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Industria y Comercio

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05-100609

54. TÍTULO Composición de Insulina Inyectable de acción prolongada y métodos de fabricación y uso del mismo

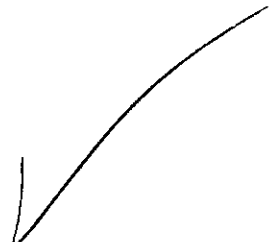
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71. SOLICITANTE the technology development company Ltd.,

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☒ Patente de invención		☐ Patente de Modelo de Utilidad	
☒ Capítulo I.		☐ Capítulo II.	
2) SOLICITANTE (71)	Nombre: THE TECHNOLOGY DEVELOPMENT COMPANY LTD. Dirección: Reid House, 31 Church Street, Hamilton HM FX, Bermuda Nacionalidad o domicilio: Hamilton, Bermuda Lugar de constitución: Bermuda		IDENTIFICACIÓN C.C. ☐ NIT: ☐ C.E. ☐ OTRO ☐ Cual _____ NÚMERO _____
	Teléfono: _____ Fax: _____ E. Mail: _____		
3) REPRESENTANTE O APODERADO	Nombre: DILIA MARÍA RODRÍGUEZ D'ALEMAN Dirección: Carrera 11 N°. 86-53 Piso 6 Bogotá D.C. Teléfono: 6181088 Fax: 6350824 E. Mail: info@clarkemodet.com.co		IDENTIFICACIÓN C.C. ☒ NIT: ☐ C.E. ☐ OTRO ☐ NÚMERO: 41.654.882 TP: 20824 CSJ
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10)

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud
- ☐ Comprobante de pago por reivindicación de prioridad
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- ☐ Poderes, si fuera el caso
- ☐ Documento de cesión del inventor al solicitante o a su causante
- ☐ Declaraciones
- ☒ Copia de la solicitud Internacional. (Resumen, descripción, reivindicación, Dibujos).
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicación, Dibujos)
- ☐ Copia del examen preliminar efectuado por la IPEA
- ☐ De ser el caso, copia del contrato de acceso
- ☐ De ser el caso, documento que acredita la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- ☐ De ser el caso, certificado de depósito del material biológico
- ☐ Arte final 12 x 12 y 6 x 6 cm
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
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NOMBRE: DILIA RODRÍGUEZ D'ALEMAN

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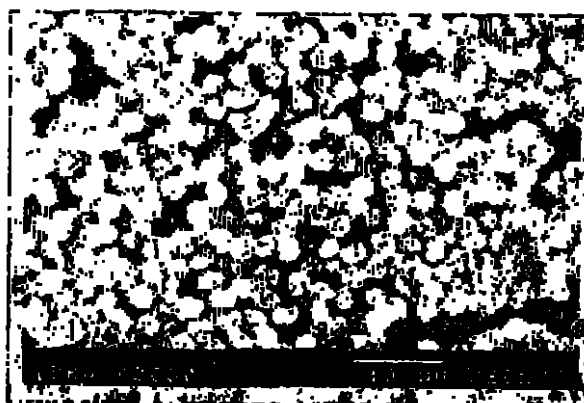
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(54) Title: LONG ACTING INJECTABLE INSULIN COMPOSITION AND METHODS OF MAKING AND USING THEREOF



(57) Abstract: A method of lowering blood glucose in a mammal includes injecting a therapeutically effective amount of crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal. The composition may be a one phase or a structured multi-phase composition for controlled release of insulin over an extended period of time.

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**LONG ACTING INJECTABLE INSULIN COMPOSITION
AND METHODS OF MAKING AND USING THEREOF**

FIELD OF THE INVENTION

[0001] This application claims benefit of the following U.S. Provisional Applications Serial Nos. 60/451,245, filed March 4, 2003; 60/467,601 filed May 5, 2003; 60/469,017 filed May 9, 2003; and 60/495,097 filed August 15, 2003, the disclosures of which are incorporated by reference herein in their entirety.

[0002] The present invention relates generally to insulin compositions and specifically to an injectable insulin composition containing insulin and crystallized dextran microparticles.

BACKGROUND OF THE INVENTION

[0003] Dextrans are high molecular weight polysaccharides synthesized by some micro organisms or by biochemical methods. Dextran with average molecular weight of about 75 kDa has a colloid osmotic pressure similar to blood plasma, so its aqueous solutions are used clinically as plasma expanders. Cross-linked dextrans in the form of beads are the basis for "Sephadex" that is used in the GPC of proteins and for "Cytodex" developed by Pharmacia to fulfill the special requirements of a micro-carrier cell culture. For example, U.S. Patent Nos. 6,395,302 and 6,303,148 (Hennink et al.) disclose attaching various biomaterials to cross-linked dextran particles. However, beads based on cross-linked dextran generally cannot be used for implant manufacturing owing to their potential toxicity due to the application of cross-linking agents (Blain J.F., Maghni K., Pelletier S. and Sirois P. Inflamm. Res. 48 (1999): 386-392).

[0004] U.S. Patent No. 4,713,249 (Schroder) describes a method of producing a depot matrix for biologically active substances. According to this patent, the depot matrix allegedly consists of carbohydrate microparticles, stabilized by crystallization, which implies using non-covalent bonds. The following process for producing the alleged crystallized carbohydrate microparticles is described by Schroder. A solution of a polymeric carbohydrate and a biologically-active substance is formed in one or more hydrophilic solvents. Then the mixture of the carbohydrate and the biologically active substance is emulsified in a liquid hydrophobic medium to form spherical droplets. The emulsion is then introduced into a crystallizing medium comprising acetone, ethanol or methanol to form spheres having a non-covalently cross-linked crystalline polymeric carbohydrate matrix, said matrix incorporating 0.001-50% by weight of the biologically-active substance. Thus, the biologically active substance is provided into the solution prior to crystallizing the spheres. Schroder does not describe the microstructure of the microparticles made by the multi-step method. Schroder's multi-step method is complex and uses organic solvents that are potentially toxic to cells and need to be removed.

BRIEF SUMMARY OF THE INVENTION

[0005] A method of lowering blood glucose in a mammal includes injecting a therapeutically effective amount of crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal. The composition may be a one phase or a structured multi-phase composition for controlled release of insulin over an extended period of time.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Figure 1 is a photograph of crystallized dextran microparticles spontaneously formed in 55.0% (W/W) aqueous solution of dextran with MW 70.0 kDa.

[0007] Figure 2A is a photograph of a cross-section of crystallized dextran microparticles shown in Figure 1.

[0008] Figure 2B is a photograph of a cross-section of a microparticle shown in Figure 2A. Microporous structure of the microparticle can be seen.

[0009] Figure 3 is a photograph of aggregates of crystallized dextran microparticles.

[0010] Figure 4 is a photograph of a slow release of the fluorescently labeled macromolecules from the implant which includes crystallized dextran microparticles into mouse muscle tissue on the 14th day after intermuscular injection.

[0011] Figure 5 is a photograph of an emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1.

[0012] Figure 6 is a photograph of an emulsion of aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1 in aqueous solution of PEG.

[0013] Figure 7 is a photograph of an intramuscular injection of emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1.

[0014] Figure 8 is a photograph of a subcutaneous injection of emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1.

[0015] Figures 9A and 9C schematically illustrate partition behavior of different types of particles and phases in an aqueous two phase system.

[0016] Figure 9B is a photograph of a cross section of an implant structure based on the two phase system.

[0017] Figures 10 and 11 schematically illustrate therapeutic agent delivery methods according to embodiments of the present invention.

[0018] Figures 12A and 12B are graphs of relative normalized of blood glucose concentrations for various insulin containing composition versus time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0019] The present inventor has discovered that a composition of crystallized dextran microparticles and insulin injected into mammals unexpectedly extended the duration of efficacy of the insulin compared to injections of the same dose of the same insulin alone. The composition may be a one phase composition or a multi-phase composition which forms a structured implant in a mammal.

[0020] The first section below describes the crystallized dextran microparticles, the second section describes formation of the structured implant from a multiphase composition, and the following sections describe specific examples of injection of the composition into mammals and methods of making the injectable composition.

A. Crystallized Dextran Microparticles

[0021] The present inventor has experimentally found that crystallized dextran microparticles with an average diameter ranging from 0.5 to 3.5 microns were spontaneously formed in concentrated aqueous solutions of dextrans (40 – 65 % W/W) with molecular weights ranging from 1.0 to 200.0 kDa, at temperature ranging from 20 – 90 °C. If it is desired to form the microparticles at room temperature, then 2 to 18 kDa dextran solutions may be used. Of course, the microparticles may also be formed from 2 to 18 kDa solutions at temperatures above room temperature, if desired. The microparticles may be spontaneously formed from higher molecular weight dextran solutions, such as 20 to 75 kDa solutions, at higher temperatures above room temperature, such as about 40 to about 70 °C. The microparticles may have any suitable shape such as a regular or an irregular shape, but are preferably spherical in shape, and are preferably 10 microns in diameter or less, such as 0.5 to 5 microns.

[0022] Transmission Electron Microscopy revealed the microporous structure of the crystallized dextran microparticles (see Figures 2A, 2B). Preferably, the microparticle porosity is at least 10 percent by volume, such as about 10 to about 50 percent, more preferably about 20 to about 40 percent. Thus, the structure comprises microporous microparticles with areas of macroporosity located between the particles.

[0023] Spray drying of aqueous suspensions of the crystallized dextran microparticles has shown the possibility to produce substantially spherical aggregates of crystallized dextran microparticles with a diameter ranging from 10.0 to 150.0 microns (see Figure 3).

[0024] A non limiting example of a method of forming the dextran microparticles is as follows. 50.0 g of dextran T40 (40 kDa molecular

weight) from Amersham Biosciences is added to 50.0 g of sterile distilled water in a 500 ml lab beaker to obtain 50% w/w solution under laminar flow. The mixture is stirred at 60°C (water bath) on a magnetic stirrer at 50 rpm until the dextran is completely dissolved and a clear solution is obtained. The solution can be vacuumed to remove all air inclusions. The clear solution is placed in lab oven at 60°C under a Tyvek® lid. 3.5 hours later, a turbid viscous suspension is developed as a result of formation of crystallized dextran microparticles.

[0025] To eliminate non-crystallized dextran, the microparticles are washed by centrifugation, for example 3,000 g, 30 min, with 3 × 250 ml of distilled sterile water, or by filtration of diluted suspension of microparticles, for example one part microparticles and 10 parts water (3 × 250 ml of distilled sterile water through sterilization filter). The centrifugation/washing is done under laminar flow. The microparticles are placed in 500 ml lab beaker under a Tyvek® lid and dried at 60°C in lab oven for 8 hours to reach a moisture level of about 5%. The resulting dry powder consists of particles with a mean diameter of about 2 microns.

[0026] The crystallized microparticles preferably are comprised of dextran molecules (i.e., polymer molecules) that are held together by a plurality of hydrogen bonds, Van Der Waals forces and/or ionic bonds and having substantially no covalent bonds between the dextran molecules. Thus, the molecules in the microparticles are preferably not intentionally cross-linked (i.e., a cross linking step is not carried out) and the microparticles contain no covalent bonds between molecules or less than 10% covalent bonds between molecules.

[0027] The slow release of macromolecules from implants has been demonstrated in experiments where macromolecules were dissolved in aqueous suspensions of crystallized dextran microparticles or their

aggregates before injections. Figure 4 shows an implant containing fluorescently labeled macromolecules (FITC-dextran, MW 500 kDa) and slow release of the macromolecules from the implant into a mouse muscle tissue on the 14th day after the intermuscular injection.

B. Two-phase system

[0028] Self assembled structures of implants based on crystallized dextran microparticles and their aggregates may be formed based on two phase systems.

[0029] Colloidal systems such as droplets of oil, liposomes, micro-and nano-particles can be dispersed in a suspension of crystallized dextran microparticles and injected to form an implant releasing therapeutic agent(s) following administration into the mammal body.

[0030] For example, in the case of oil, a special kind of implant structure can be formed where the oil core is surrounded with a shell composed of crystallized dextran microparticles or aggregates thereof dispersed in water or aqueous solutions of organic polymers such as polysaccharides (e.g. dextrans). The structure described can be designated as a capsule. It should be noted that the shell may comprise a roughly spherical shaped shell which results when the capsule is surrounded by tissue. However, when the capsule is located near a barrier, such as a substrate, bone or intestine wall, the capsule may comprise a core located between one or more walls of microparticles on one side and the barrier on the other side. Furthermore, while oil is used as an illustrative example, the core may comprise other materials, such as other polymers, cells, etc.

[0031] To form the capsule structure, two-phase aqueous systems are applied. When aqueous solutions of different polymers are mixed above

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certain concentrations they frequently form immiscible-liquid two-phase solutions. Each of the phases usually consists of more than 90% water and can be buffered and made isotonic. If a cell or particle suspension is added to such a system, the cells or particles are frequently found to have partitioned unequally between phases. This preferential partition behavior can be used as a basis for separation procedures for differing cell populations or particles since partition in these systems is determined directly by cell or particle surface properties. Cells or particles which do not have identical surface properties exhibit sufficiently different partition behavior.

[0032] The competitive adsorption of the two polymer phase depends on the chemical nature of the polymers. A two-phase polymer method has been applied to separate or partition cells, proteins, nucleic acids and minerals ("Partitioning in Aqueous Two-Phase Systems", 1985, eds., H. Walter, D. Brooks, and D. Fisher, publs. Academic Press).

[0033] The experiments with the distribution of crystallized dextran microparticles in phase systems derived from, for instance, dextran/polyethylene glycol (PEG) mixtures, revealed that the dextran microparticles prefer to be in the dextran phase, while another PEG phase can be dispersed in this dextran phase to form a W/W emulsion and vice versa in the case when the volume of the PEG phase is bigger than the volume of the dextran phase, as shown in Figures 5 and 6.

[0034] Figure 5 is a photograph of an emulsion of aqueous solution of PEG in aqueous solution of dextran containing crystallized dextran microparticles. In the structure of Figure 5, the volume of the PEG phase is less than the volume of the dextran phase. The dextran phase contains the dextran and the crystallized dextran microparticles. Thus, the PEG

phase forms into one or more sphere shaped cores surrounded by dextran / dextran microparticle shells (i.e., a closed pore structure).

[0035] Figure 6 is a photograph of an emulsion of aqueous solution of dextran containing crystallized dextran microparticles in aqueous solution of PEG, where the volume of the PEG phase is greater than the volume of the dextran phase. In this case, the dextran phase forms into one or more sphere shaped cores containing the dextran microparticles surrounded by a PEG phase (i.e., an open pore structure that is forming in vivo while PEG dissolves in tissue liquid). As can be seen in Figure 6, the smaller volume (droplet) dextran phase forms into a large spherical dextran / dextran microparticle core (bottom right of Figure 6) to which smaller spheres comprising dextran / dextran microparticles are joining and fuse with.

[0036] Thus, when the ratio of the volume of the first phase (such as the PEG phase and its inclusions, such as a therapeutic agent) to the volume of the second phase (such as the dextran phase and its inclusions, such as the dextran microparticles) is less than one, then the capsule forms by self assembly with a first phase core surrounded by a second phase shell. If the composition contains a therapeutic agent, such as insulin, which prefers to partition into the PEG phase, and the dextran microparticles which prefer to partition into the dextran phase, then the therapeutic agent selectively partitions into the PEG core while the microparticles selectively partition into and form the shell around the PEG core by self assembly.

[0037] The emulsion can be prepared by the mixing of separately prepared dextran and PEG phases and both can be suspensions of different types of particles that prefer to be in the PEG phase or in the dextran phase respectively. The principle is that the partition of particles

into different polymer phases depends on their surface structure and interfacial energy of the particles in the polymer solutions.

[0038] Injection of aqueous two phase systems containing crystallized dextran microparticles into tissues of experimental animals revealed the formation of implants with the capsule structure as shown in Figures 7 and 8. The volume of the dextran phase is greater than the volume of the PEG phase in the two-phase system. Both Figures 7 and 8 show that a capsule with a PEG core and a dextran/dextran microparticle shell forms by self assembly in vivo (i.e., after injection into mammal tissue). The shell comprises macroporous regions between adjacent microparticles as well as microporous regions in the microparticles themselves.

[0039] A non limiting example of a method of forming a capsule structure from a two phase system is as follows. 10 g of dextran T40 (40 kDa molecular weight) and 2 g of PEG are dissolved in 88 ml of (Actrapid®) Insulin solution containing 1,000 IU to which 25 g of crystallized dextran microparticles are added. These steps are performed under laminar flow conditions. The mixture is stirred on a magnetic stirrer at 100 rpm at room temperature for 30 minutes to form a homogeneous mixture (i.e., a suspension). 1.0 g of the suspension contains 8 IU of insulin.

[0040] It should be noted that the dextran microparticles may be prepared from a different molecular weight dextran solution than the dextran solution which is provided in the two phase system. Thus, the crystallized dextran microparticles may be formed in a lower molecular weight dextran solution, such as a 2 to 20 kDa solution, than the dextran solution which is provided into the two phase system, which may be a 40 to 500 kDa dextran solution, such as a 40 to 75 kDa solution. This is

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advantageous because the higher molecular weight dextran solutions, such as 40 and 70 kDa solutions, have received wider regulatory approval and can be used to form a shell of a capsule at lower concentrations. The lower molecular weight solutions may be used to decrease the crystallization time without the lower molecular weight dextran solution actually being provided in vivo. Furthermore, lower molecular weight microparticles may dissolve easier in vivo.

[0041] The capsule structure formed from a two phase system is advantageous because it allows for a more even and prolonged release of the therapeutic agent from the core than from a composition comprising a single phase containing the microparticles. Furthermore, it is believed that by using the capsule structure, a lower amount of microparticles may be needed to achieve the same or better timed release of a therapeutic agent than if a single phase system is used. Furthermore, by controlling the amount of microparticles in the two phase system, it is believed that the thickness of the microparticle shell may be controlled. A thicker shell results from a larger amount of microparticles in the two phase system. Thus, the amount, duration and/or timing of the release of the therapeutic agent from the capsule core may be controlled by controlling the thickness of the shell. Therefore, the release profile of the therapeutic agent may be customized for each patient or groups of patients.

[0042] It should be noted that while PEG and dextran are used as examples of the materials of the two phases, any other suitable materials which show the following partition behavior may be used instead. Figure 9A schematically illustrates partition behavior of different types of particles in an aqueous two phase system. For example, three types of molecules or molecular aggregates, which are preferably particles 10, 12 and 14, and two phases 16 and 18 are shown in Figure 9A. However, there may be two, or more than three types of particles. The particles

may be microparticles such as microspheres or nanospheres prepared from organic and/or inorganic materials, liposomes, living cells, viruses and macromolecules. The first type particles 10 preferentially segregate into the first phase 16. The second type particles 12 preferentially segregate to the boundary of the first 16 and second 18 phases. The third type particles 14 preferentially segregate into the second phase 18. Thus, by analogy to the previous non-limiting example, the first particles 10 may comprise a therapeutic agent, the second 12 and/or the third 14 particles may comprise crystallized dextran microparticles, the first phase 16 may comprise a PEG phase and the second phase 18 may comprise a dextran phase.

[0043] If a smaller amount of the first phase 16 is provided into a larger amount of the second phase 18, as shown in area 20 of Figure 9A, then a capsule type structure forms comprising discreet spheres of the first phase 16 containing a concentration of the first type particles 10, located in a second phase 18. The second type particles 12 may be located at the interface of the phases 16, 18 and act as a shell of the capsule. Particles 14 are dispersed in the second phase 18 and/or form a shell of the capsule.

[0044] In contrast, if a smaller amount of the second phase 18 is provided into a larger amount of the first phase 16, as shown in area 22 of Figure 9A, then a capsule type structure forms comprising discreet spheres of the second phase 18 containing a concentration of the third type particles 14, located in a first phase 16. The second type particles 12 may be located at the interface of the phases 16, 18 and act as a shell of the capsule. Particles 10 are dispersed in the first phase 16 and/or form a shell of the capsule. The two phase systems 20 and 22 may be used as an implant, such as by being injected into a mammal, such as an animal or human. Thus, the capsule forms a structured, three

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dimensional implant, with the core acting as a reservoir or depot for controlled release of the therapeutic agent through the shell. In contrast, an implant with an even distribution of microparticles is an unstructured implant.

[0045] Furthermore, particles 10, 12 and 14 may be substituted by a liquid material (e.g. oils) or macromolecules which selectively partition into one of the phases. For example, a therapeutic agent, such as insulin, may be partitioned in PEG phase of the PEG/dextran two phase system. Since insulin selectively partitions into the PEG phase, the PEG phase forms an insulin containing core of a capsule structure. It should be noted that while certain particles and therapeutic agents selectively partition, the term "selectively partitioned" does not necessarily mean that 100 percent of the particles or therapeutic agent partition into one of the phases. However, a majority of the selectively partitioned species, preferably 80% of the partitioned species, partitions into one of the phases. For example, while a majority of insulin partitions into the PEG phase, a portion of insulin may remain in the dextran phase.

[0046] Figure 9B illustrates a scanning electron microscope image of a cross section of an implant structure based on the two phase system schematically illustrated in Figure 9A. A two phase aqueous composition comprising a first dextran phase, a second PEG phase and crystallized dextran microparticles was injected into sepharose gel. This gel's composition mimics mammal tissue by stopping crystallized dextran microparticles diffusion from the injection side. The image in Figure 9B illustrates the formation of a core-shell implant structure. The core comprises regions 30 and 32 surrounded by a shell 34. Region 30 is a void that is filled with a PEG phase region prior to cutting the gel for cross sectional SEM imaging. The PEG phase region drips out of the gel when the gel is cut during cross sectioning. Region 32 is an outer portion of

the core comprising PEG droplets located in the crystallized dextran microparticles. Region 34 is the shell comprising the crystallized dextran microparticles which surrounds and holds in place the PEG containing core.

[0047] Without wishing to be bound by a particular theory, the present inventor believes that the core-shell structure shown in Figure 9B forms by self assembly as shown schematically in Figure 9C. While the first 16 and second 18 phases, such as aqueous solutions of different, incompatible polymers, are in a suitable storage container 19, such as in a glass beaker or vial, one phase 16 rises above the other phase 18. When the two phase composition is injected into a material which restricts free flow of the phases 16 and 18, such as mammal tissue or a substrate material, such as a gel which mimics the tissue, the composition self assembles into the core-shell structure. First, the phase that is present in the smaller volume forms into approximate spherical shapes, as shown in the middle portion of Figure 9C. Then the spherical shapes join to form approximately spherical cores of one phase surrounded by shells of the other phase, as shown in the bottom of Figure 9C. While a two phase system example of a multiphase system has been illustrated, the multiphase system may have more than two phases if desired.

C. Injectable insulin delivery vehicle

[0048] The present inventor has discovered that a composition of crystallized dextran microparticles and insulin injected into mammals, such as mice and rabbits, unexpectedly extended the duration of efficacy of the insulin compared to injections of the same dose of the same insulin alone. Figure 10 schematically illustrates the formation of an implant 40 in a mammal 53 by injection of a one phase composition comprising the microparticles 12, 14 and insulin 46 using a syringe 56. Figure 11

schematically illustrates the formation of a structured implant 40 in a mammal 53 by injection of a two phase composition comprising a dextran phase 18 containing selectively partitioned crystallized dextran microparticles 12, 14 and a PEG phase 16 containing selectively partitioned therapeutic agent 10 comprising insulin. The dextran phase 18 forms a shell around the PEG phase 16 core. Since mice and rabbits are a common model for humans in drug testing, the present inventor believes that the composition comprising crystallized dextran microparticles and insulin would also be effective in extending the duration of efficacy of the insulin when injected into human adults and children.

[0049] Examples 1-8 illustrate the advantage of using crystallized dextran microparticles as an injectable insulin delivery vehicle compared to injected insulin alone. The experiment involved mice and the observation was made of their response to a subcutaneously injected aqueous suspension consisting of crystallized dextran microparticles and human recombinant insulin (NovoNordisk Actrapid HM Penfill®, 40 U/ml).

