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(54) **Sterilised powders of Poly (3-hydroxybutyrate)**

(57) A medical dusting powder comprises a sterilised, particulate, 3-hydroxybutyrate polymer. The sterilisation may be effected by treatment with steam or by γ -irradiation. The polymer may include up to 60 mole % of other hydroxy-carboxylate units.

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SPECIFICATION

Powders

- 5 This invention relates to powders and in particular to finely divided powders for use as medical dusting powders:

In US-A-3846382 a review is given of various materials proposed for use as medical dusting powders, especially as surgical glove powders. In that specification, polyglycolic acid is proposed as such a dusting powder, but reference is made to the difficulty of obtaining that material in a sufficiently finely divided form in view of its hardness and

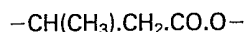
15 toughness.

The present invention provides an alternative material for use as a medical dusting powder which can more readily be produced in the desired particulate form and which also satisfies the other criteria of a satisfactory dusting powder, viz non-toxicity, biodegradability, no adverse effect on the body, capability of sterilisation, lubricity, non-irritability, and ready availability at an acceptable cost.

Accordingly the present invention provides a sterile medical dusting powder comprising a sterilised particulate 3-hydroxybutyrate polymer.

It also provides a process of making such a dusting powder comprising sterilising a particulate 3-hydroxybutyrate polymer.

- 30 Poly(3-hydroxybutyrate) is a thermoplastic polyester consisting of repeat units of the formula



35 which is accumulated by many micro-organisms, particularly bacteria, for example of the genera *Alcaligenes*, *Athiorhodium*, *Azotobacter*, *Bacillus*, *Nocardia*, *Pseudomonas*, *Rhizobium*, and *Spirillum*, as an energy reserve material.

40 The polymer is accumulated by the micro-organism in the form of granules within the micro-organism cell: typically the granules are spherical and have a diameter within the range 0.2 to 0.7 μm (see Senior et al "Advances in Microbial Physiology" (1973) 10 203-266 at pages 209-212). The use of poly(3-hydroxybutyrate) for absorbable medical applications, e.g. sutures and prosthetic devices is described in GB-A-1034123.

The polymer is conveniently prepared by cultivating the micro-organism in an aqueous medium on a suitable substrate, such as a carbohydrate or methanol, as an energy and carbon source. The substrate must, of course, be one that is assimilable by the micro-organism. In order to promote accumulation of the polymer, at least part of the cultivation is preferably conducted under conditions wherein there is a limitation of a nutrient that is essential for growth of the micro-organism but which is not required for polymer accumulation. Examples of suitable processes are described in EP-A-15669 and 46344.

Polymers containing both 3-hydroxybutyrate units and other hydroxy-carboxylate units, such as 3-hydroxyvalerate units, can also be produced micro-biologically. Thus a micro-biologically produced

heteropolymer containing 3-hydroxybutyrate and 3-hydroxyvalerate residues is described by Wallen et al in "Environmental Science and Technology" 8 (1974) 576-9. Also, as described in EP-A-52459 and 69497, various copolymers can be produced by cultivating the micro-organism on certain substrates, such as propionic acid which gives rise to 3-hydroxyvalerate units in the copolymer.

In the following description therefore, by the term HB polymer we mean not only 3-hydroxybutyrate homopolymer, but also copolymers as described above, provided that the 3-hydroxybutyrate residues form at least 40 mole %, and preferably at least 80 mole %, of the polymer chain.

- 80 For use as a dusting powder the particulate HB polymer preferably has an average particle size below 30 μm , particularly below 20 μm .

The HB polymer can be produced in a suitable particulate form by the procedure described in EP-A-46335 involving heating an aqueous suspension of micro-organism cells containing HB polymer granules under pressure to above 100°C, particularly to above 150°C, and then releasing the pressure. This process causes the cell walls to rupture enabling the HB polymer granules to be separated from the cell debris by conventional methods.

Another method for producing the polymer in a suitable particulate form involves digesting dried cells, obtained for example by spray drying an aqueous cell suspension, with hypochlorite, for example as described in J. Gen. Microbiol. 19 (1958) 198-209.

By such processes the HB polymer can be separated from the cell residue as granules having an average size of less than 1 μm , or easily deagglomerated clusters of such granules.

Alternatively the HB polymer may be extracted from the micro-organism cells, e.g. by a process involving breakage of the cells by methods such as treatment with acetone, followed by extraction of the polymer from the broken cells by treatment with a solvent in which the polymer is soluble. Examples of such processes are described in US-A-3036959 and 3044942 in which the solvents employed are pyridine or mixture of methylene chloride and ethanol. Other extraction solvents for the polymer in the form in which it is produced in the cells include cyclic carbonates such as 1,2-propylene carbonate (see US-A-4101533; chloroform (see US-A-3275610); and 1,2-dichloroethane (as disclosed in EP-A-15123).

