

The test results are plotted in the graph shown in FIG. 15. In the graph, the data at -15 hours represents the glucose level of each mouse before fasting, and the data at -1 hours represents the glucose level of each mouse after 17 hours of fasting. The data at 0 represents the glucose level of each mouse 1 hour after glucose administration, and the data at +1 hour represents the glucose level of each mouse after 1 hour of insulin treatment. The test results show that the calcium phosphate/PEG/human insulin particles were effective in controlling glucose levels in the rat up to at least 4 hours after treatment.

EXAMPLE 12

Particles having a surface modifying agent (2), such as polyethylene glycol (PEG), impregnated within the core calcium phosphate particle (4) and having a material (6), such as a therapeutic protein or peptide, and more particularly human insulin, at least partially coated on the surface are shown in FIG. 13C. Such particles having at least a partial coating of human insulin were prepared by simultaneously injecting 5 mL of 125 mM CaCl_2 and 1 mL of 156 mM sodium citrate into a 250 mL beaker containing 100 mL of 1% polyethylene glycol (PEG), under constant stirring. Precipitate was formed following the addition of 5 mL of 125 mM Na_2HPO_4 . Mixing was continued for 48 hours at room temperature. The resulting particle suspension was sonicated at maximum power for 15 minutes and stored at room temperature until ready for insulin attachment.

A therapeutic protein or peptide, such as human insulin at final 0.9 mg/mL, was incubated with batches of 20 mL PEG-entrapped particle suspension for 1 hour at room temperature by gentle mixing on a rocking platform. Finished particles were washed twice in distilled water and stored either at 4° C. (preferably not longer than 1 month) for lyophilized to dryness for future use. Illustrative particles are shown in FIG. 13C. Incorporating a surface modifying agent such as PEG in the particle structure results in increased insulin loading capacity, measured as mg bound-insulin/100 mg particle ($44\pm 4\%$ w/w), increased insulin per particle (12.5 U/mg particle, based on recombinant insulin unit by HPLC (high-performance liquid chromatography)=28.4 U/mg protein), and increased loading efficiency of $40.0\pm 3.6\%$ w/w, measured by mg bound-insulin/100 mg insulin originally added during binding.

EXAMPLE 13

Particles having both a surface modifying agent (2) and a material (6), such as a therapeutic protein or peptide impregnated within the core calcium phosphate particle (4) are shown in FIG. 14. Such particles having human insulin impregnated therein were prepared by adding one mL of 20 mg/mL of human insulin into a 50 mL beaker containing 20 mL of 1% PEG and mixed thoroughly for about 1 min. Sodium citrate at 156 mM (0.2 mL) and CaCl_2 at 125 mM concentrations (1 mL) were injected into PEG-human insulin solution simultaneously while stirring. One mL of 125 mM Na_2HPO_4 was added to initiate the particle formation. Stirring was continued for 48 hours at room temperature. The resulting particle suspension was sonicated at maximum power setting for 15 minutes. Finished particles were washed twice in distilled water and kept refrigerated at 4° C. (no more than one month) or lyophilized to dryness for further use. Illustrative particles are shown in FIG. 14. The resulting formulation has an increased loading capacity, measured as mg bound-insulin/100 mg particle ($77\pm 7\%$ w/w), increased insulin per particle (21.2 U/mg particle, based on recombinant insulin unit by HPLC (high-performance liquid chromatography)=28.4 U/mg protein),

and increased loading efficiency of $89.5\pm 8.1\%$ w/w, measured by mg bound-insulin/100 mg insulin originally added during binding.

EXAMPLE 14

Calcium phosphate core particles of the present invention, (CAP), were tested in comparison to calcium phosphate particles manufactured by Superfos Biosector a/s, referred to as "Accurate CAP," to study the effectiveness of the CAP particles of the present invention as an adjuvant.

HSV-2 CAP was prepared by co-crystallizing the viral protein with the CAP similar to the procedure described in Example 3. The resulting CAP with HSV-2 dispersed therein was then subsequently treated with cellobiose and the surface coated with antigen as described above.

Five mice each were immunized by i.p. injection with one of the following antigens: HSV-2+CAP or HSV-2+Accurate CAP. The mice were immunized with a primary injection and given two or three booster injections at two-week intervals. Blood was collected and IgG, IgG1, and IgG2a antibody titers in immunized mice were measured by ELISA. The results are presented in FIGS. 7A-C, which show different levels of antibodies between the different groups of immunized mice. Of particular importance is the result shown in FIG. 7C. FIG. 7C shows that the IgG2a antibody titer for the CAP particles of the present invention triggered a strong IgG2a response.

The procedures described above and exemplified above can be modified by those having skill in the art to yield other embodiments of the invention. For example, the material to be dispersed throughout the particle can be co-crystallized and impregnated within the particle as described above, and the resulting particles can be coated with the same or different material, using the coating methods described above. The core particles may also have a partial coating of one or a mixture of surface modifying agents described above to help adhere material coating the particle to the surface thereof.

The present invention has been described above with respect to certain specific embodiments thereof, however it will be apparent that many modifications, variations, and equivalents thereof are also within the scope of the invention.

What is claimed is:

1. A method for preparing one or more particles of calcium phosphate having diameters between about 300 nm to about 4000 nm, comprising reacting a soluble calcium salt with a soluble phosphate salt, wherein the reacting comprises:

- (a) mixing an aqueous solution of calcium chloride with an aqueous solution of sodium citrate to form a mixture,
- (b) adding an aqueous solution of sodium phosphate to the mixture to form a solution,
- (c) stirring the solution until particles of the desired size and comprising calcium phosphate are obtained.

2. The method of claim 1, wherein the concentrations of each of the aqueous calcium chloride, the aqueous sodium citrate, and the aqueous sodium phosphate solutions are independently between about 5 mM and about 100 mM.

3. The method of claim 1, further comprising adding an antigenic material along with one or more of the aqueous solutions forming the particle, to form one or more particles comprising calcium phosphate that are at least partially co-crystallized with the antigenic material.

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