[0050] The suspension was prepared as follows. 5.0 g of Dextran T10 (Pharmacia, Uppsala, Sweden) was dissolved in 20.0 g of water. The solution was filtered through a 0.22 μ m filter (Millipore, Bedford, MA) and freeze dried. 3.0 g of the resulting powder was dissolved in 3.0 g of sterile water and placed in box at temperature 60° C. 6 hours later, crystallized dextran microparticles were washed by centrifugation at 3,000 g with 3 x 5.0 ml of sterile water. Finally, the produced crystallized dextran microparticles suspension was mixed with aqueous insulin solution and used in the experiment with mice. Samples of the suspension were introduced into the mice's legs and samples of animal blood were taken from each mouse's tail and analyzed for glucose concentrations. Blood glucose was measured using the glucose oxidase method on a One-

touch system glucose analyzer (Lifescan, Johnson & Johnson, Milpitas, CA, USA) after proper calibration.

[0051] In comparative example 1, no insulin was injected into the mouse. In comparative examples 2, 3 and 7, insulin alone (0.5 UI) was injected into the three mice. In examples 4-6 and 8, Insulin (0.5 UI) and a crystallized dextran microparticles implant was injected into the four mice. The results are summarized in Table I.

Table I

Ex #		0min glucose mmol/l	18min glucose mmol/l	30min glucose mmol/l	48min glucose mmol/l	120min glucose mmol/l	210min glucose mmol/l	270min glucose mmol/l	380min glucose mmol/l
1	Intact mouse	7.9	8.1	8.2	8.4	-	-	-	-
2	Insulin 0.5 UI	6.9	3.3	2.7	1.8	0.9	3.5	3.0	3.2
3	Insulin 0.5 UI	8.1	3.8	2.8	1.9	0.9	3.7	3.4	3.5
4	Insulin 0.5 UI with crystal- lized dextran micro- particles	6.0	4.3	3.2	2.6	0.8	0.8	0.9	0.7
5	Insulin 0.5 UI with crystal- lized dextran micro- particles	6.9	5.6	4.1	3.4	-	1.2	-	1.6
8	Insulin 0.5 UI	5.9	3.5	2.9	1.9	1.2	1.0	1.0	0.7

	with crystal- lized dextran micro- particles								
7	Insulin 0.5 UI (average)	7	3.6	2.8	1.9	0.9	3.6	3.2	3.4
8	Insulin 0.5 UI with crystal- lized dextran micro- particles (average)	6.3	4.5	3.4	2.6	1.0	1.0	1.0	1.0

[0052] The average reduction of sugar in the blood (i.e., blood glucose) of animals is very different when 0.5 UI i.m. were applied with and without crystallized dextran microparticles. As shown in Table I, the glucose level in the mice of comparative examples 2, 3 and 7 is about the same or lower than the glucose level in mice of examples 4-6 and 8 during the first 45 minutes after injection. The glucose level is about the same in mice of both comparative examples 2, 3 and 7 and examples 4-6 and 8, 120 minutes after injection. However, the glucose level in the mice of comparative examples 2, 3 and 7 is about three times higher than the glucose level in mice of examples 4-6 and 8 from 210 to 390 minutes after injection. In fact, the blood glucose level in mice in examples 4-6 and 8 did not substantially increase (i.e., did not increase by more than 10%, remained the same or decreased) from 120 minutes to 390 minutes after injection. In contrast, the blood glucose level in mice in the comparative examples 2, 3 and 7 injected with the same amount of insulin did substantially increase from 120 to 390 minutes after injection.

The crystallized dextran microparticles/insulin injection decreases blood glucose for a longer time than an injection of insulin of the same dose alone. Thus, the composition containing crystallized dextran microparticles and insulin may be dosed for injection.

[0053] The following experiments on rabbits also demonstrate how the crystallized dextran microparticles/insulin injection decreases blood glucose and maintains a basal level of blood insulin for a longer time than an injection of the same insulin of the same dose alone. A subcutaneously injected composition comprising Actrapid HM® short-acting insulin and crystallized dextran microparticles was unexpectedly found to extend the duration of efficacy of this short-acting insulin to exceed that of subcutaneously injected, long-acting insulin Monotard HM® alone.

[0054] The term duration of efficacy means decreasing blood glucose concentration and/or maintaining a basal level of blood insulin concentration to desired levels independent of external events that cause spikes in blood glucose, such as eating. Thus, the term duration of efficacy is a relative term comparing the efficacy of the insulin and microparticle composition to that of the same dose of the same insulin alone. In other words, the duration of efficacy is a duration of action or a duration of pharmacological effect, which may be measured in a patient in a fasting state to compare the efficacy of the insulin and microparticle composition to that of the same dose of the same insulin alone.

[0055] As shown in Figures 12A and 12B, the composition comprising the Actrapid HM® short-acting insulin and crystallized dextran microparticles prolonged the absorption of insulin and extended the hypoglycemic effect (i.e., the duration of efficacy of the insulin) to at least twenty four hours, such as about twenty eight to about thirty one

hours, as compared to about two to about eight hours for Actrapid HM[®] insulin alone (Figure 12B) and about seventeen to about twenty-four hours for long-acting "Monotard HM[®]" insulin alone (Figure 12A). Both Actrapid HM[®] and Monotard HM[®] insulins are products of Novo Nordisk and the advertised duration of efficacy of these insulin compositions in humans obtained from company information are eight and twenty four hours, respectively.

[0056] In Figures 12A and 12B, the upper line illustrates the control line for intact rabbits to which no insulin was administered. The y-axis of Figures 12A and 12B is a relative normalized scale of blood glucose concentration for the same 8 IU dose of insulin. The data in the Figures was adjusted to be shown in one plot for each figure and shows blood glucose levels in blood of animals following insulin injections.

[0057] The data shown in Figures 12A and 12B was obtained as follows. Chinchilla rabbits (2.3 ± 0.3 kg) were monitored for their response to injections of a formulation consisting of crystallized dextran microparticles and short-acting insulin Actrapid HM[®]. Samples of the formulation were subcutaneously injected into the rabbits. Long acting insulin Monotard HM[®] (40 IU/ml) and short acting insulin Actrapid HM[®] were subcutaneously injected into separate rabbits without the microparticles and used as controls. Samples of animal blood were taken from the rabbit's ear vein and analyzed for glucose concentration. Blood glucose concentration was measured with a glucose analyzer (One-Touch[®] Lifescan, Johnson & Johnson, Milpitas, CA, USA) after proper calibration.

[0058] In comparative examples 9 and 10, two intact rabbits were not provided any insulin. In comparative examples 11 and 12 an aqueous solution of long-acting insulin Monotard HM[®] was introduced

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subcutaneously to two rabbits in a dose of 8 IU. In examples 13-15, a suspension of crystallized dextran microparticles with short-acting insulin Actrapid HM® was introduced subcutaneously to three rabbits in a dose of 8 IU. The results of the experiments are summarized in Table II.

TABLE II

Ex #	Insulin dose	0 hours glucose mmol/L	0.5 hours glucose mmol/L	1 hour glucose mmol/L	1.5 hours glucose mmol/L	2 hours glucose mmol/L	2.5 hours glucose mmol/L	16 hours glucose mmol/L	24 hours glucose mmol/L	31 hours glucose mmol/L
9	0.0	5.4	5.0	5.2	5.2	5.2	5.4	4.7	5.4	5.4
10	0.0	6.0	6.3	6.3	6.3	6.3	6.4	5.7	5.6	5.8
11	8 IU	5.4	5.8	3.8	3.2	2.4	2.6	3.9	5.6	N.A
12	8 IU	5.4	5.0	4.2	2.9	2.5	2.4	4.0	5.1	N.A
13	8 IU	5.8	3.7	1.9	1.9	1.9	2.8	4.1	4.3	4.1
14	8 IU	6.6	5.7	4.3	3.9	3.7	3.9	4.6	4.1	3.9
15	8 IU	6.2	5.1	3.6	3.2	3.1	2.9	4.2	4.4	4.7

[0059] The above examples 13-15 illustrate that the composition of crystallized dextran microparticles with short-acting insulin Actrapid HM® provides a prolonged effect that exceeds the effect of long acting insulin Monotard HM® and is believed to be comparable to the effect of long acting (once daily dosing) insulin glargine Lantus® from Aventis (see www.aventis-us.com/Pla/lantus_TXT.html). In addition, Lantus® insulin must not be diluted or mixed with any other insulin or solution. If Lantus® insulin is diluted or mixed, the pharmacokinetic/pharmacodynamic profile (e.g., onset of action, time to peak effect) of Lantus® and/or the mixed insulin may be altered in an unpredictable manner. In contrast, the composition of crystallized dextran microparticles with insulin is not so limited because any suitable insulin, such as human insulin, may be used. In the composition of crystallized

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dextran microparticles and insulin, the ratio of insulin and microparticles can be varied as desired. Furthermore, any suitable insulin may be used to custom fit an insulin therapy to an individual patient. Thus, Actrapid HM® was used in the composition as an illustrative example of a typical insulin and the composition is not limited to this brand of insulin.

[0080] As shown in examples 9-15, the composition containing the crystallized dextran microparticles and insulin is effective in maintaining a duration of efficacy of the insulin for at least 30% longer, such as at least 100% longer, preferably 100 to 400% longer than the same dose of the same insulin without the microparticles. The microparticle containing insulin composition is effective in maintaining a desired basal level of blood insulin and blood glucose concentration for at least 30% longer, such as 100% to 400% longer, than the same dose of the same insulin without the microparticles. Thus, the duration of efficacy of the microparticle containing composition is at least 24 hours, which allows it to be injected only once daily into the mammal, such as a human in need thereof.

[0081] The long lasting insulin crystallized dextran microparticle composition is safer than prior art long lasting insulin compositions because it can achieve the long lasting efficacy without using a higher dose of insulin as in the prior art compositions. For example, if a 8 IU dose of short acting insulin has been determined medically safe for a patient without a significant risk of overdose, then the composition comprising the same short acting insulin and the crystallized dextran microparticles can provide longer acting duration efficacy at the same 8 IU dose of short acting insulin without a significant risk of overdose, even if all the insulin is released into the patient at once. Furthermore, this composition provides a cost saving compared to the prior art compositions because it extends the efficacy without increasing the

amount of insulin. Current prior art long-acting diabetes therapies are made with analogs of insulin, such as the Lantus® insulin from Aventis. In contrast, the crystallized dextran microparticle-containing composition preferably contains human recombinant insulin whose safety profile is established. Thus, this composition reduces the risk of adverse reaction(s) and number of injections to diabetics, thereby enhancing the quality of life of the diabetics.

[0062] The injectable composition may comprise a single phase system comprising insulin and microparticles or a two phase system which forms a PEG and insulin core and a dextran and dextran microparticle shell for an even greater duration of efficacy. Furthermore, the composition comprises a flowable one phase or multiphase colloidal system (i.e., a suspension or an emulsion) which is relatively easy to inject into a mammal.

[0063] The following example illustrates the use of an injectable two phase composition comprising a dextran phase, a PEG phase, insulin and crystallized dextran microparticles. It is believed that when injected into a mammal, this composition forms a structured reservoir type implant having a three dimensional capsule structure. In the capsule structure, the microparticles selectively partition into the dextran phase and the insulin selectively partitions into the PEG phase. The dextran phase containing the microparticles forms a shell around a core comprising the PEG phase containing the insulin. This structured implant allows for controlled release from the core through the shell.

[0064] In comparative example 16, 0.5 IU of Actrapid HM® Insulin (100 IU/ml) is subcutaneously injected into a mouse. In example 17, 0.4 g of crystallized dextran microparticles are dispersed in 0.6 ml of 20% (W/W) aqueous solution of dextran having a molecular weight of 70 kDa

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(Pharmacia, Sweden) to form a suspension. 10 mg of PEG having a molecular weight 6 kDa (Fluka) is dissolved in 0.1 ml of Actrapid HM® insulin (100 IU/ml) to form a solution. 0.05 ml of the PEG and insulin solution is mixed with 0.15 ml of the microparticle and dextran suspension to form a two phase composition or mixture. 0.02 ml of the two phase mixture containing 0.5 IU of Insulin is injected subcutaneously into mouse. The results are shown in Table III.

Table III

Example #	0 min glucose mmol/L	15 min glucose mmol/L	30 min glucose mmol/L	45 min glucose mmol/L	60 min glucose mmol/L	120 min glucose mmol/L
16	7.8	3.7	2.3	1.7	2.9	6.7
17	7.9	5.9	4.3	4.1	4.3	4.0

[0065] As can be seen in Table III, the two phase composition duration of efficacy was longer than that of the insulin alone. Furthermore, the two phase composition decreased the blood glucose concentration more gradually than insulin alone. Without wishing to be bound by a particular theory, these effects are believed due to the controlled insulin release from the core of capsule structure.

[0066] Furthermore, the microparticle containing composition may be individually tailored for each patient by adjusting the amount of insulin and/or microparticles to allow the patient to inject the composition at the same time every day (i.e., once every 24 hours, once every 48 hours, etcetera). Thus, the duration of efficacy of the composition is adjustable for each patient. For a two phase system, the insulin release profile from the core of the capsule may be adjusted by controlling the amount of microparticles to control the shell thickness of the capsule.

[0087] While the inventor does not wish to be bound by any particular theory, it is believed that the long lasting effect of the same dose of insulin in mice and rabbits with crystallized dextran microparticles can be explained by the diffusion of the insulin molecules from the crystallized dextran microparticles based Implant (i.e., a self controlled release of Insulin). Since mice and rabbits are a common model for humans in drug testing, the data shown in the above tables I to III suggests that the use of crystallized dextran microparticles based implants makes it possible to develop controlled release delivery systems with improved pharmacokinetic and dynamics characteristics and that better meet the needs of basal insulin patients, such as humans.

D. Materials

[0088] In the preferred embodiments of the present invention, the therapeutic agent comprises insulin. In other words, the therapeutic agent may consist essentially of insulin alone or comprise insulin in combination with another agent. The term "insulin" shall be interpreted to encompass insulin analogs, natural extracted human insulin, recombinant produced human insulin, insulin extracted from bovine and/or porcine sources, recombinant produced porcine and bovine insulin and mixtures of any of these insulin products. The term is intended to encompass the polypeptide normally used in the treatment of diabetics in a substantially purified form but encompasses the use of the term in its commercially available pharmaceutical form, which includes additional excipients. The insulin is preferably recombinant produced and may be dehydrated (completely dried) or in solution.

[0089] The terms "insulin analog," "monomeric insulin" and the like are used interchangeably herein and are intended to encompass any form of "insulin" as defined above, wherein one or more of the amino acids

within the polypeptide chain has been replaced with an alternative amino acid and/or wherein one or more of the amino acids has been deleted or wherein one or more additional amino acids has been added to the polypeptide chain or amino acid sequences, which act as insulin in decreasing blood glucose levels. In general, the term "insulin analogs" of the preferred embodiments of the present invention include "insulin lispro analogs," as disclosed in U.S. Pat. No. 5,547,929, incorporated herein by reference in its entirety; insulin analogs including LysPro Insulin and humalog insulin, and other "super insulin analogs", wherein the ability of the insulin analog to affect serum glucose levels is substantially enhanced as compared with conventional insulin as well as hepatoselective insulin analogs which are more active in the liver than in adipose tissue. Preferred analogs are monomeric insulin analogs, which are insulin-like compounds used for the same general purpose as insulin, such as insulin lispro, i.e., compounds which are administered to reduce blood glucose levels.

[0070] The term "analog" refers to a molecule, which shares a common functional activity with the molecule to which it is deemed to be comparable and typically shares common structural features as well.

[0071] The term "recombinant" refers to any type of cloned therapeutic expressed in prokaryotic cells or a genetically engineered molecule, or combinatorial library of molecules which may be further processed into another state to form a second combinatorial library, especially molecules that contain protecting groups which enhance the physicochemical, pharmacological, and clinical safety of the therapeutic agent.

[0072] The term dextran microparticles includes unsubstituted dextran microparticles and substituted dextran microparticles. For example, substituted dextran microparticles include dextran substituted

with a suitable group, such as a methyl group, up to a degree which does not hamper crystallization of the dextran microparticles, such as up to 3.5 or less percent branching. The average microparticle diameter is preferably about 0.5 to about 5 microns, more preferably about 1 to about 2 microns.

[0073] Furthermore, while porous non cross-linked dextran microparticles, such as crystallized microparticles, are preferably used with the therapeutic agent, other suitable organic or inorganic microparticles may be used instead, such as other polymer microparticles including polyaccharides, PLA, PLGA, PMMA, polyimides, polyesters, acrylates, acrylamides, vinyl acetate or other polymeric materials, biomaterial particles such as alginate and cells, or inorganic particles, such as silica, glass or calcium phosphates. Preferably the microparticles are biodegradable. Preferably, porous microparticles are used. Most preferably, the microparticles have sufficient porosity to contain the therapeutic agent within the pores and to provide a timed release of the therapeutic agent from the pores. In other words, the therapeutic agent is released over time from the pores, such as in over 5 minutes, preferably in over 30 minutes, most preferably in over one hour, such as in several hours to several days, rather than all at once. Thus, the particle material, pore size and pore volume can be selected based on the type of therapeutic agent used, the volume of therapeutic agent needed for delivery, the duration of the delivery of the therapeutic agent, the environment where the therapeutic agent will be delivered and other factors.

[0074] Thus, in a preferred aspect of the present invention, the therapeutic agent is located at least partially in the pores of the porous microparticles. Preferably, the therapeutic agent is not encapsulated in the microparticle (i.e., the microparticle does not act as a shell with a

therapeutic agent core inside the shell) and is not attached to the surface of the microparticle. However, if desired, a portion of the therapeutic agent may also be encapsulated in a microparticle shell and/or is attached to the surface of the microparticle in addition to being located in the pores of the microparticle. The location of the therapeutic agent in the pores provides an optimum timed release of the therapeutic agent. In contrast, the therapeutic agent attached to the surface of the microparticle is often released too quickly, while the therapeutic agent encapsulated in the microparticle is often not released soon enough and is then released all at once as the microparticle shell disintegrates. In a two phase system, at least 80% of the therapeutic agent is preferably located in a core surrounded by a wall or shell comprising the microparticles.

E. Methods of Making

[0075] The microparticles may be formed by any suitable method. Preferably, the microparticles are combined with the therapeutic agent after the microparticles are formed. Thus, the microparticles, such as the crystallized dextran microparticles are formed by any suitable method and then the therapeutic agent and the microparticles are combined by any suitable method. In contrast, in some prior art methods, the therapeutic agent is encapsulated into a microparticle shell by providing the particle precursor material and the therapeutic agent into a solution and then crystallizing or cross-linking the precursor material, such as a monomer or oligomer material, to encapsulate a therapeutic agent core into a microparticle shell.

[0076] Preferably, the therapeutic agent is provided into the pores of the porous microparticles after the microparticles are formed. Thus, the porous microparticles are first formed and then the therapeutic agent is provided into a solution containing the microparticles to allow the

therapeutic agent to permeate into the pores of the microparticles. Of course, some of the therapeutic agent may also become attached to the surface of the microparticle in this process.

[0077] Thus, a method to manufacture non cross-linked, porous crystallized dextran microparticles includes preparation of a dextran solution, such as an aqueous dextran solution, conducting a crystallization process to form crystallized porous dextran microparticles, and if desired, isolating crystallized porous dextran microparticles from the solution. A therapeutic agent is then permeated into the pores of the microparticles by providing the therapeutic agent into the crystallization solution containing the microparticles or by providing the isolated microparticles and the therapeutic agent into a second solution, such as a second aqueous solution. For example, crystallized dextran microparticles may be formed in a first, low molecular weight dextran aqueous solution, such as a 2 to 20 kDa dextran solution. The microparticles are then removed from the first solution and then placed into a second dextran aqueous solution having a higher molecular weight dextran, such as a 40 to 500 kDa solution, for example, a 40 to 75 kDa solution. The second solution may comprise a first phase of a two phase system, which is then combined with a second phase, such as a PEG phase containing a therapeutic agent. A similar method may be used with other porous microparticles, where a therapeutic agent is then permeated into the pores of the microparticles after the porous microparticles are formed by any suitable microparticle formation method, including, but not limited to crystallization. The components of the composition such as insulin, microparticles and one or more aqueous phases may be combined in any suitable order sequentially or simultaneously.

[0078] Preferably, the microparticles are formed by self assembly from a solution that does not contain organic solvents and organic

reaction promoters which leave an organic residue in the microparticles. Thus, for example, the dextran microparticles are preferably formed by self assembly from an aqueous dextran solution. However, if desired, organic solvents and/or organic reaction promoters may also be used. In this case, the microparticles may be purified prior to subsequent use to remove the harmful organic residue.

[0079] As described above, the capsule structure having a first phase core and a second phase wall or shell may be formed in vivo or in vitro from a two phase composition. The composition may be dried powder, such as freeze dried and stored as a powder or porous cake. When the composition is ready to be administered to a mammal, it is hydrated and administered to a mammal by injection.

[0080] Preferably, the composition which includes the microparticles and the therapeutic agent is a flowable colloidal system when the composition is dosed for injection. Examples of flowable colloidal systems include emulsions and suspensions which may be injected into a mammal using a common gage syringe or needle without undue difficulty. In contrast, some prior art compositions include a therapeutic agent in a dextran hydrogel or in a cross-linked dextran matrix. A dextran hydrogel and a cross-linked dextran matrix are not flowable compositions if not specifically prepared.

[0081] In another preferred aspect of the present invention, the microparticles comprise microparticles which are adhesive to mammalian mucosa. Preferably the adhesive microparticles are porous microparticles described above. This further improves the effective delivery of the therapeutic agent.

[0082] In another preferred aspect of the present invention, the microparticles comprise microparticles whose surface has been specially

modified to enhance the adhesion of the therapeutic agent to the microparticle surface and to optimize the delivery of the therapeutic agent. The microparticle surface may contain any suitable modification that would increase the adhesion of the therapeutic agent.

[0083] The foregoing description of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed, and modifications and variations are possible in light of the above teachings or may be acquired from practice of the invention. The drawings and description were chosen in order to explain the principles of the invention and its practical application. It is intended that the scope of the invention be defined by the claims appended hereto, and their equivalents.

[0084] All of the publications and patent applications and patents cited in this specification are herein incorporated in their entirety by reference.

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I claim:

1. A method of lowering blood glucose in a mammal, comprising injecting a therapeutically effective amount of a composition comprising crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal, wherein the microparticles are formed prior to combination of the insulin and the microparticles in the composition.
2. The method of claim 1, wherein the composition comprises a flowable colloidal composition and the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.
3. The method of claim 2, wherein:
the composition comprises a two phase composition comprising a dextran phase and a PEG phase;
the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and
the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into a mammal body.
4. The method of claim 3, further comprising controlling a thickness of the shell based on the body of the mammal receiving the composition to control release of insulin from the implant.
5. The method of claim 1, wherein the composition is provided to a human suffering from diabetes to lower the blood glucose concentration in the human.
6. A dosed pharmaceutical composition, comprising crystallized dextran microparticles and a therapeutically effective amount of insulin, wherein the composition is dosed for injection to a human and the

microparticles are formed prior to combination of the insulin and the microparticles in the composition.

7. The composition of claim 6, wherein:
 - the composition comprises a flowable colloidal composition; and
 - the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.
8. The composition of claim 7, wherein:
 - the composition comprises a two phase composition comprising a dextran phase and a PEG phase;
 - the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and
 - the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into the human body.
9. A dosed pharmaceutical composition, comprising crystallized dextran microparticles and a therapeutically effective amount of first insulin, wherein the composition is dosed for injection to a mammal and a duration of efficacy of the composition when injected into a mammal is at least 30% longer than a duration of efficacy in the mammal of the same dose of the same first insulin without the microparticles.
10. The composition of claim 9, wherein:
 - the composition comprises a flowable colloidal composition;
 - the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns; and
 - the microparticles are formed prior to combination of the first insulin and the microparticles in the composition.
11. The composition of claim 10, wherein:

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the composition comprises a two phase composition comprising a dextran phase and a PEG phase;

the first insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and

the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into a mammal body.

12. The composition of claim 9, wherein:

the duration of efficacy of the composition when injected into the mammal is at least 24 hours; and

the duration of efficacy of the composition when injected into the mammal is at least 100% longer than a duration of efficacy in a mammal of the same dose of the same first insulin without the microparticles.

13. A method of lowering blood glucose in a mammal, comprising injecting a therapeutically effective amount of a composition comprising crystallized dextran microparticles and first insulin to the mammal to lower blood glucose of the mammal, wherein a duration of efficacy of the composition in the mammal is at least 30% longer than a duration of efficacy in the mammal of the same dose of the same first insulin without the microparticles.

