(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 June 2008 (26.06.2008)

PCT

(10) International Publication Number
WO 2008/077155 A1

(51) International Patent Classification:
A61L 2/00 (2006.01) *A61L 2/20* (2006.01)

(21) International Application Number:
PCT/US2007/088728

(22) International Filing Date:
21 December 2007 (21.12.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/871,426 21 December 2006 (21.12.2006) US

(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LAM, Xanthe, M.** [US/US]; 662 Orchid Drive, South San Francisco, CA 94080 (US). **NGUYEN, Tue** [US/US]; 16 Woodleaf Ave., Redwood City, CA 94061 (US).

(74) Agent: **KALINOWSKI, Grant**; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

WO 2008/077155 A1

(54) Title: STERILIZATION OF OBJECTS CONTAINING BIOLOGICAL MOLECULES

(57) Abstract: The invention relates to methods for surface-sterilizing objects containing ethylene-oxide-sensitive, temperature-sensitive compounds, including biological molecules.

STERILIZATION OF OBJECTS CONTAINING BIOLOGICAL MOLECULES

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/871,426, filed December 22, 2007, which is incorporated by reference in its entirety.

Field of Invention

The invention relates to methods for surface-sterilizing objects containing ethylene-oxide-sensitive, temperature-sensitive compounds, including biological molecules.

Background of Invention

Objects used in medical applications are generally sterilized before use. Sterilization can be accomplished by a variety of methods including, *e.g.*, steam sterilization, radiation sterilization, gas sterilization (*e.g.* with ethylene oxide), and chemical sterilization. However, these treatments cannot be used for objects containing pharmaceutical compositions because their active ingredients are typically sensitive to them. For example, steam and gas sterilization are generally performed at high temperatures (approx. 45°C to 55°C or higher) that damage certain active ingredients in pharmaceutical compositions. Similarly, the agents used for radiation or chemical sterilization generally cause chemical damage to the active ingredients. Consequently, pharmaceutical compositions are generally sterilized by an alternative method, *e.g.* by filtration, and then packaged into separately sterilized objects. Because of the complexity of this process, it is difficult to also ensure the sterility of the surfaces of the objects.

In many circumstances it would be advantageous to sterilize the surfaces of these objects in order to reduce the risk of contamination during subsequent handling. For example, there is an increased risk of endophthalmitis after intraocular injection if the surface of the syringe used for injection is not sterilized. Thus, there remains a need for efficient and cost-effective methods of surface-sterilizing objects containing ethylene-oxide-sensitive, temperature-sensitive compounds, such as biological molecules, without a significant adverse effect on their activity or integrity.

Summary of the Invention

The invention relates to methods for surface-sterilizing objects containing ethylene-oxide-sensitive, temperature-sensitive compounds, such as biological molecules. The invention is based, in part, on the surprising discovery of ethylene-oxide-based sterilization conditions that will effectively sterilize the surface of an object but which do not significantly damage ethylene-oxide-sensitive, temperature-sensitive compounds contained inside.

In one aspect, the invention provides a method for surface-sterilizing an object having an ethylene-oxide(EtO)-impermeable interior space containing a compound with a temperature-sensitive and EtO-sensitive activity by exposing the object to EtO under conditions such that the object is surface-sterilized and the compound retains at least 50% of said activity. In some embodiments, the conditions comprise: a) temperature between 25°C and 35°C; b) EtO concentration of between 300 mg/L and 800 mg/L; and c) relative humidity between 45% and 60%; for between 1 and 6 hours. In some embodiments, the conditions comprise: a) temperature between 27°C and 33°C; b) EtO concentration of between 300 mg/L and 600 mg/L; and c) relative humidity between 48% and 52%; for between 1 and 6 hours. In some embodiments, the conditions comprise: a) temperature of 30°C; b) EtO concentration of 600 mg/L; and c) relative humidity of 50%; for 1, 1.5 or 2 hours.

In some embodiments, the compound retains at least 90% of said activity. In some embodiments, the compound is a polypeptide, *e.g.* an antibody, which includes monoclonal antibodies, chimeric antibodies, humanized antibodies or human antibodies. In some embodiments where the compound is a polypeptide, the percent alkylation of the polypeptide is not statistically different from a control polypeptide not exposed to EtO. In some embodiments, the antibody is an antigen-binding fragment, *e.g.* a Fab fragment. In some embodiments, the Fab fragment binds VEGF, *e.g.* ranibizumab (LUCENTIS®).