US-A-3275610 discloses other methods of cell breakage viz. ultrasonic vibration, grinding, French pressing, freezing/thawing cycles and lysozyme treatment, while as described in the aforementioned EP-A-15123, spray or flash drying of the suspension of cells as produced by culturing the micro-organism can also cause sufficient cell breakage to enable the polymer to be extracted from the cells.

HB polymer powder can be prepared from the solution of the polymer in the extraction solvent by, for example, spray drying the solution, to give a very fine powder.

An alternative extraction method involves enzymatic digestion of the non-HB polymer material in the micro-organism cells, for example by digesting

an aqueous suspension of the cells with a proteolytic enzyme composition. In this way the non-HB polymer cell material can be solubilised, leaving the HB polymer in particulate form. In order to assist separation of the HB polymer from the aqueous medium, the aqueous suspension is preferably heated, particularly to above 100°C, prior to enzyme digestion.

Where necessary the particles of the HB polymer, eg spray dried powder, or agglomerates of granules, can be ground to the requisite particle size.

The HB polymer particles may be sterilized with steam under conventional sterilizing conditions. Where the sterilisation process involves heating to above 140°C, it is preferred that the HB polymer is the homopolymer, poly(3-hydroxybutyrate), since copolymers tend to have lower melting points and hence are more susceptible to agglomeration in the sterilisation process.

HB polymers, unlike polyglycolic acid, can also be sterilised by γ -irradiation. Suitably a γ -irradiation dose of the order of 2.5 Mrad can be employed.

The invention is illustrated by the following examples.

Example 1

100 g of spray dried cells of *Alcaligenes eutrophus* containing approximately 50% by weight of 3-hydroxybutyrate homopolymer were added slowly with cooling to 1.5 litres of 15% by weight sodium hypochlorite solution. The maximum temperature attained was 40°C. The resulting suspension was centrifuged at 4000 rpm for 10 minutes and the supernatant discarded. The pellet was resuspended in 1 litre of deionised water and recentrifuged under the same conditions. This washing procedure was then repeated first with 0.5 M sodium thiosulphate and then again with deionised water.

After drying the resulting washed pellets of polymer granules in an oven at 40°C for 48 hours, they were Soxhlet extracted with diethyl ether for four hours and redried in a vacuum oven at 60°C for 12 hours. This produced a white, odourfree, free flowing powder with an average particle size of less than 100 μ m. Electron microscopy revealed that these particles were agglomerates of the original 0.2 – 0.5 μ m diameter polymer granules that are produced within the bacterial cell. The agglomerates could be broken down to smaller units quite easily by mild milling or grinding procedures using, for example, a micronising mill. Powders made by milling the free-flowing powder showed excellent lubricity as surgical glove dusting powders and could be sterilized by γ -irradiation at a dose of 2.5 Mrad.

Example 2

A fermenter culture containing 22 g/l of cells of *Alcaligenes eutrophus* containing approximately 72% by weight of a random copolymer of 83 mol % 3-hydroxybutyrate and 17 mol % 3-hydroxybutyrate units, was heated at 135°C for 10 minutes in a sterilizer. The culture flocculated and was then digested for 1 hour at pH 8.5 at 55°C with 0.2 g/l of a proteolytic enzyme composition ("Alcalase" 0.6 L, supplied by Novo Industries). The mixture was then

centrifuged and the separated solids were suspended in de-ionised water to give a solids contents of approximately 20 g/l. The suspension was again digested using 0.2 g/l of the proteolytic enzyme composition at 55°C, pH 8.5, for 1 hour.

The solids were separated by centrifugation and washed by re-suspending in de-ionised water. The suspension was then spray dried to give a free-flowing powder having an average particle size of less than 20 μ m and having an HB polymer of at least 96% by weight. Electron microscopy revealed the fine structure of the original 0.2 – 0.5 μ m HB polymer granules. The powder could be sterilized by γ -irradiation for use as a surgical dusting powder.

CLAIMS

1. A process of making a medical dusting powder comprising sterilising a particulate 3-hydroxybutyrate polymer.

2. A process according to claim 1 wherein the particulate polymer has an average particle size below 30 μ m.

3. A process according to claim 1 or claim 2 wherein the 3-hydroxybutyrate polymer contains at least 80 mole % of 3-hydroxybutyrate units.

4. A process according to claim 3 wherein the 3-hydroxybutyrate polymer is a 3-hydroxybutyrate homopolymer.

5. A process according to claim 4 wherein the sterilisation is effected by heating to a temperature above 140°C.

6. A process according to any one of claims 1 to 4 wherein sterilisation is effected by γ -irradiation.

7. A sterile medical dusting powder comprising a sterilised particulate 3-hydroxybutyrate polymer.