14. The method of claim 13, wherein:

the composition comprises a flowable colloidal composition;

the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns; and

the microparticles are formed prior to combination of the first insulin and the microparticles in the composition

15. The method of claim 14, wherein:

the composition comprises a two phase composition comprising a dextran phase and a PEG phase;

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the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and
the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into the mammal body.

16. The method of claim 13, wherein:

the duration of efficacy of the composition when injected into a mammal is at least 24 hours; and

the duration of efficacy of the composition in the mammal is at least 100% longer than a duration of efficacy in a mammal of the same dose of the same first insulin without the microparticles.

17. The method of claim 16, wherein the duration of efficacy of the composition in the mammal is 100 to 400 % longer than a duration of efficacy in a mammal of the same dose of the same first insulin without the microparticles.

18. A method of making a dosed pharmaceutical composition, comprising:

providing crystallized dextran microparticles;

combining a therapeutically effective amount of insulin and the crystallized dextran microparticles in a solution after the microparticles have been crystallized to form a composition of insulin and crystallized dextran microparticles; and

dosing the composition for injection to a mammal.

19. The method of claim 18, wherein:

the composition comprises a flowable colloidal composition; and

the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.

20. The method of claim 19, wherein:

the composition comprises a two phase composition comprising a dextran phase and a PEG phase;

the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and

the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into the mammal body.

21. The method of claim 18, wherein:

a duration of efficacy of the composition when injected into a mammal is at least 24 hours; and

the duration of efficacy of the composition in the mammal is at least 100% longer than a duration of efficacy in a mammal of the same dose of the same insulin without the microparticles.

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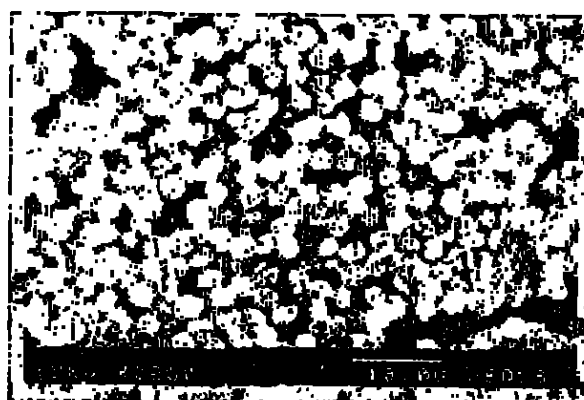


FIG. 1



FIG. 2A

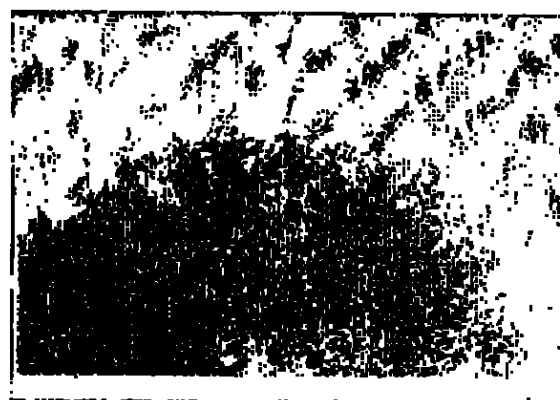


FIG. 2B

41. 7/8

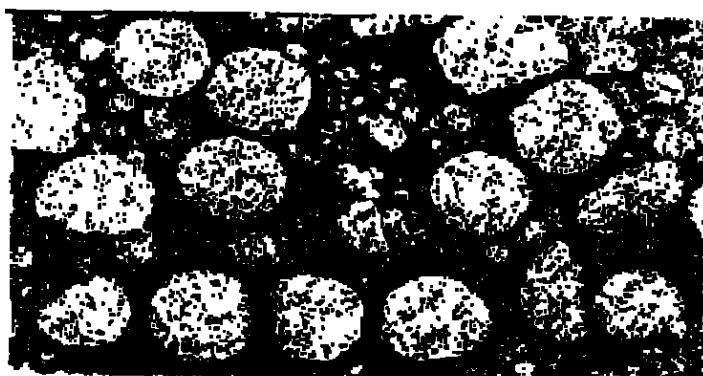


FIG. 3



FIG. 4



FIG. 5

92 177



FIG. 6

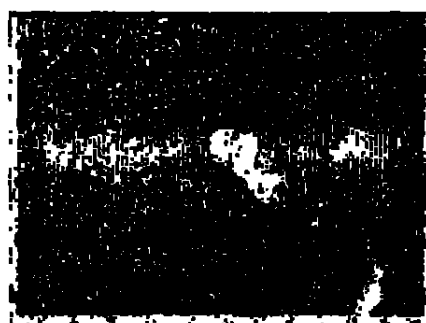
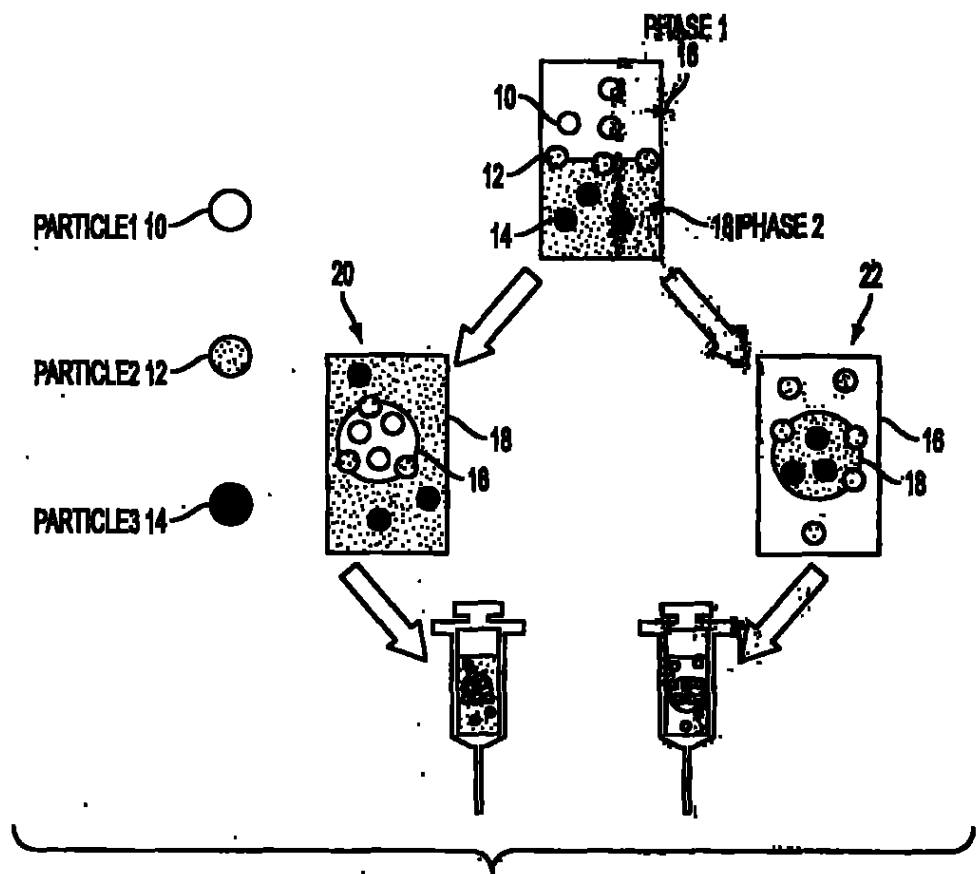


FIG. 7



FIG. 8

A3 7



44 5/9

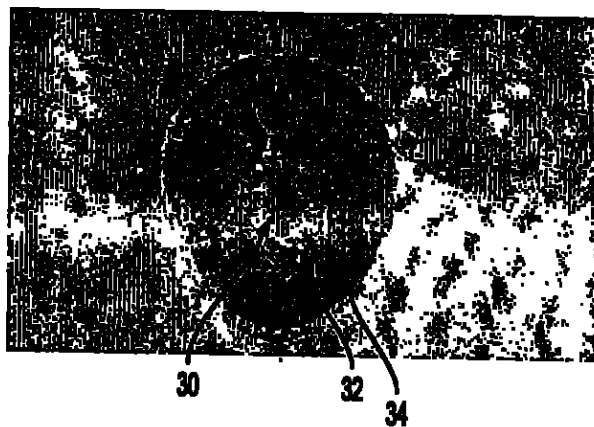


FIG. 9B

45 ~~46~~

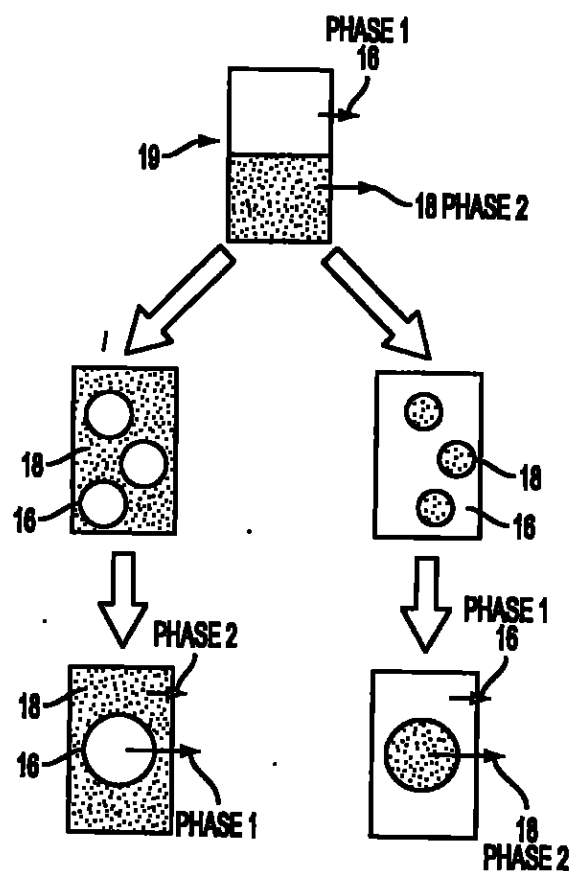


FIG. 9C

96 46

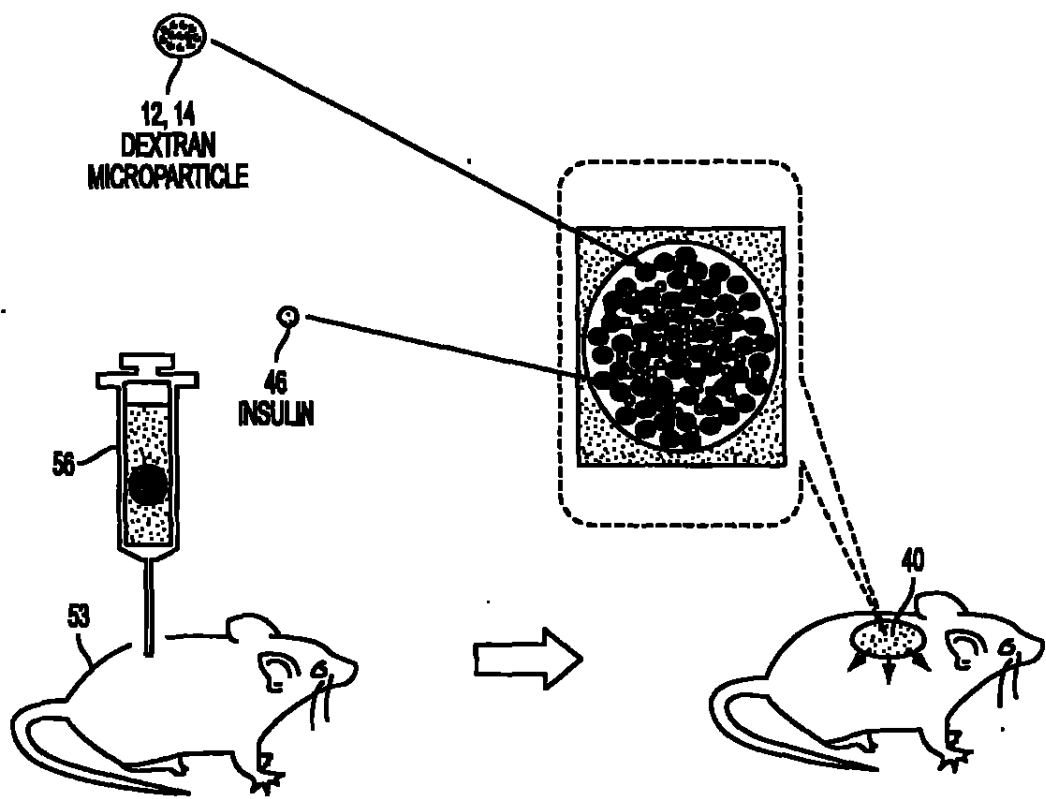


FIG. 10

47 48

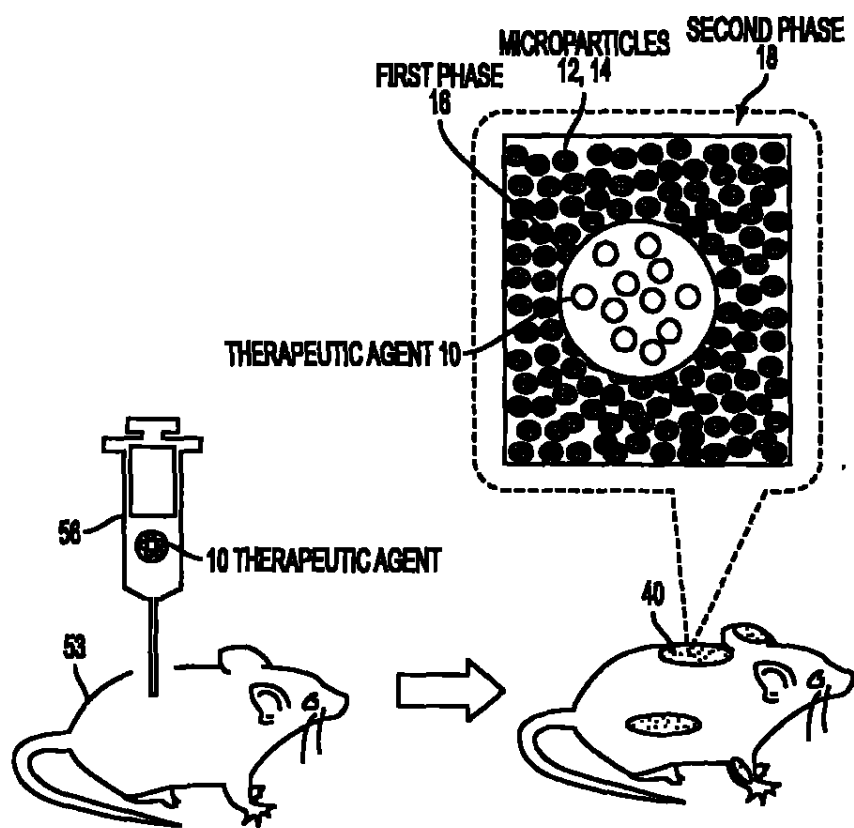


FIG. 11

90 75-

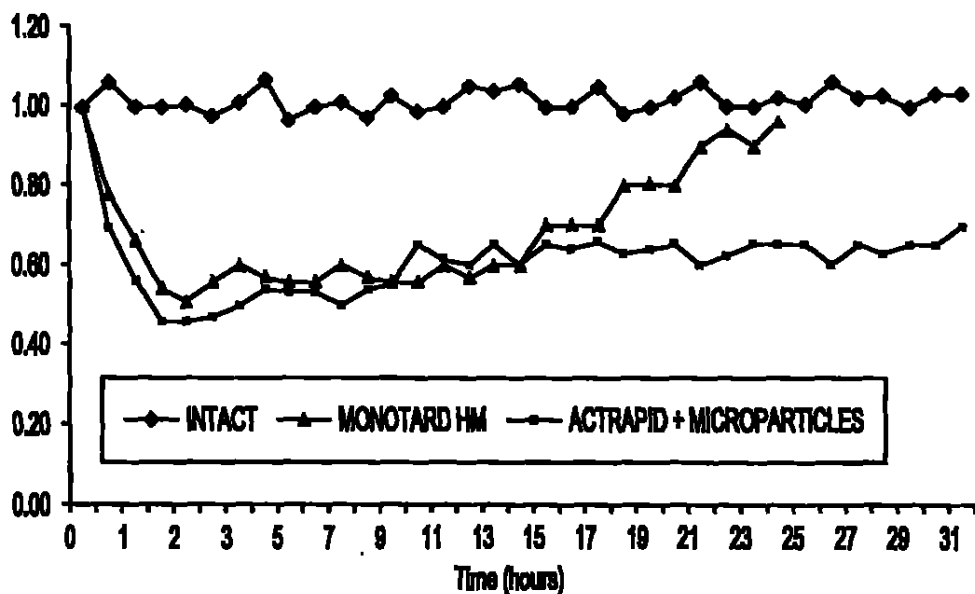


FIG. 12A

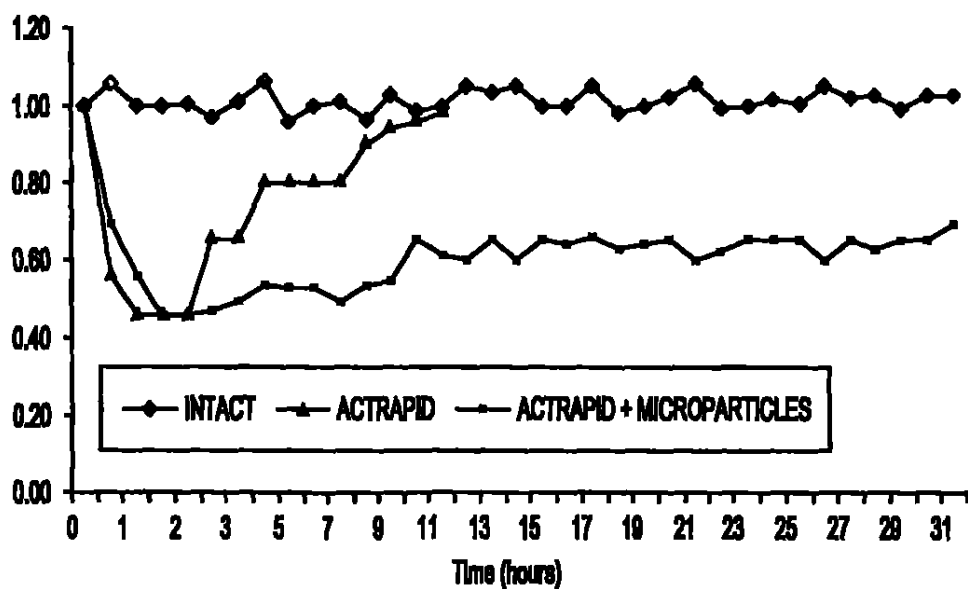


FIG. 12B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB2004/000984

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/28 A61K47/36 A61K9/00

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)

IPC 7 A61K

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 practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 713 249 A (SCHROEDER ULF) 15 December 1987 (1987-12-15) cited in the application column 6; claims 1,4,7; example 13	1-17
X	MORIYAMA K ET AL: "Regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol)" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 42, no. 3, 1 December 1996 (1996-12-01), pages 237-248, XP004069859 ISSN: 0168-3659 page 240 page 238, column 1	1-21
	-/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "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 (see specification)
- "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

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to underlain the principle or theory underlying the invention
- "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
- "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.
- "A" document member of the same patent family

Date of the actual completion of the international search

15 July 2004

Date of mailing of the international search report

21/07/2004

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Bochelen, D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2004/000984

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STENEKES R J H ET AL: "Formation of dextran hydrogels by crystallization" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 22, no. 13, July 2001 (2001-07), pages 1891-1898, XP004245950 ISSN: 0142-9612 page 1892, column 1	1-21
Y	EP 1 184 032 A (OCTOPLUS B V) 6 March 2002 (2002-03-06) page 3, paragraphs 15,16 page 4, line 46 - page 5, line 28	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2004/000984

Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search 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:
Although claims 1-5,13-17 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the composition.
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 III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

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

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.

INTERNATI NAL SEARCH REPORT

International Application No
PCT/IB2004/000984

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
US 4713249	A	15-12-1987	AU	9127482 A	01-06-1983
			EP	0093757 A1	16-11-1983
			WO	8301738 A1	26-05-1983
			US	4501726 A	26-02-1985
			AT	34659 T	15-06-1988
			AU	567434 B2	19-11-1987
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			WO	0217884 A1	07-03-2002

32 52

COMPOSICIÓN DE INSULINA INYECTABLE DE ACCIÓN PROLONGADA Y**MÉTODOS DE FABRICACIÓN Y****USO DEL MISMO****CAMPO DE LA INVENCION**

[0001] Esta solicitud reivindica el beneficio de las siguientes solicitudes provisionales estadounidenses números de series 60/451,245, presentada en Marzo 4, 2003; 60/467,601, presentada en Mayo 5, 2003; 60/469,017 presentada en Mayo 9, 2003; y 60/495,097 presentada en Agosto 15, 2003, las descripciones de las cuales se incorporan aquí en su totalidad.

[0002] La presente invención se relaciona generalmente con composiciones de insulina y específicamente a una composición de insulina inyectable que contiene insulina inyectable y micropartículas de dextrán cristalizadas.

ANTECEDENTE DE LA INVENCION

[0003] Los dextranos son polisacáridos de alto peso molecular sintetizados por algunos microorganismos o por métodos bioquímicos. El dextrán con peso molecular promedio

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de cerca de 75kDa tiene una presión osmótica coloide similar al plasma sanguíneo, de tal forma que sus soluciones acuosas son utilizadas químicamente como expansores de plasma. Los dextranos reticulados en la forma de granulados son la base para el "Sephadex"® que es usado en el GPC de proteínas y para el "citodex"® desarrollado por Pharmacia para cumplir los requerimientos especiales de un cultivo celular microportador. Por ejemplo, las patentes estadounidenses Números 6,396,302 y 6.303,148 (Hennink. et al.) describe varios biomateriales adheridos a partículas de dextrán reticuladas. Sin embargo, los granulados reticulados de dextrán generalmente no pueden ser usados para fabricación de implantes debido a su toxicidad potencial de aplicación de los agentes de reticulación (Blain J.F., Maghni., Pelletier S y Sirios P. inflamm. Res. 48 (1999): 386-392).

[0004] La solicitud de patente estadounidense No. 4,713,249 (Schroder) describe un método para producir una matriz pretendida para sustancias biológicamente activas. De acuerdo a esta patente, la pretendida matriz de depósito consiste de micropartículas carbohidratadas, estabilizadas por cristalización, que implican el uso de uniones no covalentes. El proceso siguiente para producir micropartículas carbohidratadas cristalizadas pretendidas es descrito por Schroder. Una solución de un carbohidrato

polimérico y una sustancia biológicamente activa se forma en uno o más solventes hidrofílicos. Luego la mezcla del carbohidrato y las sustancias biológicamente activas es emulsificada en un medio hidrófobo líquido para formar gotitas esféricas. La emulsión es luego introducida en un medio de cristalización que comprende acetona, etanol o metanol para formar esferas que tienen una matriz de carbohidrato polimérico reticulado no covalente, dicha matriz reincorpora 0,001-50% por peso de la sustancia biológicamente activa. Así, la sustancia biológicamente activa es colocada dentro de la solución antes de cristalizar las esferas. Schroder no describe la microestructura de las micropartículas hecho por el método multi-etapa. El método multi-etapa de Schroder es complejo y utiliza solventes orgánicos que son potencialmente tóxico para la célula y necesita ser removido.

BREVE RESUMEN DE LA INVENCION

[0005] Un método para reducir la glucosa sanguínea en un mamífero incluye inyectar una cantidad terapéuticamente efectiva de micropartículas de dextrán cristalizada e insulina al mamífero para reducir la glucosa sanguínea del mamífero. La composición puede ser una fase o una composición

go \$

multi-fase estructurada para liberación controlada de insulina durante un periodo de tiempo extendido.

BREVE DESCRIPCIÓN DE LOS DIBUJOS

[0006] La figura 1 es una fotografía cristalizada de micropartículas de dextrán formadas espontáneamente en 55,0% (P/P) de solución acuosa de dextrán con PM 70,0 kDa.