In some embodiments, the compound is present in an aqueous pharmaceutical composition, *e.g.* a composition comprising at least one of: an amino acid, a disaccharide and a non-ionic surfactant. In some embodiments the pharmaceutical composition comprises histidine, trehalose and polysorbate 20.

In some embodiments, the object is a syringe. In some embodiments the syringe comprises glass and comprises a stopper comprising D777-7 laminated with FluroTec®; and a tip cap comprising D777-7 laminated with FluroTec® or D21-7H laminated with FluroTec®. In some embodiments, the object is contained within a package comprising an EtO-permeable material, e.g. TYVEK®.

In another aspect, the invention provides an object produced by a method of the invention.

Detailed Description of the Invention

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the relevant art. All patents and publications mentioned herein are expressly incorporated by reference in their entireties for all purposes.

Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," indicate the inclusion of any recited integer or group of integers but not the exclusion of any other integer or group of integers.

As used herein, a "compound" is any molecule that has an activity which can be measured. Compounds according to the present invention generally have a pharmacological activity. For example, the activity of a compound may include the ability to bind to a particular molecule, to inhibit (or enhance) an enzymatic activity, induce a particular physiological response, *etc.*

As used herein, an "ethylene-oxide-impermeable" or "EtO-impermeable" object is one in which no more than 0.5 ppm EtO is present inside the object after EtO sterilization. As EtO-impermable object my comprise, e.g., glass and/or certain plastics.

As used herein, an activity is "ethylene-oxide-sensitive" or "EtO-sensitive" when the activity is reduced following exposure to ethylene oxide (EtO). In some embodiments, the exposure is to 10 ppm EtO at 30ºC for 3 days. In some embodiments, the activity is reduced by at least 90% following EtO exposure. In some embodiments, the activity is reduced by less that 90%, e.g. at least 80%, 70%, 60% or 50%.

As used herein, "percent alkylation" in the context of a polypeptide is the percentage of polypeptide that is in the basic peak relative to polypeptide that is in the acidic or main peaks as measured by IEC.

As used herein, an activity is "temperature-sensitive" when the activity is reduced following exposure to a high temperature, *e.g.* above room temperature. In some embodiments, the exposure is for 2 hours. In some embodiments, the activity is reduced following exposure to temperatures of at least 30ºC, *e.g.* at least 35ºC, 40ºC, 45ºC, 50ºC, 55ºC or 60ºC. In some embodiments, the activity is reduced by at least 90% following exposure to a high temperature. In some embodiments, the activity is reduced by less that

90%, e.g. at least 80%, 70%, 60% or 50%. In some embodiments, the activity is reduced by at least 5% or at least 1%.

As used herein, the surfact of an object is "sterilized" when the amount of at least one biological contaminant present on the surface of the object being treated according to the present invention is reduced following the treatment. Typically, the amount is reduced by at least one log (*i.e.* by at least 10-fold). In some embodiments of the invention, the amount is reduced by 2 logs, 3 logs, 4 logs, 5 logs, or 6 logs.

As used herein, a "biological contaminant" is a contaminant that, upon direct or indirect contact with a biological material, may have a deleterious effect on the biological material. Examples of biological contaminants include viruses; bacteria or bacterial spores; parasites; yeasts; molds; mycoplasmas; and prions. Further, a biological contaminant need not be naturally or accidentally present. For example, a biological contaminant may be *Bacillus subtilis* spores deliberately placed on the surface of an object to be sterilized in order to monitor the success of the sterilization.

As used herein, a "subject" is a human subject or patient.

As used herein, a "polypeptide" is broadly defined and includes both short polypeptides as well as longer polypeptides such as proteins and protein fragments. For example, the term polypeptide may include from dipeptides, tripeptides, and the like to enzymes, hormones, antibodies or any fragments of these that has an activity.

As used here, the term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity.

The term "monoclonal antibody" as used herein refers to an antibody from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope(s), except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Such monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones or recombinant DNA clones. It should be understood that the selected target binding sequence can be further altered, for example, to improve affinity

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