of the international search report
OCT 28 1993

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/05519

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

Please See Extra Sheet.

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

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

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

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

LIT Search: starch-iodine (p) plasma or blood
starch-iodine (p) microbes
starch (2) iodine (p) PVP or polyvinyl pyrrolidone-iodide

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1 and 2, drawn to an invention, classified in class 424, subclass 520.

Group II, claims 3-9, drawn to a method of disinfecting biological materials, classified in class 422, subclass 28.

Group III, claim 10, drawn to a method of treating plasma, classified in class 424, subclass 530.

Group IV, claims 11-16, drawn to an apparatus for treatment of liquid to kill microbes, classified in class 210, subclass 266.

Group V, claims 17-19, drawn to a method of sterilizing an implantable tissue, classified in class 422, subclass 28.