[0007] La figura 2A es una fotografía de una sección transversal de micropartículas de dextrán cristalizada mostrada en la figura 1.

[0008] La figura 2B es una fotografía de una sección transversal de micropartícula mostrada en la figura 2A. La estructura microporosa de la micropartícula puede ser vista.

[0009] La figura 3 es una fotografía de agregados de micropartículas de dextrán cristalizado.

[0010] La figura 4 es una fotografía de una liberación lenta de las macromoléculas fluorescentes del implante que incluye partículas de dextrán cristalizadas dentro del tejido muscular de ratón en el día 14 después de la inyección intramuscular.

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[0011] La figura 5 es una fotografía de una emulsión de solución acuosa de PEG en una solución acuosa de dextrán (PM 500 kDa) que contiene micropartículas de dextrán cristalizadas mostradas en la figura 1.

[0012] La figura 6 es una fotografía de una emulsión de solución acuosa de dextrán (PM 500 kDa) que contiene micropartículas cristalizadas de dextrán mostrados en la figura 1 en solución acuosa de PEG.

[0013] La figura 7 es una fotografía de una inyección intramuscular de emulsión de solución acuosa de PEG en solución acuosa de dextrán (PM 500 kDa) que contiene micropartículas de dextrán cristalizadas mostradas en la figura 1.

[0014] La figura 8 es una fotografía de una inyección subcutánea de emulsión de una solución acuosa de PEG en solución acuosa de dextrán (PM 500 kDa) que contiene micropartículas de dextrán cristalizada mostrada en la figura 1.

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[0015] La figura 9A y 9C ilustran esquemáticamente el comportamiento de partición de diferentes tipos de partículas y fases en un sistema de dos fases acuosas.

[0016] La figura 9B es una fotografía de una sección transversal de una estructura de implante basada en un sistema de dos fases.

[0017] Las figuras 10 y 11 ilustran esquemáticamente los diferentes métodos de administración de agentes terapéuticos de acuerdo con las modalidades de la presente invención.

[0018] Las figuras 12A y 12B son gráficos de concentraciones de glucosa sanguínea relativamente normalizadas de varias composiciones que contienen insulina versus tiempo.

DESCRIPCIÓN DETALLADA DE LAS MODALIDADES PREFERIDAS

[0019] El presente inventor ha descubierto que una composición de micropartículas de dextrán cristalizado e insulina inyectada a mamíferos extiende inesperadamente la duración de la eficacia de la insulina comparado con las inyecciones de la misma dosis de la misma insulina sola. La composición puede ser una composición de fase o una

composición multi-fase la cual forma un implante estructurado en un mamífero.

[0020] La primera sección adelante describe las micropartículas de dextrán cristalizado, la segunda sección describe la formación del implante estructurado de una composición multi-fase, y las siguientes secciones describen ejemplos específicos de inyección de la composición en mamíferos y métodos de fabricación de la composición inyectable.

A. Micropartículas Cristalizadas de Dextrán

[0021] El presente inventor ha encontrado experimentalmente que las micropartículas de dextrán cristalizado con un diámetro promedio que varía de 0,5 a 3,5 micrones fueron formados espontáneamente en soluciones acuosas de dextrán (40-65% P/P) con pesos moleculares que varían de 1,0 a 200,0 kDa, a temperaturas que varían entre 20-90° C. Si se desea formar las micropartículas a temperatura ambiente, entonces 2 a 18 kDa de soluciones dextrán pueden ser usadas. Por supuesto, también las micropartículas pueden ser formadas de soluciones 2 a 18 KDa a temperaturas por encima de la temperatura ambiente, si se desea. Las micropartículas pueden ser formadas de soluciones

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de dextrán de alto peso molecular, tales como soluciones de 20 a 75 kDa, a temperaturas mayores de la temperatura ambiente, tales como cerca de 40 a 70° C. Las micropartículas puede tener cualquier forma adecuada tal como una forma regular o una forma irregular, pero solo preferiblemente de forma esférica, pero son preferiblemente en forma esférica, y son preferiblemente de 10 micrómetros en diámetro o menos, tales como 0,5 a 5 micrones.

[0022] La microscopía de transmisión de electrones reveló la estructura microporosa de micropartículas de dextrán cristalizado (ver figuras 2A, 2B). Preferiblemente, la porosidad de la micropartículas es al menos de 10 por ciento de volumen, tal como de 10 a 50 por ciento, más preferiblemente cerca de 20 a 40 por ciento. Así, la estructura comprende micropartículas microporosas con áreas de microporosidad localizada entre las partículas.

[0023] El secado por rociado de las suspensiones acuosas de las micropartículas de dextrán cristalizado han mostrado la posibilidad de producir sustancialmente agregados esféricos de micropartículas de dextrán cristalizado con un diámetro que varía de 10,0 a 150,0 micrones(ver figura 3).

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[0024] Un ejemplo no limitativo de un método para formar las partículas de dextrán es como sigue. 50,0 g de dextrán T40 (peso molecular 40kDa) de Amersham Biosciences es agregado a 50,0 g de agua destilada estéril en un beaker de laboratorio de 500 ml para obtener solución 50% P/P bajo flujo laminar. La mezcla se agita a 60° C (baño de agua) en un agitador magnético a 50 rpm hasta que el dextrán es disuelto completamente y se obtiene una solución clara. La solución se puede exponer al vacío para remover todas las inclusiones de aire. La solución clara es colocada en un horno de laboratorio a 60° C bajo una cubierta Tyvek®. 3,5 horas después, una suspensión viscosa turbia se desarrolló como un resultado de formación de micropartículas de dextrán cristalizado.

[0025] Para eliminar el dextrán no cristalizado, las micropartículas se lavan por centrifugación, por ejemplo 3,000 g, 30 minutos, con 3 x 250 ml de agua destilada estéril, o por filtración de suspensión diluida de micropartículas, por ejemplo una parte de micropartículas y 10 parte de agua (3 x 250 ml de agua destilada estéril mediante filtro de esterilización). La centrifugación/lavado se hace bajo flujo laminar. Las micropartículas son colocadas en un beaker de 500 ml bajo una tapa Tyvek® y secado a 60°C en un horno de laboratorio por 8 horas para buscar el nivel

A
B

de humedad cerca de un 5%. El polvo seco resultante consiste de partículas con un diámetro medio de cerca de 2 micrones.

[0026] Las micropartículas cristalizadas preferiblemente comprenden moléculas de dextrán (es decir, moléculas de polímeros) que son mantenidas juntas por una pluralidad de enlaces de hidrógeno, fuerzas de Van Der Waals y/o enlaces iónicos y no tienen sustancialmente enlaces covalentes entre las moléculas de dextrán. Así, las moléculas en las micropartículas son preferiblemente no intencionalmente reticuladas (es decir, una etapa de reticulación no se lleva a cabo) y las micropartículas no contienen uniones covalentes entre las moléculas o menos de 10% de las uniones covalentes entre las moléculas.

[0027] La liberación lenta de las macromoléculas de los implantes se ha demostrado en experimentos en donde macromoléculas fueron disueltas en suspensiones acuosas de micropartículas de dextrán cristalizado o sus agregados antes de inyecciones. La figura 4 muestra un implante que contiene macromoléculas fluorescentes etiquetadas (FITC-dextrán, PM 500 kDa) y liberación lenta de macromoléculas del implante dentro del tejido muscular de ratón en el día 14 después de la inyección intermuscular.

B. Sistema de dos fases

[0028] Las estructuras autoensambladas de implantes basados en micropartículas de dextrán cristalizado y sus agregados puede ser formado basado en sistemas de dos fases.

[0029] Sistemas coloidales tales como gotitas de aceites, liposomas, micro-y nano-partículas pueden ser dispersadas en una suspensión de micropartículas de dextrán cristalizado e inyectado para formar un implante de un agente terapéutico de liberación luego de la administración al cuerpo del mamífero.

[0030] Por ejemplo, en el caso del aceite, una clase especial de estructura de implante puede ser formada donde el núcleo del aceite está formado con una concha de micropartículas de dextrán cristalizado o agregados del mismo dispersados en agua o soluciones acuosas de polímeros orgánicos tales como polisacáridos (por ejemplo, dextranos). La estructura descrita puede ser designada como una cápsula. Se debe notar que la concha puede comprender una concha de forma rugosa esférica que resulta cuando la cápsula se rodea por el tejido, sin embargo, cuando la cápsula se ubica cerca de una barrera, tal como un sustrato, hueso o pared de intestino, la cápsula puede comprender un núcleo ubicado entre una o mas paredes de micropartículas en un lado y la

64 \$

barrera en otro lado. Adicionalmente, mientras se utiliza aceite como un ejemplo ilustrativo, el núcleo puede comprender otros materiales, tales como otros polímeros, células, etc.

[0031] Para formar la estructura de la cápsula, los sistemas acuosos de dos fases se aplican. Cuando las soluciones acuosas de polímeros diferentes se mezclan por encima de ciertas concentraciones ellos frecuentemente forman soluciones de dos fases de líquido inmiscible. Cada una de las fases consiste usualmente de mas del 90% de agua y puede ser amortiguada y hecha isotónica. Si una célula o suspensión de partículas se agrega a cada sistema, las células o partículas se ha encontrado frecuentemente que tienen división desigual entre fases. Este comportamiento de partición preferencial puede ser usado como unas bases para procedimientos de separación para diferenciar poblaciones de células o partículas en razón de la partición en esos sistemas se determina directamente por células o propiedades de superficie de partícula. Las células o partículas que no tienen propiedades de superficie idéntica exhiben comportamiento de partición suficientemente diferente.

[0032] La absorción competitiva de las dos fases de polímeros depende de la naturaleza química de los polímeros.

65

Un método de polímero de las dos fases ha sido aplicado para separar o dividir células, proteínas, ácidos nucleicos y minerales ("Partitioning in Aqueous Two-Phase Systems", 1985, eds., H. Walter, D. Brooks, and D. Fisher, publs, Academia Press).

[0033] Los experimentos con la distribución de micropartículas de dextrán cristalizado en sistemas de fase derivados de, por ejemplo, dextrán/mezclas PEG polietilenglicol, reveló que las micropartículas de dextrán prefieren estar en la fase dextrán, mientras que otras fases de PEG pueden estar dispersas en esta fase de dextrán para formar una emulsión P/P y viceversa en el caso cuando el volumen de la fase PEG es mayor que el volumen de la fase dextrán, como se muestra en la figura 5 y 6.

[0034] La figura 5 es una fotografía de una emulsión de una solución acuosa de PEG en solución acuosa de dextrán que contiene micropartículas de dextrán cristalizado. En la estructura de la figura 5, el volumen de la fase PEG es menor que el volumen de la fase dextrán. La fase dextrán contiene el dextrán y micropartículas de dextrán cristalizado. Así, las formas de fase PEG en uno o más núcleos con forma de esfera redondeados por conchas de micropartículas de dextrán/dextrán (es decir, una estructura de poros cerrados).

b

[0035] La figura 6 es una fotografía de una emulsión de solución acuosa de dextrán que contiene micropartículas de dextrán cristalizado en solución acuosa de PEG, en donde el volumen de la fase PEG es mas grande que el volumen de la fase dextrán. En este caso, las formas de la fase dextrán dentro de uno o más núcleos de forma esférica que contiene las micropartículas de dextrán rodeados por una fase PEG (es decir, una estructura de poro abierto que se forma in vivo mientras el PEG se disipa en tejido líquido). Como se puede ver en la figura 6, el volumen más pequeño (gotita) en formas de fase dextrán dentro de un largo dextrán esférico/gran núcleo de micropartícula (inferior derecho de la figura 6) cuyas esferas mas pequeñas comprenden dextrán/se unen y se fusionan con micropartículas de dextrán.

[0036] Así, cuando la proporción de volumen de la primera fase (tal como la fase PEG y sus inclusiones, tales como un agente terapéutico) a el volumen de la segunda fase (tal como la fase dextrán y sus inclusiones, tal como micropartículas de dextrán) es menor de uno, entonces la cápsula forma por sí mismo un ensamble con un núcleo de la primera fase rodeado por una concha de la segunda fase. Si la composición contiene un agente terapéutico, tal como insulina que prefiere dividirse en fase PEG y las micropartículas de dextrán que

prefieren dividirse en la fase dextrán, entonces el agente terapéutico se divide selectivamente en el núcleo PEG mientras que las micropartículas se dividen selectivamente dentro y forman alrededor del núcleo el PEG mediante autoensamble.

[0037] La emulsión puede ser preparada al mezclar el dextrán preparado y las fases separadamente PEG y ambos pueden ser suspensiones de diferentes tipos de partículas que prefieren estar en la fase PEG o en la fase dextrán respectivamente. El principio es que la partición de las partículas en diferentes fases de polímeros depende de su estructura de superficie y de la energía interfacial de las partículas en la solución de polímeros.

[0038] La inyección de sistema de dos fases acuosas que contiene micropartículas de dextrán cristalizada dentro de tejidos en animales experimental reveló que la formación de implantes con la estructura de la cápsula como se mostró en la figura 7 y 8. El volumen de la fase dextrán es más grande que el volumen de la fase PEG en el sistema de dos fases. Ambas figuras 7 y 8 muestran que una cápsula con el núcleo PEG y un dextrán/conchas de micropartículas dextrán formadas por autoensamble in vivo (es decir, después de inyección dentro del tejido de un mamífero). Las conchas comprenden

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regiones macroporosas entre micropartículas microporosas como también regiones microporosas en las mismas micropartículas.

[0039] Un ejemplo no limitativo de un método para formar una estructura de cápsula a partir de un sistema de dos fases es como el siguiente. 10 g de dextrán T40 (40 kDa de peso molecular) y 2 g de PEG son disueltos en 88 ml de (Actrapid) solución de insulina que contiene 1.000 y UI del cual 25 g de micropartículas de dextrán cristalizado son agregados. Estas etapas se desarrollan bajo condiciones de flujo laminar. La mezcla se agita sobre un agitador magnético a 100 rpm a temperatura ambiente por 30 minutos para formar una mezcla homogénea (por ejemplo, una suspensión). 1,0 g de la suspensión contiene 8 UI de insulina.

[0040] Se debe notar que las micropartículas de dextrán pueden ser preparadas a partir de una solución de diferente peso molecular que la solución de dextrán la cual se suministró en el sistema de dos fases. Así, las micropartículas de dextrán cristalizadas pueden ser formadas en una solución de dextrán de bajo peso molecular, tal como una solución de 2 a 20 kDa, que la solución de dextrán que se suministró en el sistema de dos fases, el cual puede ser una solución de dextrán de 40 a 500 kDa, como también una solución de 40 a 75 kDa. Esto es una ventaja debido al alto

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peso molecular de las soluciones de dextrán, tal como soluciones de 40 a 70kDa, han recibido aprobación ampliamente regulatoria y puede ser usado para formar una concha de una cápsula a concentraciones menores. Las soluciones de bajo peso molecular pueden ser usadas para disminuir el tiempo de cristalización sin la solución de dextrán de bajo peso molecular que actualmente está siendo suministrado en vivo. Además las micropartículas de alto peso molecular pueden ser disueltas in vivo.

[0041] La estructura de la cápsula formada a partir de un sistema de dos fases es ventajoso debido a que esta permite mas liberación y liberación prolongada de un agente terapéutico del núcleo de una composición que comprende una fase simple que contiene las micropartículas. Adicionalmente se cree que al utilizar una estructura de cápsula, una cantidad inferior de micropartículas se pueden necesitar para alcanzar el mismo mejor tiempo de liberación que si se utiliza un sistema de fase simple. Además, para controlar la cantidad de micropartículas en el sistema de dos fases, se cree que el espesor de la concha de micropartícula pueda ser controlado. Una concha mas gruesa resulta de una cantidad más grande de micropartículas. Así, la cantidad, duración y/o tiempo de liberación del agente terapéutico a partir del núcleo de la cápsula puede ser controlado al controlar el

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espesor de la concha. Por lo tanto, el perfil de liberación del agente terapéutico puede ser utilizado por cada paciente o grupos de pacientes.

[0042] Se debe notar que mientras el PEG y el dextrán son usados como ejemplos de materiales de las dos fases, cualquier otro material adecuado que muestre el siguiente comportamiento de partición puede ser utilizado. La figura 9A ilustra sistemáticamente la partición de diferentes tipos de partículas en su sistema acuoso de dos fases. Por ejemplo, tres tipos de moléculas o agregados moleculares, que son preferiblemente partículas 10, 12 y 14, y dos fases 16 y 18 son mostradas en la figura 9A. Sin embargo, puede haber dos, o más que tres tipos de partículas. Las partículas pueden ser micropartículas tales como microesferas o nanoesferas preparadas de materiales orgánicos y/o inorgánicos, liposomas, células vivientes, virus y macromoléculas. El primer tipo de partículas 10 preferencialmente segrega dentro de la primer fase 16. El segundo tipo de partículas 12 segrega preferencialmente al límite de la primera fase y segundas fases 18. El tercer tipo de partículas 14 preferencialmente segrega dentro de la segunda fase 18. Así, por analogía al ejemplo previo no limitativo, las primeras partículas 10 pueden comprender un agente terapéutico, el segundo 12 y/o el tercero partículas 14 pueden comprender

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micropartículas de dextrán cristalizado, la primera fase 16 puede comprender una fase PEG y la segunda fase 18 puede comprender una fase dextrán.

[0043] Sí una pequeña cantidad de la primera fase 16 se suministra dentro de una cantidad mas grande de la segunda fase 18, como se muestra en un área 20 de la figura 9^a, entonces una estructura de tipo cápsula forma comprendiendo esferas discretas de la primer fase 16 que contiene una concentración del primer tipo de partículas 10, localizado en una segunda fase 18. El segundo tipo de partículas 12 puede ser ubicado en la interfase de la fase 16, 18 y actuar como una concha de la cápsula. Las partículas 14 son dispersadas en la segunda fase 18 y/o formar una concha de la cápsula.

[0044] En contraste, si una pequeña cantidad de la segunda fase 18 se suministra a lo largo de una cantidad de la primer fase 16, como se muestra en el área 22 de la figura 9A, entonces una estructura tipo cápsula se forma comprendiendo esferas discretas de la segunda fase 18 que comprende una concentración del tercer tipo de partículas 14, ubicado en una primer fase 16. El segundo tipo de partículas 12 puede ser ubicado en la interfase de las fases 16, 18 y actuar como una concha de la cápsula. Las partículas 10 están dispersas en la primer fase 16 y/o forman una concha de la cápsula. Los

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dos sistemas de fase 20 y 22 pueden ser usados como un implante, tal como al ser inyectado dentro de un mamífero, como un animal o un humano. Así, la cápsula forma un implante tridimensional estructurado, con el núcleo que actúa como un reservorio o depósito para liberación controlada del agente terapéutico a través de la concha. En contraste, un implante con una distribución de micropartículas es un implante no estructurado.

[0045] Adicionalmente, las partículas 10, 12 y 14 pueden ser sustituidas por un material líquido (por ejemplo, aceite) o macromoléculas que selectivamente se particionan en una de las fases. Por ejemplo, un agente terapéutico, tal como insulina, puede ser particionado en fase PEG del sistema de dos fases PEG/dextrán. En razón a que la insulina selectivamente se particiona en la fase PEG, forma un núcleo que contiene insulina de una estructura de cápsula. Se debe notar que mientras ciertas partículas o agentes terapéuticos selectivamente particionan, el término "selectivamente particionado" no necesariamente significa que el cien por ciento de las partículas o agente terapéutico se particionan en una de las fases. Sin embargo, una mayoría de las especies particionadas, preferiblemente el 80% de la especie particionada, se particiona en una de las fases. Por ejemplo, mientras una de la mayoría de particiones de insulina en la

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fase PEG, una porción de la insulina puede permanecer en la fase dextrán.

[0046] La figura 9B ilustra una imagen de microscopía de electrón de exploración de una sección transversal de una estructura de implante basada en el sistema de dos fases ilustrado esquemáticamente en la figura 9A. Una composición acuosa de dos fases que comprende una primer fase de dextrán, una segunda fase de PEG y micropartículas de dextrán cristalizado fue inyectada en el gel sefarosa. Esta composición de gel imita el tejido del mamífero al detener la difusión de micropartículas de dextrán cristalizados de la inyección. La imagen de la figura 9B ilustra la formación de una estructura de implante de núcleo de concha. El núcleo comprende regiones 30 y 32 rodeadas por una concha 34. La región 30 es un vacío que se llena con una región de fase PEG antes de cortar el gel para una formación de imagen SEM transversal seccional. La región de fase PEG gotea hacia fuera del gel cuando el gel se corta durante el seccionamiento transversal. La región 32 es una porción exterior del núcleo que comprende las gotitas PEG ubicadas en las micropartículas de dextrán cristalizado. La región 34 es la concha que comprende las micropartículas del dextrán cristalizado que rodean y se mantienen en el lugar del núcleo que contiene PEG.

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[0047] Sin desear unirse a una teoría en particular, el presente inventor cree que la estructura de núcleo de concha mostrado en la figura 9B formado mediante el autoensamble como se muestra esquemáticamente en la figura 9C, mientras que las primeras fases 16 y segunda fase 18, tal como una solución acuosa de polímero diferente incompatible, están en un contenedor de almacenamiento adecuado 19, tal como en un beaker de vidrio, una fase 16 se eleva sobre la otra fase 18. Cuando la composición de dos fases se inyecta dentro de un material que restringe el flujo libre de las fases 16 y 18, tal como el tejido de un mamífero o un material de un sustrato, tal como un gel que imita el tejido, la composición se autoensambla dentro de la estructura del núcleo de concha. Primero, la fase que está presente en el volumen más pequeño se forma dentro de las formas esféricas aproximadas, como se muestra en la porción media de la figura 9C. Luego las formas esféricas se unen para formar núcleos aproximadamente esféricos de una fase rodeada por conchas de la otra fase, como se muestra en el fondo de la figura 9C. Mientras un ejemplo de un sistema de dos fases de un sistema multifase, ha sido ilustrado, el sistema multifase puede tener más de dos fases si se desea.

C. Vehículo de administración de insulina inyectable.

[0048] El siguiente inventor ha descubierto que una composición de micropartículas de dextrán cristalizado e insulina inyectado a mamíferos, tal como ratones y conejos, inesperadamente extendió la duración de la eficacia de la insulina comparado con inyecciones de la misma dosis de la misma insulina sola. La figura 10 ilustra esquemáticamente la formación de un implante 40 en un mamífero 53 mediante la inyección de una composición de fase que comprende las micropartículas 12, 14 e insulina 46 usando una jeringa 56. La figura 11 ilustra esquemáticamente la formación de un implante 40 en un mamífero 53 mediante la inyección de una composición de dos fases que comprende una fase dextrán 18 que contiene selectivamente micropartículas de dextrán cristalizado 12, 14 y una fase PEG 16 que contiene selectivamente agente terapéutico particionado que comprende insulina. La fase dextrán 18 forma una concha alrededor de núcleo 16 de la fase PEG. En razón que los ratones y conejos son modelos comunes para los humanos en ensayos de drogas, el presente inventor cree que la composición que comprende micropartículas de dextrán cristalizadas e insulina podría también ser efectivo extendiendo la duración de la eficacia de la insulina cuando se inyecta dentro de humanos adultos y niños.

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[0049] Ejemplos 1-8 ilustran la ventaja de usar micropartículas de dextrán cristalizado como un vehículo de administración de insulina inyectable comparado con la insulina inyectada sola. El experimento involucró ratones y la observación se hizo de su respuesta a una suspensión acuosa inyectada subcutáneamente de micropartículas de dextrán cristalizada e insulina recombinante humana (NovoNordisk Actrapid Penfill®, 40 UI/ml).

[0050] La suspensión fue preparada como sigue. 5,0 de Dextrán T10 (Pharmacia, Uppsala, Suecia) fue disuelto en 20,0 g de agua. La solución fue filtrada a través de un filtro de 0,22 µm (Millipore, Bedford, MA) y secada por congelamiento. 3,0 g del polvo resultante se disolvió en 0,3 g de agua estéril y colocada en una caja a temperatura de 60° C. 6 horas después, las micropartículas de dextrán cristalizadas fueron lavadas mediante centrifugación a 3.000 g con 3 x 5,0 ml de agua estéril. Finalmente, la suspensión de micropartículas de dextrán cristalizadas fue mezclada con solución de insulina acuosa y utilizada en experimentos con ratones. Las muestras de las suspensiones fueron introducidas en las piernas de los ratones y las muestras de sangre de ratón fueron tomadas de las colas de cada ratón y analizadas para concentraciones de glucosa. La glucosa sanguínea fue

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medida utilizando el método de la glucosa oxidasa en un analizador de glucosa de sistema de un toque (Lifescan, Johnson & Johnson, Milpitas, CA, USA) después de calibración apropiada.

[0051] En un ejemplo comparativo 1, no se inyectó insulina dentro del ratón. En ejemplos comparativos 2, 3 y 7, insulina sola (0,5 UI) fue inyectada en tres ratones. En ejemplos 4-6 y 8, se inyectó insulina (0,5UI) e implante de micropartículas de dextrán cristalizadas en cuatro ratones. Los resultados se resumen en la tabla 1.

Tabla I

Ex #		0min Glucosa mmol/l	15min Glucosa mmol/l	30min Glucosa mmol/l	45min Glucosa mmol/l	120min Glucosa mmol/l	210min Glucosa mmol/l	270min Glucosa mmol/l	390min Glucosa mmol/l
1	Ratón intacto	7,9	8,1	8,2	8,4				
2	Insulina 0,5 UI	5,9	3,3	2,7	1,8	0,9	3,5	3,0	3,2
3	Insulina 0.5 UI	8,1	3,8	2,8	1,9	0,9	3,7	3,4	3,5
4	Insulina 0.5 UI con micro Partícul as de dextrán cristali	6,0	4,3	3,2	2,5	0,8	0,8	0,9	0,7

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	zado								
5	Insulina 0,5 Ul Con micro- Particul as de dextrán cristali zado	6,9	5,6	4,1	3,4	-	1,2	-	1,6
6	Insulina 0,5 Ul	5,9	3,5	2,9	1,9	1,2	1,0	1,0	0,7

[0052] La reducción del promedio de azúcar en la sangre (es decir, glucosa sanguínea) de animales es muy diferente cuando se aplicó 0,5 Ul i.m. con y sin micropartículas de dextrán cristalizado. Como se muestra en la tabla 1, el nivel de glucosa en los ratones de los ejemplos comparativos 2, 3 y 7 es cerca de la misma o menor que el nivel de glucosa en ratones de ejemplos 4-6 y 8 durante los primeros 45 minutos después de la inyección. El nivel de glucosa es el mismo en los ratones de ambos ejemplos comparativos 2, 3 y 7 y los ejemplos 4-6 y 8, 120 minutos después de la inyección. Sin embargo, el nivel de glucosa en los ratones de los ejemplos comparativos 2, 3 y 7 es cerca de 3 veces mayor que el nivel de glucosa en los ratones de ejemplos 4-6 y 8 a partir de 210 a 390 minutos después de la inyección. De hecho, el nivel de glucosa sanguínea en ratones en los ejemplos 4-6 y 8 no se

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incrementó sustancialmente (es decir, no se incrementó en mas del 10%, mantenido igual o disminuido) a partir de 120 minutos a 390 minutos después de la inyección. Por el contrario, el nivel de glucosa sanguínea en los ratones de los ejemplos comparativos 2, 3 y 7 inyectados con la misma cantidad de insulina sustancialmente se incrementa de 120 a 390 minutos después de la inyección. La inyección de insulina/micropartículas de dextrán cristalizado reduce la glucosa sanguínea durante un tiempo mayor que una inyección de insulina de la misma dosis sola. Así, la composición que contiene micropartículas de dextrán cristalizado e insulina pueden ser dosificadas para inyección.

[0053] Los siguientes experimentos en conejos también demuestran la inyección de insulina/ micropartículas de dextrán cristalizado reduce la glucosa sanguínea y mantiene un nivel basal de insulina sanguínea durante un tiempo mayor que la misma insulina de la misma dosis sola. Una composición inyectada subcutáneamente que comprende Actrapid HM® insulina de acción corta y micropartículas de dextrán cristalizado fueron inesperadamente encontradas para alcanzar la duración de eficacia de su insulina de acción corta para acceder la que se inyectó subcutáneamente, insulina de acción larga Monotard HM® sola.

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[0054] El término duración de la eficacia significa la reducción de concentración de glucosa sanguínea y/o mantenimiento del nivel basal de la concentración de insulina sanguínea a niveles deseados independiente de eventos externos que causan picos en la glucosa sanguínea, tales como comer. Así, el término de duración de la eficacia es un término relativo que compara la eficacia de la insulina y la composición de micropartículas al igual que la misma dosis de la misma insulina sola. En otras palabras, la duración de la eficacia es una duración de acción o una duración del efecto farmacológico, que puede ser medido en un paciente en un listado de ayuno para comparar la eficacia de la insulina y la composición de micropartícula al igual que la dosis de la misma insulina sola.

[0055] Como se muestra en las figuras 12A y 12B, la composición que comprende la insulina de acción corta Actrapid HM® y las micropartículas de dextrán cristalizado prolongaron la absorción de insulina y alcanzaron el efecto hipoglicémico (es decir, la duración de la eficacia de la insulina) al menos 24 horas, tal como cerca de 28 a 31 horas, según se comparó cerca de 2 a 8 horas para la insulina Actrapid® HM sola (figura 12B) y cerca de diez y siete y cerca de veinte y cuatro horas para la insulina "Monotard HM"® insulina sola (Figura 12A). Ambas insulinas Actrapid® HM

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y "Monotard HM"® son productos de Novo Nordisk y la duración de la eficacia de estas composiciones de insulina en humanos obtenidos de la información de la compañía son ocho y veinte y cuatro horas, respectivamente.

[0056] En las figuras 12A y 12B, la línea superior ilustra la línea de control para conejos intactos a los que no se administró insulina. El eje Y de las figuras 12A y 12B es una escala normalizada relativa de concentraciones de glucosa sanguínea para la misma dosis 8 IU de insulina. Los datos de las figuras fueron ajustados para ser mostrados en un diagrama para cada figura y los datos muestra los niveles de glucosa sanguínea luego de inyecciones de insulina.

[0057] Los datos mostrados en las Figuras 12A y 12B fueron obtenidos como sigue. Los conejos chinchilla ($2,3 \pm 0,3$ kg) fueron monitoreados por su respuesta a inyecciones de una formulación que consiste de micropartículas de dextrán cristalizado e insulina de corta duración Actrapid®. Las muestras de la formulación fueron inyectadas subcutáneamente en los conejos. La insulina de acción prolongada Monotard® HM (40 IU/ml) y la insulina de acción reducida Actrapid® HM fueron inyectadas subcutáneamente en conejos separados sin las micropartículas y utilizados como controles. Las muestras de sangre animal fueron tomadas de las orejas de los conejos

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y analizadas para la concentración de glucosa. La concentración de glucosa sanguínea fue medida con un analizador de glucosa (One-touch® Lifescan, Johnson & Johnson, Milpitas, CA, USA) después de calibración apropiada.

[0058] En los ejemplos comparativos 9 y 10, dos conejos intactos no se les suministró ningún tipo de insulina. En los ejemplos comparativos 11 y 12 en solución acuosa de insulina de acción prolongada Monotard® HM fue introducida subcutáneamente en dos conejos en una dosis de 8 IU. En los ejemplos 13-15, una suspensión de micropartículas de dextrán cristalizado con insulina de acción reducida Actrapid® HM fue introducida subcutáneamente en una dosis de 8IU. Los resultados de los experimentos se resumen en la tabla II.

TABLA II

Ex #	Dosis de insulina	0 horas Glucosa Mmol/L	0,5 horas Glucosa Mmol/L	1 horas Glucosa Mmol/L	1,5 horas Glucosa Mmol/L	2 horas Glucosa Mmol/L	2,5 horas Glucosa Mmol/L	16 horas Glucosa Mmol/L	24 horas Glucosa Mmol/L	31 horas Glucosa Mmol/L
9	0,0	5,4	5,0	5,2	5,2	5,2	5,4	4,7	5,4	5,4
10	0,0	6,0	6,3	6,3	6,3	6,3	6,4	5,7	5,6	5,8
11	8IU	5,4	5,8	3,8	3,2	2,4	2,6	3,9	5,6	N.A
12	8IU	5,4	5,0	4,2	2,9	2,5	2,4	4,0	5,1	N.A
13	8IU	5,8	3,7	1,9	1,9	1,9	2,8	4,1	4,3	4,1
14	8IU	6,6	5,7	4,3	3,9	3,7	3,9	4,6	4,1	3,9
15	8IU	6,2	5,1	3,6	3,2	3,1	2,9	4,2	4,4	4,7

[0059] Los anteriores ejemplos 13-15 ilustran que la composición de micropartículas de dextrán cristalizado con insulina de acción corta Actrapid HM ® suministra un efecto prolongado que excede el efecto de acción prolongada de insulina Monotard HM® y se cree que es comparable con el efecto de acción prolongada de insulina glargine Lantus® (una dosis diaria) de Aventis (ver www.aventis-us.com/Pls/lantus-TXT.html).Adicionalmente, la insulina Lantus® no debe ser diluida o mezclada con otra insulina o solución. Si la insulina lantus® se diluyó o se mezcló, el perfil farmacocinético/farmacodinámico (por ejemplo, inicio de la acción, tiempo para el efecto pico) de Lantus® y/o insulina mezclada pueden ser alterados en una forma impredecible. Por el contrario, la composición de micropartículas de dextrán cristalizado con insulina no se limita porque ninguna insulina adecuada, tal como insulina humana, puede ser utilizado. En la composición de micropartículas de dextrán cristalizado e insulina, la proporción de insulina y de micropartículas puede varia según se desee. Adicionalmente, cualquier insulina adecuada puede ser utilizada para personalizar una terapia de insulina a un paciente individual. Así, el Actrapid HM® fue usado en la composición como un ejemplo ilustrativo de una insulina típica y la composición no se limita a esta marca de insulina.

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[0060] Como se muestra en los ejemplos 9-15, la composición que contiene las partículas de dextrán cristalizado e insulina es efectiva en mantener una duración de la eficacia de insulina durante al menos un 30% mayor, tal como al menos 100% mayor, preferiblemente 100 a 400% mayor que la misma dosis de la misma insulina sin las micropartículas. La composición de insulina que contiene micropartículas es efectiva en mantener un nivel basal deseado de insulina sanguínea y concentración de glucosa sanguínea durante al menos 30% mayor, tal como 100% a 400% mayor, que la misma dosis de la misma insulina sin las micropartículas. Así, la duración de la eficacia de la composición que contiene micropartículas es al menos 24 horas, lo cual permite ser inyectada una sola vez al mamífero, tal como un humano en necesidad de esta.

[0061] La composición de micropartículas de dextrán cristalizado e insulina duradera es más segura que las composiciones de insulina duradera de la técnica anterior. En razón a que esta alcanza una eficacia duradera sin utilizar una dosis mayor de insulina como en las composiciones de la técnica anterior. Por ejemplo, si una dosis de 8 IU de insulina de acción corta ha sido administrada médicamente segura a un paciente sin un riesgo importante de sobredosis,

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entonces la composición que comprende la misma insulina de acción corta y las micropartículas de dextrán cristalizado pueden suministrar una eficacia de acción de duración prolongada en la misma dosis de 8 IU de insulina de acción corta sin un riesgo significativo de sobredosis, aún si toda la insulina es liberada en el paciente de una sola vez. Adicionalmente, esta composición suministra un ahorro de costo comparado con las composiciones de la técnica anterior en razón a que esta extiende la eficacia sin incrementar la cantidad de insulina. Las terapias de diabetes de acción prolongada de la técnica anterior actuales están hechos con análogos de insulina, tal como insulina Lantus® de Aventis. En contraste, la micropartículas de dextrán cristalizado que contiene la composición preferiblemente contiene insulina recombinante humana cuyo perfil de seguridad se establece. Así, esta composición reduce el riesgo de reacciones adversas y el número de inyecciones a diabéticos, mejorando por tanto la calidad de vida de los diabéticos.

[0062] La composición inyectable puede comprender un sistema de fase simple que comprende insulina o micropartículas o un sistema de dos fases que forma un núcleo de PEG e insulina y un dextrán y una concha de micropartícula de dextrán para una duración aún mas grande de eficacia. Adicionalmente, la composición comprende un sistema coloidal

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multifase o una fase fluida (por ejemplo, una suspensión o una emulsión) la cual es relativamente fácil de inyectar en un mamífero.

[0063] El siguiente ejemplo ilustra el uso de una composición de dos fases inyectables que comprende una fase dextrán, una fase PEG, insulina y micropartículas de dextrán cristalizado. Se cree que cuando se inyecta en un mamífero, esta composición forma un implante de tipo reservorio estructurado que tiene una estructura de cápsula tridimensional. En la estructura de la cápsula, las micropartículas selectivamente se particionan en la fase dextrán y la insulina selectivamente se particiona en la fase PEG. La fase dextrán que contienen las micropartículas forma una concha alrededor de un núcleo que comprende la fase PEG que contiene la insulina. Este implante estructurado permite la liberación controlada del núcleo a través de la concha.

[0064] En el ejemplo comparativo 16, 0,5 IU de insulina Actrapid HM® (100 IU) se inyecta subcutáneamente en un ratón. En el ejemplo 17, 0,4 g de micropartículas de dextrán cristalizado están dispersos en 0,6 ml de 20% (P/P) de solución acuosa de dextrán que tiene un peso molecular de 70 kDa (Pharmacia, Suecia) para formar una suspensión. 10 mg de PEG que tiene un peso molecular de 6 kDa (Fluka) es disuelto

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en 0,1 ml de insulina Actrapid HM® (100 IU/ml) para formar una solución. 0,05 ml de la solución PEG e insulina se mezcla con 0,15 ml de la micropartícula y suspensión de dextrán para formar una composición de dos fases o mezcla. 0,02 ml de la mezcla de dos fases que contiene 0,5 IU de insulina es inyectada subcutáneamente en un ratón. Los resultados son mostrados en la Tabla III.

TABLA III

Ejemplo #	0 min Glucosa Mmol/L	15 min Glucosa Mmol/L	30 min Glucosa Mmol/L	45 min Glucosa Mmol/L	60 min Glucosa Mmol/L	120 min Glucosa Mmol/L
16	7,8	3,7	2,3	1,7	2,9	6,7
17	7,9	5,9	4,3	4,1	4,3	4,0

[0065] Como puede verse en la tabla III, la duración de la composición de dos fases de eficacia fue mayor que aquella de la insulina sola. Adicionalmente, la composición de dos fases disminuyó la glucosa sanguínea más gradualmente que la insulina sola. Sin desear unirse a una teoría en particular, estos efectos se cree que se deben a la liberación de insulina controlada del núcleo de la estructura de la cápsula.



[0066] Adicionalmente, la composición que contiene micropartículas puede ser adaptado para cada paciente al ajustar la cantidad de insulina y/o micropartículas para permitir al paciente inyectar la composición al mismo tiempo cada día (es decir, una vez cada 24 horas, una vez cada 48 horas, etcétera). Así, la eficacia de la composición se ajusta a cada paciente. Para un sistema de dos fases, el perfil de la liberación de insulina a partir del núcleo de la cápsula puede ser ajustada controlando la cantidad de micropartículas, para controlar el espesor de la concha de la cápsula.

[0067] Mientras el inventor no desea unirse a ninguna teoría en particular, se cree que el efecto duradero de la misma dosis de insulina en ratones y conejos con micropartículas de dextrán cristalizado se puede explicar por la difusión de las moléculas de insulina de las micropartículas de dextrán cristalizado basadas en implante (es decir, una autoliberación controlada de insulina). En razón a que los ratones y conejos son un modelo común para los humanos en ensayo de drogas, los datos mostrados en las tablas I a III anteriormente sugieren que el uso de micropartículas de dextrán cristalizado basado en implantes hace posible desarrollar sistemas de liberación controlada con características farmacocinéticas y dinámicas mejoradas y

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que presentan mejores necesidades en pacientes de insulina basal, tales como humanos.

D. Materiales

[0068] En las modalidades preferidas de la invención, el agente terapéutico comprende insulina. En otras palabras, el agente terapéutico puede consistir esencialmente de insulina sola o comprende insulina en combinación con otro agente. El termino "insulina" debe ser interpretado para abarcar análogos de insulina, insulina humana extraída naturalmente, insulina humana producida recombinante, insulina extraída de fuentes porcinas y/o bovinas, insulina bovina y porcina producida recombinante y mezclas de cualquiera de estos productos de insulina. El término se pretende que abarque el polipéptido utilizado normalmente en el tratamiento de diabéticos en una forma sustancialmente purificada pero que abarca el uso del término en su forma farmacéutica disponible comercialmente, que incluye excipientes adicionales. La insulina es producida preferiblemente recombinante y puede ser deshidratada (secada completamente) o en solución.

[0069] El término "análogo de insulina", "insulina monomérica" y los similares son usados intercambiabilmente aquí y se pretende que abarque alguna forma de "insulina"

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como se definió anteriormente, en donde uno o más de los aminoácidos dentro de la cadena de polipéptido ha sido reemplazado con un aminoácido alternativo y/o en donde uno o mas de los aminoácidos ha sido eliminado o donde uno o mas de los aminoácidos adicional ha sido agregado a la cadena de polipéptido o secuencia de aminoácido, que actúan como insulina en la reducción de los niveles de glucosa. En general, el término "análogos de insulina" de las modalidades preferidas de la presente invención incluye "análogos de lispro insulina", como se describe en la patente Estadounidense No. 5,547,929, incorporada aquí como referencia en su totalidad; los análogos de insulina incluyen insulina LysPro e insulina humalog, y otra "análogos super insulina", en donde la habilidad del análogo de insulina para afectar los niveles de glucosa de suero es sustancialmente mejorado como se comparó con la insulina convencional así como también con análogos de insulina hepatoselectivos que son mas activos en el hígado que en el tejido adiposo. Análogos preferidos son análogos de insulina monoméricos, que son compuestos similares a insulina para algún propósito general como la insulina, tal como insulina lispro, es decir, compuestos que son administrados para reducir los niveles de glucosa sanguínea.

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[0070] El término "análogo" se refiere a una molécula, que comparte una actividad funcional común con la molécula la cual se juzga para ser comparable y comparte típicamente características comunes también.

[0071] El término "recombinante" se refiere a cualquier tipo terapéutico clonado expresado en células procariotas o una molécula construida genéticamente o librería combinatoria de moléculas que pueden ser además procesadas en otro estado para formar una segunda librería combinatoria, especialmente moléculas que contiene grupos protectores que mejoran la seguridad fisicoquímica, farmacológica y clínica del agente terapéutico.

[0072] El término micropartículas de dextrán incluye micropartículas de dextrán no sustituidas y micropartículas de dextrán sustituidas. Por ejemplo, micropartículas de dextrán sustituidas incluyen dextrán sustituido con un grupo adecuado, tal como un grupo metilo, hasta un grado que no obstaculiza la cristalización de las micropartículas de dextrán, tales como hasta 3,5 o menos porcentaje de ramificación. El diámetro de micropartículas promedio es preferible de 0,5 a cerca de 5 micrones, más preferiblemente cerca de 1 a 2 micrones.

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[0073] Adicionalmente, mientras las micropartículas de dextrán no reticuladas porosas, tales como micropartículas cristalizadas, son preferiblemente usadas con el agente terapéutico, otras micropartículas orgánica e inorgánicas adecuadas puede ser utilizadas en cambio, tales como otras micropartículas de polímero que incluye polisacáridos, PLA, PLGA, PMMA, poliimidas, poliésteres, acrilatos, archilamidas, acetato vinilo u otros materiales poliméricos, partículas biomateriales tales como alginato y células, o partículas inorgánicas, tal como sílice, vidrio o fosfatos de calcio. Preferiblemente las micropartículas son biodegradables. Preferiblemente las micropartículas porosas son utilizadas. Más preferiblemente, las micropartículas tienen suficiente porosidad para contener el agente terapéutico dentro de los poros y suministrar una liberación a tiempo del agente terapéutico desde los poros. En otras palabras, el agente terapéutico se libera a tiempo desde los poros, tal como cerca de 5 minutos, preferiblemente cerca de 30 minutos, Más preferiblemente sobre una hora, tal como de varias horas a varios días, en diferencia de todo de una vez. Así, el material de partícula, el tamaño del poro y el volumen del poro puede ser seleccionado basado en el tipo de agente terapéutico utilizado, el volumen del agente terapéutico necesario para la administración, la duración de la

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administración del agente terapéutico, el ambiente en donde el agente terapéutico será liberado y otros factores.

[0074] Así, en un aspecto preferido de la presente invención, el agente terapéutico es localizado al menos parcialmente en los poros de las micropartículas de poro. Preferiblemente, el agente terapéutico no se encapsula en la micropartícula (es decir, la micropartícula no actúa como una concha con un núcleo de agente terapéutico en lugar de la concha) y no se adhiere a la superficie de la micropartícula. Sin embargo, si se desea, una porción del agente terapéutico puede ser encapsulado en una concha de micropartícula y ellas se adhiere a la superficie de la micropartícula en adición a los poros de la micropartícula. La ubicación del agente terapéutico en los poros suministra una liberación temporizada óptima del agente terapéutico. En contraste, el agente terapéutico adherido a la superficie de la micropartícula es a menudo liberado demasiado rápido, mientras que el agente terapéutico encapsulado en la micropartícula a menudo no se libera suficientemente pronto y es luego liberado todo de una vez cuando la concha de micropartícula se desintegra. En un sistema de dos fases, al menos un 80% del agente terapéutico es ubicado preferiblemente en un núcleo rodeado por una pared o concha que comprende las micropartículas.

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E. Métodos de Fabricación

[0075] Las micropartículas pueden ser formadas por algún método adecuado. Preferiblemente, las micropartículas son combinadas con los agentes terapéuticos después de que las micropartículas son formadas. Así, las micropartículas, tales como micropartículas de dextrán cristalizado se forman mediante cualquier método adecuado luego de que el agente terapéutico y las micropartículas se combinan mediante cualquier método adecuado. En contraste, algunos métodos de la técnica anterior, el agente terapéutico se encapsula en una concha de micropartícula al administrar el material precursor de partícula y al agente terapéutico dentro de una solución y luego cristalizar o reticulizar el material precursor, tal como un monómero o material oligómero, para encapsular un núcleo de agente terapéutico dentro de una concha de micropartícula.

[0076] Preferiblemente, el agente terapéutico se suministra en los poros de micropartículas porosos después de que las micropartículas son formadas. Así, las micropartículas porosas son primero formados y luego el agente terapéutico se suministra dentro de una solución que contiene las micropartículas para permitir al agente

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terapéutico permanecer dentro de los poros de las micropartículas. Por supuesto, algunos de los agentes terapéuticos pueden también ser atacados en la superficie de la micropartícula en este proceso.

[0077] Así, un método para fabricar poros de micropartículas de dextrán cristalizado no reticulado incluye la preparación de una solución de dextrán, tal como una solución de dextrán acuosa que conduce un proceso de cristalización para formar micropartículas de dextrán de poro cristalizado y si se desea, aislar las micropartículas de dextrán de la solución. Un agente terapéutico es luego permeable dentro de los poros de las micropartículas al suministrar el agente terapéutico dentro de la solución de cristalización que contiene las micropartículas o al suministrar las micropartículas aisladas y el agente terapéutico dentro de una segunda solución, tal como una segunda solución acuosa. Por ejemplo, las micropartículas de dextrán cristalizado pueden ser formadas en una primera, solución acuosa de dextrán de bajo peso molecular, tal como una solución de dextrán de 2 a 20 kDa. Las micropartículas son luego removidas a partir de la primera solución y luego colocadas dentro de una segunda solución acuosa de dextrán que tiene un dextrán de peso molecular mayor, tal como una solución de 40 a 500 kDa, por ejemplo, una solución de 40 a

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75 kDa. La segunda solución puede comprender una primera fase de un sistema de dos fases, que es luego combinada con una segunda fase, tal como una fase PEG que contiene un agente terapéutico. Un método similar puede ser utilizado con otras micropartículas de poro, en donde un agente terapéutico es luego permeable dentro de los poros de las micropartículas después de que las micropartículas de poro se forman mediante cualquier método de formación de micropartícula adecuado, que incluye, pero no está limitado a cristalización. Los componentes tales como insulina, micropartículas y una o más fases acuosas pueden ser combinadas en cualquier orden adecuado secuencialmente o simultáneamente.

[0078] Preferiblemente, las micropartículas se forman por autoensamble de una solución que no contiene solventes orgánicos y promotores de reacción orgánico que dejan un residuo orgánico en las micropartículas. Así, por ejemplo, las micropartículas de dextrán se forman preferiblemente por autoensamble de una solución de dextrán acuosa. Sin embargo, si se desea, los solventes orgánicos y/o promotores de reacción orgánica pueden ser también usados. En este caso, las micropartículas pueden ser purificadas antes de uso posterior para remover el residuo orgánico dañino.

[0079] Como se describió anteriormente, la estructura de la cápsula que tiene un primer núcleo de fase y una segunda pared de fase o concha puede ser formada in vivo o in Vitro a partir de una composición de dos fases. La composición puede ser polvo seco, tal como polvo seco por congelamiento y almacenado como un polvo o torta porosa. Cuando la solución esté lista para ser administrada a un mamífero, se hidrata y se administra a un mamífero mediante inyección.

[0080] Preferiblemente, la composición que incluye las micropartículas y el agente terapéutico es un sistema coloidal fluible en donde la composición se dosifica para la inyección. Ejemplos de sistemas coloides fluibles incluyen emulsiones y suspensiones que pueden ser inyectadas dentro de un mamífero utilizando una jeringa común o aguja sin dificultad. Por el contrario, algunas composiciones de la técnica anterior incluye un agente terapéutico en un hidrogel de dextrán o en una matriz de dextrán reticulada. Un hidrogel de dextrán y una matriz de dextrán no son composiciones fluibles sino específicamente preparadas.

[0081] En otro aspecto preferido de la presente invención, las micropartículas comprenden micropartículas que son adhesivas a la mucosa del mamífero. Preferiblemente las micropartículas adhesivas son micropartículas porosas

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descritas anteriormente. Esto mejora además la liberación efectiva del agente terapéutico.

[0082] En otro aspecto preferido de la presente invención, las micropartículas comprenden micropartículas cuya superficie han sido especialmente modificadas para mejorar la adhesión del agente terapéutico a la superficie de la micropartícula y para optimizar la liberación del agente terapéutico. La superficie de micropartícula puede contener cualquier modificación adecuada que puede incrementar la adhesión del agente terapéutico.

[0083] La anterior descripción de la invención ha sido presentada para propósitos de ilustración y descripción. No se pretende ser exhaustivo o limitar la invención para precisar la forma descrita, y las modificaciones y variaciones son posibles en claridad de las anteriores enseñanzas o pueden ser adquiridas de la práctica de la invención. Los dibujos y la descripción se escogieron con el fin de explicar los principios de la invención y su aplicación práctica. Se pretende que el alcance, de la invención se defina por las reivindicaciones adjuntas y sus equivalentes.

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[0084] Todas las publicaciones y solicitudes de patentes citadas en esta especificación se incorporan aquí en su totalidad para referencia.

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REIVINDICACIONES

1. Un método para reducir la glucosa sanguínea en un mamífero, que comprende inyectar una cantidad efectiva de una composición que comprende micropartículas de dextrán cristalizado e insulina al mamífero para reducir la glucosa sanguínea en donde las micropartículas se forman antes para combinación de la insulina y las micropartículas en la composición.
2. El método de la reivindicación 1, en donde la composición comprende una composición coloidal fluible y las micropartículas comprenden micropartículas de dextrán cristalizado que contienen un diámetro de 0,5 a 5 micrones.
3. El método de la reivindicación 2, en donde:

La composición comprende una composición de dos fases que comprende una fase dextrán y una fase PEG;

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La insulina es particionada selectivamente en la fase PEG y las micropartículas son particionadas selectivamente en la fase dextrán; y

La composición forma un implante estructurado que comprende una fase de PEG y una concha de fase dextrán después de inyección en el cuerpo de un mamífero.

4. El método de la reivindicación 3, además comprende controlar un espesor de la concha basado en el cuerpo del animal que recibe la composición para liberación controlada de insulina desde el implante.
5. El método de la reivindicación 1, en donde la composición se administra a un humano para reducir la concentración de glucosa sanguínea en el humano.
6. Una composición farmacéutica dosificada, que comprende micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina, en donde la composición se dosifica para un humano y las micropartículas que se forman antes para combinación de la insulina y las micropartículas en la composición.

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7. La composición de la reivindicación 6, en donde:

La composición comprende una composición coloidal fluible; y las micropartículas comprenden micropartículas de dextrán cristalizado que tienen un diámetro promedio de 0,5 a 5 micrones.

8. La composición de la reivindicación 7, en donde:

La composición comprende una composición de dos fases que comprende una fase dextrán y una fase PEG;

La insulina es selectivamente particionada en la fase PEG y las micropartículas se particionan selectivamente en la fase dextrán; y

La composición forma un implante estructurado que comprende un núcleo de fase PEG y una concha de fase dextrán después de inyección en el cuerpo humano.

9. Una composición farmacéutica dosificada, que comprende micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de la primera insulina, en donde la composición se dosifica para

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inyección a un mamífero y una duración de la eficacia de la composición cuando se inyecta en un mamífero es al menos un 30% mayor que una duración de eficacia en el mamífero de la misma dosis de la misma primera insulina sin las micropartículas.

10. La composición de la reivindicación 9, en donde:

La composición comprende una composición coloidal fluible; las micropartículas comprenden micropartículas de dextrán cristalizado que tienen un diámetro promedio de 0,5 a 5 micrones; y

Las micropartículas se forman antes para la combinación con la primera insulina y la micropartícula en la composición.

11. La composición de la reivindicación 10, en donde:

La composición comprende una composición de dos fases que comprende una fase dextrán y una fase PEG;

La primera insulina es particionada selectivamente en la fase PEG y las micropartículas son selectivamente particionadas en la fase dextrán; y

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La composición forma un implante estructurado que comprende un núcleo de fase PEG y una concha de fase dextrán después de inyección en un cuerpo de mamífero.

12. La composición de la reivindicación 9, en donde:

La duración de la eficacia de la composición cuando se inyecta dentro de un mamífero es al menos de 24 horas; y

La duración de la eficacia de la composición cuando se inyecta a un mamífero es al menos 100% mayor que una duración de eficacia en un mamífero de la misma dosis de la primera insulina sin las micropartículas.

13. Un método para reducir la glucosa sanguínea en un mamífero que comprende inyecta una cantidad terapéuticamente efectiva de una composición que comprende micropartículas de dextrán cristalizado y primera insulina para reducir la glucosa sanguínea del mamífero, en donde una duración de la eficacia de la composición en el mamífero es al menos 30%

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mayor que una duración de la eficacia del mamífero de la misma dosis de la misma primera insulina sin las micropartículas.

14. El método de la reivindicación 13, en donde:

La composición comprende una composición coloidal fluible; las micropartículas comprenden micropartículas de dextrán cristalizado que tienen un diámetro promedio de 0,5 a 5 micrones; y

Las micropartículas se forman antes para la combinación de la primera insulina y las micropartículas en la composición.

15. El método de la reivindicación 14, en donde:

La composición comprende una composición de dos fases que comprende una fase dextrán y una fase PEG;

La primera insulina es particionada selectivamente en la fase PEG y las micropartículas son selectivamente particionadas en la fase dextrán; y

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La composición forma un implante estructurado que comprende un núcleo de fase PEG y una concha de fase dextrán después de inyección en un cuerpo de mamífero.

16. El método de la reivindicación 13, en donde:

La duración de la eficacia de la composición cuando se inyecta dentro de un mamífero es al menos de 24 horas; y

La duración de la eficacia de la composición cuando se inyecta a un mamífero es al menos 100% mayor que una duración de eficacia en un mamífero de la misma dosis de la primera insulina sin las micropartículas.

17. El método de la reivindicación 16, en donde:

La duración de la eficacia de la composición cuando se inyecta a un mamífero es al menos 100 a 400% mayor que una duración de eficacia en un mamífero de la misma dosis de la primera insulina sin las micropartículas.

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18. Un método de fabricar una composición farmacéutica dosificada, comprende:

Suministrar micropartículas de dextrán cristalizado;

Combinar una cantidad terapéuticamente efectiva de insulina y las micropartículas de dextrán cristalizado en una solución después de que las micropartículas han sido cristalizadas para formar una composición de insulina y las micropartículas de dextrán cristalizado; y

Dosificar la composición por inyección a un mamífero.

19. El método de la reivindicación 18, en donde:

La composición comprende una composición coloidal fluible; las micropartículas comprenden micropartículas de dextrán cristalizado que tienen un diámetro promedio de 0,5 a 5 micrones.

20. El método de la reivindicación 19, en donde:

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La composición comprende una composición de dos fases que comprende una fase dextrán y una fase PEG;

La insulina es particionada selectivamente en la fase PEG y las micropartículas son selectivamente particionadas en la fase dextrán; y

Las composiciones forman un implante estructurado que comprende un núcleo de fase PEG y una concha de fase dextrán después de inyección en un cuerpo de mamífero.

21. El método de la reivindicación 18, en donde:

La duración de la eficacia de la composición cuando se inyecta dentro de un mamífero es al menos de 24 horas; y

La duración de la eficacia de la composición cuando se inyecta a un mamífero es al menos 100% mayor que una duración de eficacia en un mamífero de la misma dosis de la primera insulina sin las micropartículas.

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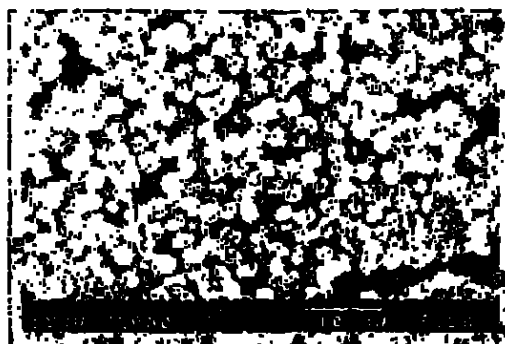


FIG. 1



FIG. 2A



FIG. 2B

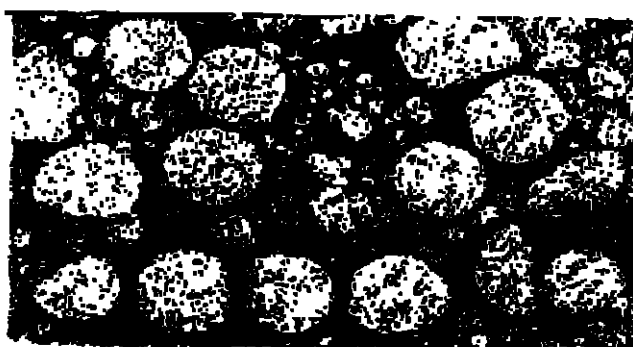


FIG. 3

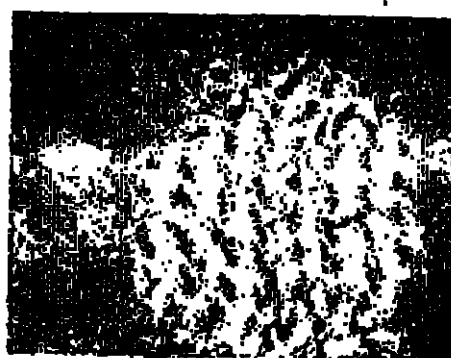


FIG. 4



FIG. 5

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FIG. 6

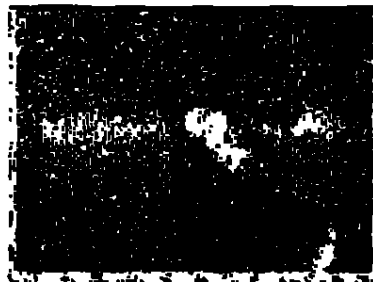


FIG. 7



FIG. 8

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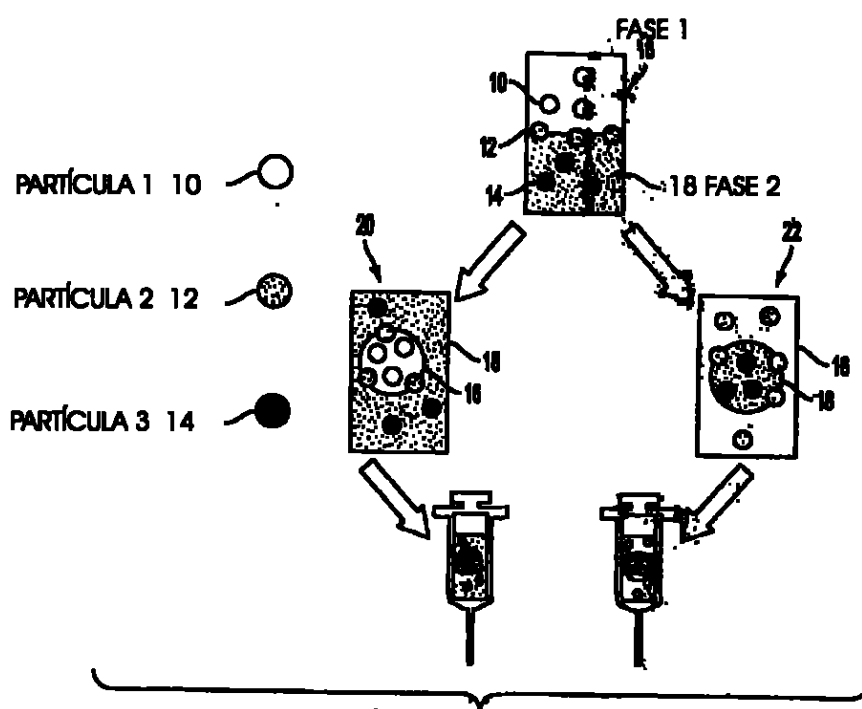




FIG. 9B

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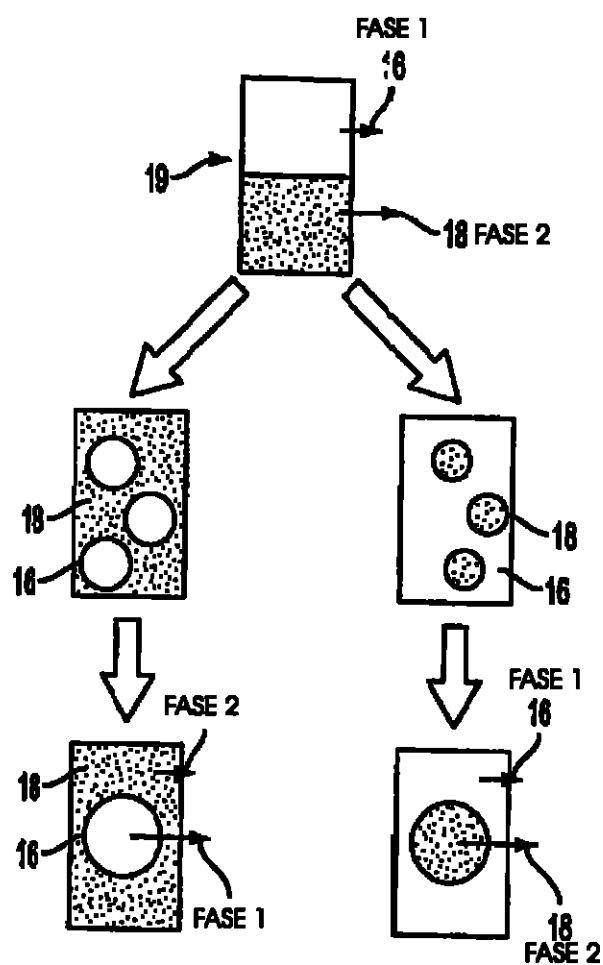


FIG. 9C

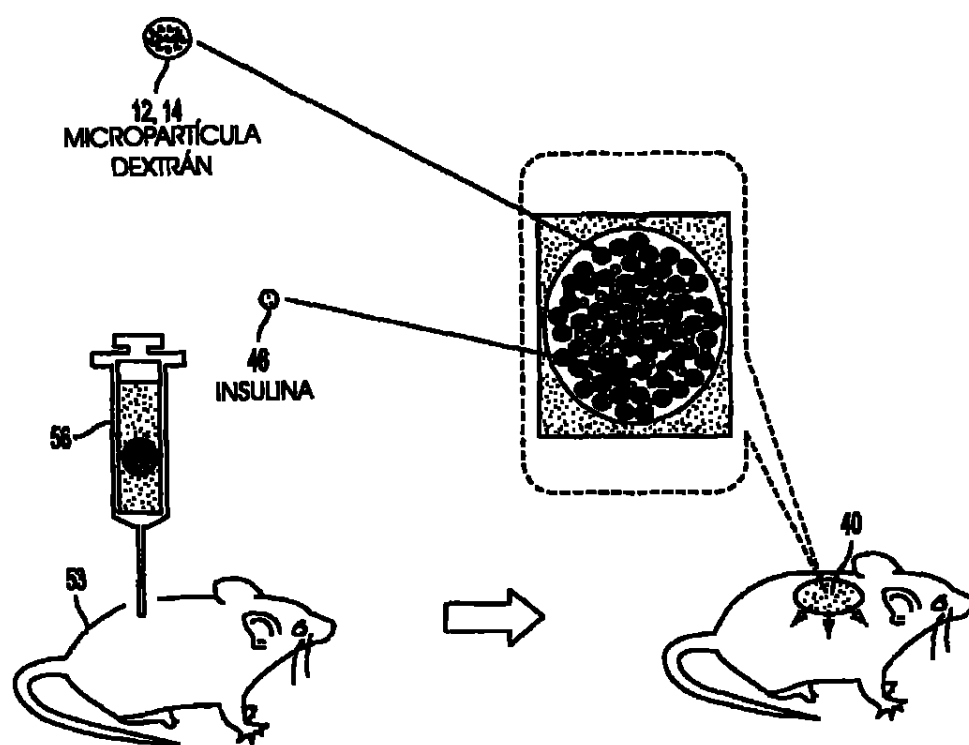


FIG. 10

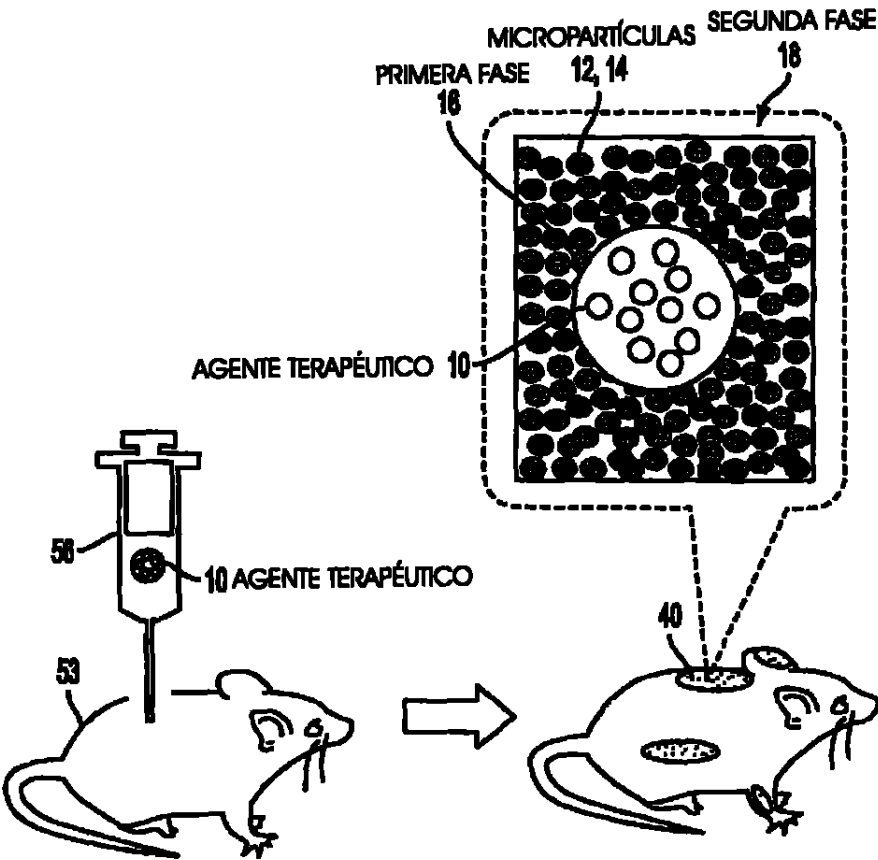


FIG. 11

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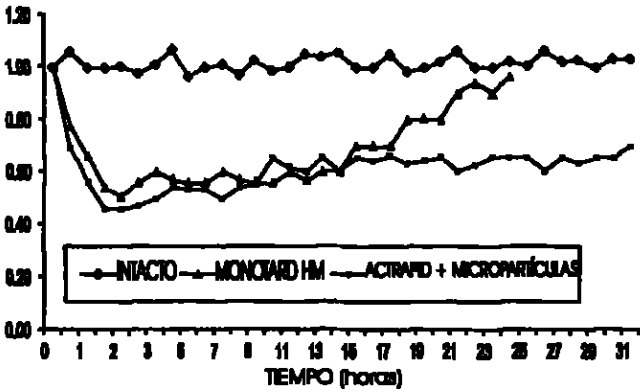


FIG. 12A

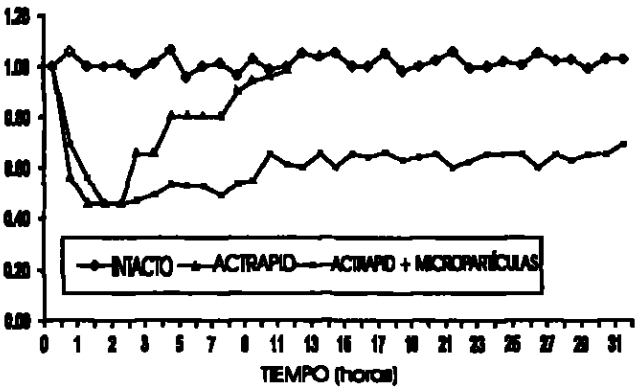


FIG. 12B

PCT REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

Sheet No. 1

297583

... receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference

(if desired) (12 characters maximum)

028093-0112

Box No. I TITLE OF INVENTION **LONG ACTING INJECTABLE INSULIN COMPOSITION AND METHODS OF MAKING AND USING THEREOF**

Box No. II APPLICANT

☐

This person is also inventor.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

THE TECHNOLOGY DEVELOPMENT COMPANY LTD.
Reid House
31 Church Street
Hamilton HM FX, Bermuda

Telephone No.

Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State (that is, country) of nationality: RU

State (that is, country) of residence: BM

This person is applicant for the purposes of:

☐

all designated States

☒

all designated States except the United States of America

☐

the United States of America only

☐

the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

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Russia

This person is:

☐

applicant only

☒

applicant and inventor

☐

inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality: RU

State (that is, country) of residence: RU

This person is applicant for the purposes of:

☐

all designated States

☐

all designated States except the United States of America

☒

the United States of America only

☐

the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent

☐

common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

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32,904

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Supplemental Box

If the Supplemental Box is not used, this sheet should not be included in the request.

1. If, in any of the Boxes, except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) If more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) If, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) If, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) If, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) If, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

2. If the applicant intends to make an indication of the wish that the international application be treated, in certain designated States, as an application for a patent of addition, certificate of addition, inventor's certificate of addition or utility certificate of addition: in such a case, write the name or two-letter code of each designated State concerned and the indication "patent of addition," "certificate of addition," "inventor's certificate of addition" or "utility certificate of addition," the number of the parent application or parent patent or other patent grant and the date of grant of the parent patent or other patent grant or the date of filing of the parent application (Rules 4.11(a)(iii) and 49bis.1(a) or (b)).

3. If the applicant intends to make an indication of the wish that the international application be treated, in the United States of America, as a continuation or continuation-in-part of an earlier application: in such case, write "continuation" or "continuation-in-part" and the number and the filing date of the parent application (Rules 4.11(a)(iv) and 49bis.1(d)).

Continuation of Box IV

PAVAN K. AGARWAL
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Box No. V DESIGNATIONS

The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

- ☐ DE Germany is not designated for any kind of national protection
- ☐ KR Republic of Korea is not designated for any kind of national protection
- ☐ RU Russian Federation is not designated for any kind of national protection

(The check-boxes above may be used to exclude (irrevocably) the designations concerned in order to avoid the costing of the effect, under the national law, of an earlier national application from which priority is claimed. See the Notes to Box No. V as to the consequences of such national law provisions in these and certain other States.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application*: regional Office	international application: receiving Office
item (1) 04 March 2003 (04/03/2003)	60/451,245	US		
item (2) 05 May 2003 (05/05/2003)	60/467,601	US		
item (3) 09 May 2003 (09/05/2003)	60/469,017	US		

☒ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as:

☒ all items ☐ item(1) ☐ item(2) ☐ item(3) ☐ item(4) ☐ item(5) ☐ other, see Supplemental Box

* Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organisation for which that earlier application was filed (Rule 4.10(b)(ii)):

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA/EP

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year) Number Country (or regional Office)

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):

		Number of declarations
☐ Box No. VIII(i)	Declaration as to the identity of the inventor	:
☐ Box No. VIII(ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐ Box No. VIII(iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☐ Box No. VIII(iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐ Box No. VIII(v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

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SUPPLEMENTAL BOX
Continuation of Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application: regional Office	international application: receiving Office
item (4) 15 August 2003 (15/08/2003)	60/495,097	US		
item (5)				
item (6)				

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Box No. IX CHECK LIST; LANGUAGE OF FILING		
This international application contains: (a) In paper form, the following number of sheets: request (including declaration sheets) : 5 descriptions (excluding sequence listings and/or tables related thereto) : 30 claims : 5 abstract : 1 drawings : 9 Sub-total number of sheets : Sequence listings : tables related thereto : 30 (For both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form, see (c) below) Total number of sheets : 50 (b) ☐ only in computer readable form (Section 801(a)(II)) (i) ☐ sequence listings (ii) ☐ tables related thereto (c) ☐ also in computer readable form (Section 801(a)(II)) (i) ☐ sequence listings (ii) ☐ tables related thereto Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the ☐ sequence listings: ☐ tables related thereto (Additional copies to be indicated under items 9(i) and/or 10(i), in right column):		This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item): 1. ☒ fee calculation sheet : 1 2. ☐ original separate power of attorney : 3. ☐ original general power of attorney : 4. ☐ copy of general power of attorney, reference number, if any : 7. ☐ statement explaining lack of signature : 6. ☐ priority document(s) identified in Box No. VI as item(s) : 7. ☐ translation of international application into (language) : 8. ☐ separate indications concerning deposited microorganism or other biological material : 9. ☐ sequence listings in computer readable form (indicate type and number of carriers) (i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application) : (ii) ☐ (only where check-box (b)(i) or (c)(i) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter : (iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listings mentioned in left column : 10. ☐ tables in computer readable form related to sequence listing (indicate type and number of carriers) (i) ☐ copy submitted for the purposes of international search under Section 802(b-quarter) only (and not as part of the international Application) : (ii) ☐ (only where check-box (b)(ii) or (c)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-quarter) together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column : (iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column : 11. ☒ other (specify): Transmittal Letter, Postcard & Check : 3
Figure of the drawings which should accompany the abstract: 1		Language of filing of the international application: English
Box No. IX SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE		
Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).  Michael Orischenko, Vice President of The Technology Development Company Ltd., Applicant		

For International Bureau use only	
1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA/	6. ☐ Transmittal of search copy delayed until search fee is paid

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Date of receipt of the record copy by the International Bureau:

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LONG ACTING INJECTABLE INSULIN COMPOSITION AND METHODS OF MAKING AND USING THEREOF

FIELD OF THE INVENTION

[0001] This application claims benefit of the following U.S. Provisional Applications Serial Nos. 60/451,245, filed March 4, 2003; 60/467,601 filed May 5, 2003; 60/469,017 filed May 9, 2003; and 60/495,097 filed August 15, 2003, the disclosures of which are incorporated by reference herein in their entirety.

[0002] The present invention relates generally to insulin compositions and specifically to an injectable insulin composition containing insulin and crystallized dextran microparticles.

BACKGROUND OF THE INVENTION

[0003] Dextran is a high molecular weight polysaccharide synthesized by some micro organisms or by biochemical methods. Dextran with average molecular weight of about 75 kDa has a colloid osmotic pressure similar to blood plasma, so its aqueous solutions are used clinically as plasma expanders. Cross-linked dextrans in the form of beads are the basis for "Sephadex" that is used in the GPC of proteins and for "Cytodex" developed by Pharmacia to fulfill the special requirements of a micro-carrier cell culture. For example, U.S. Patent Nos. 6,395,302 and 6,303,148 (Hennink et al.) disclose attaching various biomaterials to cross-linked dextran particles. However, beads based on cross-linked dextran generally cannot be used for implant manufacturing owing to their potential toxicity due to the application of cross-linking agents (Blain J.F., Maghni K., Pelletier S. and Sirois P. Inflamm. Res. 48 (1999): 386-392).

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[0004] U.S. Patent No. 4,713,249 (Schroder) describes a method of producing a depot matrix for biologically active substances. According to this patent, the depot matrix allegedly consists of carbohydrate microparticles, stabilized by crystallization, which implies using non-covalent bonds. The following process for producing the alleged crystallized carbohydrate microparticles is described by Schroder. A solution of a polymeric carbohydrate and a biologically-active substance is formed in one or more hydrophilic solvents. Then the mixture of the carbohydrate and the biologically active substance is emulsified in a liquid hydrophobic medium to form spherical droplets. The emulsion is then introduced into a crystallizing medium comprising acetone, ethanol or methanol to form spheres having a non-covalently cross-linked crystalline polymeric carbohydrate matrix, said matrix incorporating 0.001-50% by weight of the biologically-active substance. Thus, the biologically active substance is provided into the solution prior to crystallizing the spheres. Schroder does not describe the microstructure of the microparticles made by the multi-step method. Schroder's multi-step method is complex and uses organic solvents that are potentially toxic to cells and need to be removed.

BRIEF SUMMARY OF THE INVENTION

[0005] A method of lowering blood glucose in a mammal includes injecting a therapeutically effective amount of crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal. The composition may be a one phase or a structured multi-phase composition for controlled release of insulin over an extended period of time.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Figure 1 is a photograph of crystallized dextran microparticles spontaneously formed in 55.0% (W/W) aqueous solution of dextran with MW 70.0 kDa.

[0007] Figure 2A is a photograph of a cross-section of crystallized dextran microparticles shown in Figure 1.

[0008] Figure 2B is a photograph of a cross-section of a microparticle shown in Figure 2A. Microporous structure of the microparticle can be seen.

[0009] Figure 3 is a photograph of aggregates of crystallized dextran microparticles.

[0010] Figure 4 is a photograph of a slow release of the fluorescently labeled macromolecules from the implant which includes crystallized dextran microparticles into mouse muscle tissue on the 14th day after intermuscular injection.

[0011] Figure 5 is a photograph of an emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1.

[0012] Figure 6 is a photograph of an emulsion of aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1 in aqueous solution of PEG.

[0013] Figure 7 is a photograph of an intramuscular injection of emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1.

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[0014] Figure 8 is a photograph of a subcutaneous injection of emulsion of aqueous solution of PEG in aqueous solution of dextran (MW 500 kDa) containing crystallized dextran microparticles shown in Figure 1.

[0015] Figures 9A and 9C schematically illustrate partition behavior of different types of particles and phases in an aqueous two phase system.

[0016] Figure 9B is a photograph of a cross section of an implant structure based on the two phase system.

[0017] Figures 10 and 11 schematically illustrate therapeutic agent delivery methods according to embodiments of the present invention.

[0018] Figures 12A and 12B are graphs of relative normalized of blood glucose concentrations for various insulin containing composition versus time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0019] The present inventor has discovered that a composition of crystallized dextran microparticles and insulin injected into mammals unexpectedly extended the duration of efficacy of the insulin compared to injections of the same dose of the same insulin alone. The composition may be a one phase composition or a multi-phase composition which forms a structured implant in a mammal.

[0020] The first section below describes the crystallized dextran microparticles, the second section describes formation of the structured implant from a multiphase composition, and the following sections describe specific examples of injection of the composition into mammals and methods of making the injectable composition.

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A. Crystallized Dextran Microparticles

[0021] The present inventor has experimentally found that crystallized dextran microparticles with an average diameter ranging from 0.5 to 3.5 microns were spontaneously formed in concentrated aqueous solutions of dextrans (40 – 65 % W/W) with molecular weights ranging from 1.0 to 200.0 kDa, at temperature ranging from 20 – 90 °C. If it is desired to form the microparticles at room temperature, then 2 to 18 kDa dextran solutions may be used. Of course, the microparticles may also be formed from 2 to 18 kDa solutions at temperatures above room temperature, if desired. The microparticles may be spontaneously formed from higher molecular weight dextran solutions, such as 20 to 75 kDa solutions, at higher temperatures above room temperature, such as about 40 to about 70 °C. The microparticles may have any suitable shape such as a regular or an irregular shape, but are preferably spherical in shape, and are preferably 10 microns in diameter or less, such as 0.5 to 5 microns.

[0022] Transmission Electron Microscopy revealed the microporous structure of the crystallized dextran microparticles (see Figures 2A, 2B). Preferably, the microparticle porosity is at least 10 percent by volume, such as about 10 to about 50 percent, more preferably about 20 to about 40 percent. Thus, the structure comprises microporous microparticles with areas of macroporosity located between the particles.

[0023] Spray drying of aqueous suspensions of the crystallized dextran microparticles has shown the possibility to produce substantially spherical aggregates of crystallized dextran microparticles with a diameter ranging from 10.0 to 150.0 microns (see Figure 3).

[0024] A non limiting example of a method of forming the dextran microparticles is as follows. 50.0 g of dextran T40 (40 kDa molecular

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weight) from Amersham Biosciences is added to 50.0 g of sterile distilled water in a 500 ml lab beaker to obtain 50% w/w solution under laminar flow. The mixture is stirred at 60°C (water bath) on a magnetic stirrer at 50 rpm until the dextran is completely dissolved and a clear solution is obtained. The solution can be vacuumed to remove all air inclusions. The clear solution is placed in lab oven at 60°C under a Tyvek® lid. 3.5 hours later, a turbid viscous suspension is developed as a result of formation of crystallized dextran microparticles.

[0025] To eliminate non-crystallized dextran, the microparticles are washed by centrifugation, for example 3,000 g, 30 min, with 3 × 250 ml of distilled sterile water, or by filtration of diluted suspension of microparticles, for example one part microparticles and 10 parts water (3 × 250 ml of distilled sterile water through sterilization filter). The centrifugation/washing is done under laminar flow. The microparticles are placed in 500 ml lab beaker under a Tyvek® lid and dried at 60°C in lab oven for 8 hours to reach a moisture level of about 5%. The resulting dry powder consists of particles with a mean diameter of about 2 microns.

[0026] The crystallized microparticles preferably are comprised of dextran molecules (i.e., polymer molecules) that are held together by a plurality of hydrogen bonds, Van Der Waals forces and/or ionic bonds and having substantially no covalent bonds between the dextran molecules. Thus, the molecules in the microparticles are preferably not intentionally cross-linked (i.e., a cross linking step is not carried out) and the microparticles contain no covalent bonds between molecules or less than 10% covalent bonds between molecules.

[0027] The slow release of macromolecules from implants has been demonstrated in experiments where macromolecules were dissolved in aqueous suspensions of crystallized dextran microparticles or their

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aggregates before injections. Figure 4 shows an implant containing fluorescently labeled macromolecules (FITC-dextran, MW 500 kDa) and slow release of the macromolecules from the implant into a mouse muscle tissue on the 14th day after the intermuscular injection.

B. Two-phase system

[0028] Self assembled structures of implants based on crystallized dextran microparticles and their aggregates may be formed based on two phase systems.

[0029] Colloidal systems such as droplets of oil, liposomes, micro-and nano-particles can be dispersed in a suspension of crystallized dextran microparticles and injected to form an implant releasing therapeutic agent(s) following administration into the mammal body.

[0030] For example, in the case of oil, a special kind of implant structure can be formed where the oil core is surrounded with a shell composed of crystallized dextran microparticles or aggregates thereof dispersed in water or aqueous solutions of organic polymers such as polysaccharides (e.g. dextrans). The structure described can be designated as a capsule. It should be noted that the shell may comprise a roughly spherical shaped shell which results when the capsule is surrounded by tissue. However, when the capsule is located near a barrier, such as a substrate, bone or intestine wall, the capsule may comprise a core located between one or more walls of microparticles on one side and the barrier on the other side. Furthermore, while oil is used as an illustrative example, the core may comprise other materials, such as other polymers, cells, etc.

[0031] To form the capsule structure, two-phase aqueous systems are applied. When aqueous solutions of different polymers are mixed above

certain concentrations they frequently form immiscible-liquid two-phase solutions. Each of the phases usually consists of more than 90% water and can be buffered and made isotonic. If a cell or particle suspension is added to such a system, the cells or particles are frequently found to have partitioned unequally between phases. This preferential partition behavior can be used as a basis for separation procedures for differing cell populations or particles since partition in these systems is determined directly by cell or particle surface properties. Cells or particles which do not have identical surface properties exhibit sufficiently different partition behavior.

[0032] The competitive adsorption of the two polymer phase depends on the chemical nature of the polymers. A two-phase polymer method has been applied to separate or partition cells, proteins, nucleic acids and minerals ("Partitioning in Aqueous Two-Phase Systems", 1985, eds., H. Walter, D. Brooks, and D. Fisher, publs. Academic Press).

[0033] The experiments with the distribution of crystallized dextran microparticles in phase systems derived from, for instance, dextran/polyethylene glycol (PEG) mixtures, revealed that the dextran microparticles prefer to be in the dextran phase, while another PEG phase can be dispersed in this dextran phase to form a W/W emulsion and vice versa in the case when the volume of the PEG phase is bigger than the volume of the dextran phase, as shown in Figures 5 and 6.

[0034] Figure 5 is a photograph of an emulsion of aqueous solution of PEG in aqueous solution of dextran containing crystallized dextran microparticles. In the structure of Figure 5, the volume of the PEG phase is less than the volume of the dextran phase. The dextran phase contains the dextran and the crystallized dextran microparticles. Thus, the PEG

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phase forms into one or more sphere shaped cores surrounded by dextran / dextran microparticle shells (i.e., a closed pore structure).

[0035] Figure 6 is a photograph of an emulsion of aqueous solution of dextran containing crystallized dextran microparticles in aqueous solution of PEG, where the volume of the PEG phase is greater than the volume of the dextran phase. In this case, the dextran phase forms into one or more sphere shaped cores containing the dextran microparticles surrounded by a PEG phase (i.e., an open pore structure that is forming in vivo while PEG dissipates in tissue liquid). As can be seen in Figure 6, the smaller volume (droplet) dextran phase forms into a large spherical dextran / dextran microparticle core (bottom right of Figure 6) to which smaller spheres comprising dextran / dextran microparticles are joining and fuse with.

[0036] Thus, when the ratio of the volume of the first phase (such as the PEG phase and its inclusions, such as a therapeutic agent) to the volume of the second phase (such as the dextran phase and its inclusions, such as the dextran microparticles) is less than one, then the capsule forms by self assembly with a first phase core surrounded by a second phase shell. If the composition contains a therapeutic agent, such as insulin, which prefers to partition into the PEG phase, and the dextran microparticles which prefer to partition into the dextran phase, then the therapeutic agent selectively partitions into the PEG core while the microparticles selectively partition into and form the shell around the PEG core by self assembly.

[0037] The emulsion can be prepared by the mixing of separately prepared dextran and PEG phases and both can be suspensions of different types of particles that prefer to be in the PEG phase or in the dextran phase respectively. The principle is that the partition of particles

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into different polymer phases depends on their surface structure and interfacial energy of the particles in the polymer solutions.

[0038] Injection of aqueous two phase systems containing crystallized dextran microparticles into tissues of experimental animals revealed the formation of implants with the capsule structure as shown in Figures 7 and 8. The volume of the dextran phase is greater than the volume of the PEG phase in the two-phase system. Both Figures 7 and 8 show that a capsule with a PEG core and a dextran/dextran microparticle shell forms by self assembly in vivo (i.e., after injection into mammal tissue). The shell comprises macroporous regions between adjacent microparticles as well as microporous regions in the microparticles themselves.

[0039] A non limiting example of a method of forming a capsule structure from a two phase system is as follows. 10 g of dextran T40 (40 kDa molecular weight) and 2 g of PEG are dissolved in 88 ml of (Actrapid®) insulin solution containing 1,000 IU to which 25 g of crystallized dextran microparticles are added. These steps are performed under laminar flow conditions. The mixture is stirred on a magnetic stirrer at 100 rpm at room temperature for 30 minutes to form a homogeneous mixture (i.e., a suspension). 1.0 g of the suspension contains 8 IU of insulin.

[0040] It should be noted that the dextran microparticles may be prepared from a different molecular weight dextran solution than the dextran solution which is provided in the two phase system. Thus, the crystallized dextran microparticles may be formed in a lower molecular weight dextran solution, such as a 2 to 20 kDa solution, than the dextran solution which is provided into the two phase system, which may be a 40 to 500 kDa dextran solution, such as a 40 to 75 kDa solution. This is

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advantageous because the higher molecular weight dextran solutions, such as 40 and 70 kDa solutions, have received wider regulatory approval and can be used to form a shell of a capsule at lower concentrations. The lower molecular weight solutions may be used to decrease the crystallization time without the lower molecular weight dextran solution actually being provided in vivo. Furthermore, lower molecular weight microparticles may dissolve easier in vivo.

[0041] The capsule structure formed from a two phase system is advantageous because it allows for a more even and prolonged release of the therapeutic agent from the core than from a composition comprising a single phase containing the microparticles. Furthermore, it is believed that by using the capsule structure, a lower amount of microparticles may be needed to achieve the same or better timed release of a therapeutic agent than if a single phase system is used. Furthermore, by controlling the amount of microparticles in the two phase system, it is believed that the thickness of the microparticle shell may be controlled. A thicker shell results from a larger amount of microparticles in the two phase system. Thus, the amount, duration and/or timing of the release of the therapeutic agent from the capsule core may be controlled by controlling the thickness of the shell. Therefore, the release profile of the therapeutic agent may be customized for each patient or groups of patients.

[0042] It should be noted that while PEG and dextran are used as examples of the materials of the two phases, any other suitable materials which show the following partition behavior may be used instead. Figure 9A schematically illustrates partition behavior of different types of particles in an aqueous two phase system. For example, three types of molecules or molecular aggregates, which are preferably particles 10, 12 and 14, and two phases 16 and 18 are shown in Figure 9A. However, there may be two, or more than three types of particles. The particles

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may be microparticles such as microspheres or nanospheres prepared from organic and/or inorganic materials, liposomes, living cells, viruses and macromolecules. The first type particles 10 preferentially segregate into the first phase 16. The second type particles 12 preferentially segregate to the boundary of the first 16 and second 18 phases. The third type particles 14 preferentially segregate into the second phase 18. Thus, by analogy to the previous non-limiting example, the first particles 10 may comprise a therapeutic agent, the second 12 and/or the third 14 particles may comprise crystallized dextran microparticles, the first phase 16 may comprise a PEG phase and the second phase 18 may comprise a dextran phase.

[0043] If a smaller amount of the first phase 16 is provided into a larger amount of the second phase 18, as shown in area 20 of Figure 9A, then a capsule type structure forms comprising discreet spheres of the first phase 16 containing a concentration of the first type particles 10, located in a second phase 18. The second type particles 12 may be located at the interface of the phases 16, 18 and act as a shell of the capsule. Particles 14 are dispersed in the second phase 18 and/or form a shell of the capsule.

[0044] In contrast, if a smaller amount of the second phase 18 is provided into a larger amount of the first phase 16, as shown in area 22 of Figure 9A, then a capsule type structure forms comprising discreet spheres of the second phase 18 containing a concentration of the third type particles 14, located in a first phase 16. The second type particles 12 may be located at the interface of the phases 16, 18 and act as a shell of the capsule. Particles 10 are dispersed in the first phase 16 and/or form a shell of the capsule. The two phase systems 20 and 22 may be used as an implant, such as by being injected into a mammal, such as an animal or human. Thus, the capsule forms a structured, three

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dimensional implant, with the core acting as a reservoir or depot for controlled release of the therapeutic agent through the shell. In contrast, an implant with an even distribution of microparticles is an unstructured implant.

[0045] Furthermore, particles 10, 12 and 14 may be substituted by a liquid material (e.g. oils) or macromolecules which selectively partition into one of the phases. For example, a therapeutic agent, such as insulin, may be partitioned in PEG phase of the PEG/dextran two phase system. Since insulin selectively partitions into the PEG phase, the PEG phase forms an insulin containing core of a capsule structure. It should be noted that while certain particles and therapeutic agents selectively partition, the term "selectively partitioned" does not necessarily mean that 100 percent of the particles or therapeutic agent partition into one of the phases. However, a majority of the selectively partitioned specie, preferably 80% of the partitioned specie, partitions into one of the phases. For example, while a majority of insulin partitions into the PEG phase, a portion of insulin may remain in the dextran phase.

[0046] Figure 9B illustrates a scanning electron microscope image of a cross section of an implant structure based on the two phase system schematically illustrated in Figure 9A. A two phase aqueous composition comprising a first dextran phase, a second PEG phase and crystallized dextran microparticles was injected into sepharose gel. This gel's composition mimics mammal tissue by stopping crystallized dextran microparticles diffusion from the injection side. The image in Figure 9B illustrates the formation of a core-shell implant structure. The core comprises regions 30 and 32 surrounded by a shell 34. Region 30 is a void that is filled with a PEG phase region prior to cutting the gel for cross sectional SEM imaging. The PEG phase region drips out of the gel when the gel is cut during cross sectioning. Region 32 is an outer portion of

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the core comprising PEG droplets located in the crystallized dextran microparticles. Region 34 is the shell comprising the crystallized dextran microparticles which surrounds and holds in place the PEG containing core.

[0047] Without wishing to be bound by a particular theory, the present inventor believes that the core-shell structure shown in Figure 9B forms by self assembly as shown schematically in Figure 9C. While the first 16 and second 18 phases, such as aqueous solutions of different, incompatible polymers, are in a suitable storage container 19, such as in a glass beaker or vial, one phase 16 rises above the other phase 18. When the two phase composition is injected into a material which restricts free flow of the phases 16 and 18, such as mammal tissue or a substrate material, such as a gel which mimics the tissue, the composition self assembles into the core-shell structure. First, the phase that is present in the smaller volume forms into approximate spherical shapes, as shown in the middle portion of Figure 9C. Then the spherical shapes join to form approximately spherical cores of one phase surrounded by shells of the other phase, as shown in the bottom of Figure 9C. While a two phase system example of a multiphase system has been illustrated, the multiphase system may have more than two phases if desired.

C. Injectable Insulin delivery vehicle

[0048] The present inventor has discovered that a composition of crystallized dextran microparticles and insulin injected into mammals, such as mice and rabbits, unexpectedly extended the duration of efficacy of the insulin compared to injections of the same dose of the same insulin alone. Figure 10 schematically illustrates the formation of an implant 40 in a mammal 53 by injection of a one phase composition comprising the microparticles 12, 14 and insulin 46 using a syringe 56. Figure 11

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schematically illustrates the formation of a structured implant 40 in a mammal 53 by injection of a two phase composition comprising a dextran phase 18 containing selectively partitioned crystallized dextran microparticles 12, 14 and a PEG phase 16 containing selectively partitioned therapeutic agent 10 comprising insulin. The dextran phase 18 forms a shell around the PEG phase 16 core. Since mice and rabbits are a common model for humans in drug testing, the present inventor believes that the composition comprising crystallized dextran microparticles and insulin would also be effective in extending the duration of efficacy of the insulin when injected into human adults and children.

[0049] Examples 1-8 illustrate the advantage of using crystallized dextran microparticles as an injectable insulin delivery vehicle compared to injected insulin alone. The experiment involved mice and the observation was made of their response to a subcutaneously injected aqueous suspension consisting of crystallized dextran microparticles and human recombinant insulin (NovoNordisk Actrapid HM Penfill®, 40 UI/ml).

[0050] The suspension was prepared as follows. 5.0 g of Dextran T10 (Pharmacia, Uppsala, Sweden) was dissolved in 20.0 g of water. The solution was filtered through a 0.22 μ m filter (Millipore, Bedford, MA) and freeze dried. 3.0 g of the resulting powder was dissolved in 3.0 g of sterile water and placed in box at temperature 60° C. 6 hours later, crystallized dextran microparticles were washed by centrifugation at 3,000 g with 3 x 5.0 ml of sterile water. Finally, the produced crystallized dextran microparticles suspension was mixed with aqueous insulin solution and used in the experiment with mice. Samples of the suspension were introduced into the mice's legs and samples of animal blood were taken from each mouse's tail and analyzed for glucose concentrations. Blood glucose was measured using the glucose oxidase method on a One-

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touch system glucose analyzer (Lifescan, Johnson & Johnson, Milpitas, CA, USA) after proper calibration.

[0051] In comparative example 1, no insulin was injected into the mouse. In comparative examples 2, 3 and 7, insulin alone (0.5 UI) was injected into the three mice. In examples 4-6 and 8, insulin (0.5 UI) and a crystallized dextran microparticles implant was injected into the four mice. The results are summarized in Table I.

Table I

Ex #		0min glucose mmol/l	15min glucose mmol/l	30min glucose mmol/l	45min glucose mmol/l	120min glucose mmol/l	210min glucose mmol/l	270min glucose mmol/l	390min glucose mmol/l
1	Intact mouse	7.9	8.1	8.2	8.4	-	-	-	-
2	Insulin 0.5 UI	5.9	3.3	2.7	1.8	0.9	3.5	3.0	3.2
3	Insulin 0.5 UI	8.1	3.8	2.8	1.9	0.9	3.7	3.4	3.5
4	Insulin 0.5 UI with crystal- lized dextran micro- particles	6.0	4.3	3.2	2.5	0.8	0.8	0.9	0.7
5	Insulin 0.5 UI with crystal- lized dextran micro- particles	6.9	5.6	4.1	3.4	-	1.2	-	1.6
6	Insulin 0.5 UI	5.9	3.5	2.9	1.9	1.2	1.0	1.0	0.7

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	with crystal- lized dextran micro- particles								
7	Insulin 0.5 UI (average)	7	3.6	2.8	1.9	0.9	3.6	3.2	3.4
8	Insulin 0.5 UI with crystal- lized dextran micro- particles (average)	6.3	4.5	3.4	2.6	1.0	1.0	1.0	1.0

[0052] The average reduction of sugar in the blood (i.e., blood glucose) of animals is very different when 0.5 UI i.m. were applied with and without crystallized dextran microparticles. As shown in Table I, the glucose level in the mice of comparative examples 2, 3 and 7 is about the same or lower than the glucose level in mice of examples 4-6 and 8 during the first 45 minutes after injection. The glucose level is about the same in mice of both comparative examples 2, 3 and 7 and examples 4-6 and 8, 120 minutes after injection. However, the glucose level in the mice of comparative examples 2, 3 and 7 is about three times higher than the glucose level in mice of examples 4-6 and 8 from 210 to 390 minutes after injection. In fact, the blood glucose level in mice in examples 4-6 and 8 did not substantially increase (i.e., did not increase by more than 10%, remained the same or decreased) from 120 minutes to 390 minutes after injection. In contrast, the blood glucose level in mice in the comparative examples 2, 3 and 7 injected with the same amount of insulin did substantially increase from 120 to 390 minutes after injection.

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The crystallized dextran microparticles/insulin injection decreases blood glucose for a longer time than an injection of insulin of the same dose alone. Thus, the composition containing crystallized dextran microparticles and insulin may be dosed for injection.

[0053] The following experiments on rabbits also demonstrate how the crystallized dextran microparticles/insulin injection decreases blood glucose and maintains a basal level of blood insulin for a longer time than an injection of the same insulin of the same dose alone. A subcutaneously injected composition comprising Actrapid HM® short-acting insulin and crystallized dextran microparticles was unexpectedly found to extend the duration of efficacy of this short-acting insulin to exceed that of subcutaneously injected, long-acting insulin Monotard HM® alone.

[0054] The term duration of efficacy means decreasing blood glucose concentration and/or maintaining a basal level of blood insulin concentration to desired levels independent of external events that cause spikes in blood glucose, such as eating. Thus, the term duration of efficacy is a relative term comparing the efficacy of the insulin and microparticle composition to that of the same dose of the same insulin alone. In other words, the duration of efficacy is a duration of action or a duration of pharmacological effect, which may be measured in a patient in a fasting state to compare the efficacy of the insulin and microparticle composition to that of the same dose of the same insulin alone.

[0055] As shown in Figures 12A and 12B, the composition comprising the Actrapid HM® short-acting insulin and crystallized dextran microparticles prolonged the absorption of insulin and extended the hypoglycemic effect (i.e., the duration of efficacy of the insulin) t at least twenty four hours, such as about twenty eight to about thirty one

hours, as compared to about two to about eight hours for Actrapid HM[®] insulin alone (Figure 12B) and about seventeen to about twenty-four hours for long-acting "Monotard HM"[®] insulin alone (Figure 12A). Both Actrapid HM[®] and Monotard HM[®] insulins are products of Novo Nordisk and the advertised duration of efficacy of these insulin compositions in humans obtained from company information are eight and twenty four hours, respectively.

[0056] In Figures 12A and 12B, the upper line illustrates the control line for intact rabbits to which no insulin was administered. The y-axis of Figures 12A and 12B is a relative normalized scale of blood glucose concentration for the same 8 IU dose of insulin. The data in the Figures was adjusted to be shown in one plot for each figure and shows blood glucose levels in blood of animals following insulin injections.

[0057] The data shown in Figures 12A and 12B was obtained as follows. Chinchilla rabbits (2.3 ± 0.3 kg) were monitored for their response to injections of a formulation consisting of crystallized dextran microparticles and short-acting insulin Actrapid HM[®]. Samples of the formulation were subcutaneously injected into the rabbits. Long acting insulin Monotard HM[®] (40 IU/ml) and short acting insulin Actrapid HM[®] were subcutaneously injected into separate rabbits without the microparticles and used as controls. Samples of animal blood were taken from the rabbit's ear vein and analyzed for glucose concentration. Blood glucose concentration was measured with a glucose analyzer (One-Touch[®] Lifescan, Johnson & Johnson, Milpitas, CA, USA) after proper calibration.

[0058] In comparative examples 9 and 10, two intact rabbits were not provided any insulin. In comparative examples 11 and 12 an aqueous solution of long-acting insulin Monotard HM[®] was introduced

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subcutaneously to two rabbits in a dose of 8 IU. In examples 13-15, a suspension of crystallized dextran microparticles with short-acting insulin Actrapid HM® was introduced subcutaneously to three rabbits in a dose of 8 IU. The results of the experiments are summarized in Table II.

TABLE II

Ex #	Insulin dose	0 hours glucose mmol/L	0.5 hours glucose mmol/L	1 hour glucose mmol/L	1.5 hours glucose mmol/L	2 hours glucose mmol/L	2.5 hours glucose mmol/L	16 hours glucose mmol/L	24 hours glucose mmol/L	31 hours glucose mmol/L
9	0.0	5.4	5.0	5.2	5.2	5.2	5.4	4.7	5.4	5.4
10	0.0	6.0	6.3	6.3	6.3	6.3	6.4	5.7	5.6	5.8
11	8 IU	5.4	5.8	3.8	3.2	2.4	2.6	3.9	5.6	N.A
12	8 IU	5.4	5.0	4.2	2.9	2.5	2.4	4.0	5.1	N.A
13	8 IU	5.8	3.7	1.9	1.9	1.9	2.8	4.1	4.3	4.1
14	8 IU	6.6	5.7	4.3	3.9	3.7	3.9	4.6	4.1	3.9
15	8 IU	6.2	5.1	3.8	3.2	3.1	2.9	4.2	4.4	4.7

[0059] The above examples 13-15 illustrate that the composition of crystallized dextran microparticles with short-acting insulin Actrapid HM® provides a prolonged effect that exceeds the effect of long acting insulin Monotard HM® and is believed to be comparable to the effect of long acting (once daily dosing) insulin glargine Lantus® from Aventis (see www.aventis-us.com/Pls/lantus_TXT.html). In addition, Lantus® insulin must not be diluted or mixed with any other insulin or solution. If Lantus® insulin is diluted or mixed, the pharmacokinetic/pharmacodynamic profile (e.g., onset of action, time to peak effect) of Lantus® and/or the mixed insulin may be altered in an unpredictable manner. In contrast, the composition of crystallized dextran microparticles with insulin is not so limited because any suitable insulin, such as human insulin, may be used. In the composition of crystallized

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dextran microparticles and insulin, the ratio of insulin and microparticles can be varied as desired. Furthermore, any suitable insulin may be used to custom fit an insulin therapy to an individual patient. Thus, Actrapid HM® was used in the composition as an illustrative example of a typical insulin and the composition is not limited to this brand of insulin.

[0060] As shown in examples 9-15, the composition containing the crystallized dextran microparticles and insulin is effective in maintaining a duration of efficacy of the insulin for at least 30% longer, such as at least 100% longer, preferably 100 to 400% longer than the same dose of the same insulin without the microparticles. The microparticle containing insulin composition is effective in maintaining a desired basal level of blood insulin and blood glucose concentration for at least 30% longer, such as 100% to 400% longer, than the same dose of the same insulin without the microparticles. Thus, the duration of efficacy of the microparticle containing composition is at least 24 hours, which allows it to be injected only once daily into the mammal, such as a human in need thereof.

[0061] The long lasting insulin crystallized dextran microparticle composition is safer than prior art long lasting insulin compositions because it can achieve the long lasting efficacy without using a higher dose of insulin as in the prior art compositions. For example, if a 8 IU dose of short acting insulin has been determined medically safe for a patient without a significant risk of overdose, then the composition comprising the same short acting insulin and the crystallized dextran microparticles can provide longer acting duration efficacy at the same 8 IU dose of short acting insulin without a significant risk of overdose, even if all the insulin is released into the patient at once. Furthermore, this composition provides a cost saving compared to the prior art compositions because it extends the efficacy without increasing the

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amount of insulin. Current prior art long-acting diabetes therapies are made with analogs of insulin, such as the Lantus® insulin from Aventis. In contrast, the crystallized dextran microparticle containing composition preferably contains human recombinant insulin whose safety profile is established. Thus, this composition reduces the risk of adverse reaction(s) and number of injections to diabetics, thereby enhancing the quality of life of the diabetics.

[0062] The injectable composition may comprise a single phase system comprising insulin and microparticles or a two phase system which forms a PEG and insulin core and a dextran and dextran microparticle shell for an even greater duration of efficacy. Furthermore, the composition comprises a flowable one phase or multiphase colloidal system (i.e., a suspension or an emulsion) which is relatively easy to inject into a mammal.

[0063] The following example illustrates the use of an injectable two phase composition comprising a dextran phase, a PEG phase, insulin and crystallized dextran microparticles. It is believed that when injected into a mammal, this composition forms a structured reservoir type implant having a three dimensional capsule structure. In the capsule structure, the microparticles selectively partition into the dextran phase and the insulin selectively partitions into the PEG phase. The dextran phase containing the microparticles forms a shell around a core comprising the PEG phase containing the insulin. This structured implant allows for controlled release from the core through the shell.

[0064] In comparative example 16, 0.5 IU of Actrapid HM® insulin (100 IU/ml) is subcutaneously injected into a mouse. In example 17, 0.4 g of crystallized dextran microparticles are dispersed in 0.6 ml of 20% (W/W) aqueous solution of dextran having a molecular weight of 70 kDa

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(Pharmacia, Sweden) to form a suspension. 10 mg of PEG having a molecular weight 6 kDa (Fluka) is dissolved in 0.1 ml of Actrapid HM® insulin (100 IU/ml) to form a solution. 0.05 ml of the PEG and insulin solution is mixed with 0.15 ml of the microparticle and dextran suspension to form a two phase composition or mixture. 0.02 ml of the two phase mixture containing 0.5 IU of insulin is injected subcutaneously into mouse. The results are shown in Table III.

Table III

Example #	0 min glucose mmol/L	15 min glucose mmol/L	30 min glucose mmol/L	45 min glucose mmol/L	60 min glucose mmol/L	120 min glucose mmol/L
16	7.8	3.7	2.3	1.7	2.9	6.7
17	7.9	5.9	4.3	4.1	4.3	4.0

[0065] As can be seen in Table III, the two phase composition duration of efficacy was longer than that of the insulin alone. Furthermore, the two phase composition decreased the blood glucose concentration more gradually than insulin alone. Without wishing to be bound by a particular theory, these effects are believed due to the controlled insulin release from the core of capsule structure.

[0066] Furthermore, the microparticle containing composition may be individually tailored for each patient by adjusting the amount of insulin and/or microparticles to allow the patient to inject the composition at the same time every day (i.e., once every 24 hours, once every 48 hours, etcetera). Thus, the duration of efficacy of the composition is adjustable for each patient. For a two phase system, the insulin release profile from the core of the capsule may be adjusted by controlling the amount of microparticles to control the shell thickness of the capsule.

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[0067] While the inventor does not wish to be bound by any particular theory, it is believed that the long lasting effect of the same dose of insulin in mice and rabbits with crystallized dextran microparticles can be explained by the diffusion of the insulin molecules from the crystallized dextran microparticles based implant (i.e., a self controlled release of insulin). Since mice and rabbits are a common model for humans in drug testing, the data shown in the above tables I to III suggests that the use of crystallized dextran microparticles based implants makes it possible to develop controlled release delivery systems with improved pharmacokinetic and dynamics characteristics and that better meet the needs of basal insulin patients, such as humans.

D. Materials

[0068] In the preferred embodiments of the present invention, the therapeutic agent comprises insulin. In other words, the therapeutic agent may consist essentially of insulin alone or comprise insulin in combination with another agent. The term "insulin" shall be interpreted to encompass insulin analogs, natural extracted human insulin, recombinant produced human insulin, insulin extracted from bovine and/or porcine sources, recombinant produced porcine and bovine insulin and mixtures of any of these insulin products. The term is intended to encompass the polypeptide normally used in the treatment of diabetics in a substantially purified form but encompasses the use of the term in its commercially available pharmaceutical form, which includes additional excipients. The insulin is preferably recombinant produced and may be dehydrated (completely dried) or in solution.

[0069] The terms "insulin analog," "monomeric insulin" and the like are used interchangeably herein and are intended to encompass any form of "insulin" as defined above, wherein one or more of the amino acids

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within the polypeptide chain has been replaced with an alternative amino acid and/or wherein one or more of the amino acids has been deleted or wherein one or more additional amino acids has been added to the polypeptide chain or amino acid sequences, which act as insulin in decreasing blood glucose levels. In general, the term "insulin analogs" of the preferred embodiments of the present invention include "insulin lispro analogs," as disclosed in U.S. Pat. No. 5,547,929, incorporated hereinto by reference in its entirety; insulin analogs including LysPro insulin and humalog insulin, and other "super insulin analogs", wherein the ability of the insulin analog to affect serum glucose levels is substantially enhanced as compared with conventional insulin as well as hepatoselective insulin analogs which are more active in the liver than in adipose tissue. Preferred analogs are monomeric insulin analogs, which are insulin-like compounds used for the same general purpose as insulin, such as insulin lispro, i.e., compounds which are administered to reduce blood glucose levels.

[0070] The term "analog" refers to a molecule, which shares a common functional activity with the molecule to which it is deemed to be comparable and typically shares common structural features as well.

[0071] The term "recombinant" refers to any type of cloned therapeutic expressed in prokaryotic cells or a genetically engineered molecule, or combinatorial library of molecules which may be further processed into another state to form a second combinatorial library, especially molecules that contain protecting groups which enhance the physicochemical, pharmacological, and clinical safety of the therapeutic agent.

[0072] The term dextran microparticles includes unsubstituted dextran microparticles and substituted dextran microparticles. For example, substituted dextran microparticles include dextran substituted

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with a suitable group, such as a methyl group, up to a degree which does not hamper crystallization of the dextran microparticles, such as up to 3.5 or less percent branching. The average microparticle diameter is preferably about 0.5 to about 5 microns, more preferably about 1 to about 2 microns.

[0073] Furthermore, while porous non cross-linked dextran microparticles, such as crystallized microparticles, are preferably used with the therapeutic agent, other suitable organic or inorganic microparticles may be used instead, such as other polymer microparticles including polysaccharides, PLA, PLGA, PMMA, polyimides, polyesters, acrylates, acrylamides, vinyl acetate or other polymeric materials, biomaterial particles such as alginate and cells, or inorganic particles, such as silica, glass or calcium phosphates. Preferably the microparticles are biodegradable. Preferably, porous microparticles are used. Most preferably, the microparticles have sufficient porosity to contain the therapeutic agent within the pores and to provide a timed release of the therapeutic agent from the pores. In other words, the therapeutic agent is released over time from the pores, such as in over 5 minutes, preferably in over 30 minutes, most preferably in over one hour, such as in several hours to several days, rather than all at once. Thus, the particle material, pore size and pore volume can be selected based on the type of therapeutic agent used, the volume of therapeutic agent needed for delivery, the duration of the delivery of the therapeutic agent, the environment where the therapeutic agent will be delivered and other factors.

[0074] Thus, in a preferred aspect of the present invention, the therapeutic agent is located at least partially in the pores of the porous microparticles. Preferably, the therapeutic agent is not encapsulated in the microparticle (i.e., the microparticle does not act as a shell with a

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therapeutic agent core inside the shell) and is not attached to the surface of the microparticle. However, if desired, a portion of the therapeutic agent may also be encapsulated in a microparticle shell and/or is attached to the surface of the microparticle in addition to being located in the pores of the microparticle. The location of the therapeutic agent in the pores provides an optimum timed release of the therapeutic agent. In contrast, the therapeutic agent attached to the surface of the microparticle is often released too quickly, while the therapeutic agent encapsulated in the microparticle is often not released soon enough and is then released all at once as the microparticle shell disintegrates. In a two phase system, at least 80% of the therapeutic agent is preferably located in a core surrounded by a wall or shell comprising the microparticles.

E. Methods of Making

[0075] The microparticles may be formed by any suitable method. Preferably, the microparticles are combined with the therapeutic agent after the microparticles are formed. Thus, the microparticles, such as the crystallized dextran microparticles are formed by any suitable method and then the therapeutic agent and the microparticles are combined by any suitable method. In contrast, in some prior art methods, the therapeutic agent is encapsulated into a microparticle shell by providing the particle precursor material and the therapeutic agent into a solution and then crystallizing or cross-linking the precursor material, such as a monomer or oligomer material, to encapsulate a therapeutic agent core into a microparticle shell.

[0076] Preferably, the therapeutic agent is provided into the pores of the porous microparticles after the microparticles are formed. Thus, the porous microparticles are first formed and then the therapeutic agent is provided into a solution containing the microparticles to allow the

therapeutic agent to permeate into the pores of the microparticles. Of course, some of the therapeutic agent may also become attached to the surface of the microparticle in this process.

[0077] Thus, a method to manufacture non cross-linked, porous crystallized dextran microparticles includes preparation of a dextran solution, such as an aqueous dextran solution, conducting a crystallization process to form crystallized porous dextran microparticles, and if desired, isolating crystallized porous dextran microparticles from the solution. A therapeutic agent is then permeated into the pores of the microparticles by providing the therapeutic agent into the crystallization solution containing the microparticles or by providing the isolated microparticles and the therapeutic agent into a second solution, such as a second aqueous solution. For example, crystallized dextran microparticles may be formed in a first, low molecular weight dextran aqueous solution, such as a 2 to 20 kDa dextran solution. The microparticles are then removed from the first solution and then placed into a second dextran aqueous solution having a higher molecular weight dextran, such as a 40 to 500 kDa solution, for example, a 40 to 75 kDa solution. The second solution may comprise a first phase of a two phase system, which is then combined with a second phase, such as a PEG phase containing a therapeutic agent. A similar method may be used with other porous microparticles, where a therapeutic agent is then permeated into the pores of the microparticles after the porous microparticles are formed by any suitable microparticle formation method, including, but not limited to crystallization. The components of the composition such as insulin, microparticles and one or more aqueous phases may be combined in any suitable order sequentially or simultaneously.

[0078] Preferably, the microparticles are formed by self assembly from a solution that does not contain organic solvents and organic

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reaction promoters which leave an organic residue in the microparticles. Thus, for example, the dextran microparticles are preferably formed by self assembly from an aqueous dextran solution. However, if desired, organic solvents and/or organic reaction promoters may also be used. In this case, the microparticles may be purified prior to subsequent use to remove the harmful organic residue.

[0079] As described above, the capsule structure having a first phase core and a second phase wall or shell may be formed in vivo or in vitro from a two phase composition. The composition may be dried powder, such as freeze dried and stored as a powder or porous cake. When the composition is ready to be administered to a mammal, it is hydrated and administered to a mammal by injection.

[0080] Preferably, the composition which includes the microparticles and the therapeutic agent is a flowable colloidal system when the composition is dosed for injection. Examples of flowable colloidal systems include emulsions and suspensions which may be injected into a mammal using a common gage syringe or needle without undue difficulty. In contrast, some prior art compositions include a therapeutic agent in a dextran hydrogel or in a cross-linked dextran matrix. A dextran hydrogel and a cross-linked dextran matrix are not flowable compositions if not specifically prepared.

[0081] In another preferred aspect of the present invention, the microparticles comprise microparticles which are adhesive to mammalian mucosa. Preferably the adhesive microparticles are porous microparticles described above. This further improves the effective delivery of the therapeutic agent.

[0082] In another preferred aspect of the present invention, the microparticles comprise microparticles whose surface has been specially

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modified to enhance the adhesion of the therapeutic agent to the microparticle surface and to optimize the delivery of the therapeutic agent. The microparticle surface may contain any suitable modification that would increase the adhesion of the therapeutic agent.

[0083] The foregoing description of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed, and modifications and variations are possible in light of the above teachings or may be acquired from practice of the invention. The drawings and description were chosen in order to explain the principles of the invention and its practical application. It is intended that the scope of the invention be defined by the claims appended hereto, and their equivalents.

[0084] All of the publications and patent applications and patents cited in this specification are herein incorporated in their entirety by reference.

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I claim:

- 1. A method of lowering blood glucose in a mammal, comprising injecting a therapeutically effective amount of a composition comprising crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal, wherein the microparticles are formed prior to combination of the insulin and the microparticles in the composition.**
- 2. The method of claim 1, wherein the composition comprises a flowable colloidal composition and the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.**
- 3. The method of claim 2, wherein:
the composition comprises a two phase composition comprising a dextran phase and a PEG phase;
the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and
the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into a mammal body.**
- 4. The method of claim 3, further comprising controlling a thickness of the shell based on the body of the mammal receiving the composition to control release of insulin from the implant.**
- 5. The method of claim 1, wherein the composition is provided to a human suffering from diabetes to lower the blood glucose concentration in the human.**
- 6. A dosed pharmaceutical composition, comprising crystallized dextran microparticles and a therapeutically effective amount of insulin, wherein the composition is dosed for injection to a human and the**

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microparticles are formed prior to combination of the insulin and the microparticles in the composition.

7. The composition of claim 6, wherein:
the composition comprises a flowable colloidal composition; and
the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.

8. The composition of claim 7, wherein:
the composition comprises a two phase composition comprising a dextran phase and a PEG phase;
the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and
the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into the human body.

9. A dosed pharmaceutical composition, comprising crystallized dextran microparticles and a therapeutically effective amount of first insulin, wherein the composition is dosed for injection to a mammal and a duration of efficacy of the composition when injected into a mammal is at least 30% longer than a duration of efficacy in the mammal of the same dose of the same first insulin without the microparticles.

10. The composition of claim 9, wherein:
the composition comprises a flowable colloidal composition;
the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns; and
the microparticles are formed prior to combination of the first insulin and the microparticles in the composition.

11. The composition of claim 10, wherein:

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the composition comprises a two phase composition comprising a dextran phase and a PEG phase;

the first insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and

the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into a mammal body.

12. The composition of claim 9, wherein:

the duration of efficacy of the composition when injected into the mammal is at least 24 hours; and

the duration of efficacy of the composition when injected into the mammal is at least 100% longer than a duration of efficacy in a mammal of the same dose of the same first insulin without the microparticles.

13. A method of lowering blood glucose in a mammal, comprising injecting a therapeutically effective amount of a composition comprising crystallized dextran microparticles and first insulin to the mammal to lower blood glucose of the mammal, wherein a duration of efficacy of the composition in the mammal is at least 30% longer than a duration of efficacy in the mammal of the same dose of the same first insulin without the microparticles.

14. The method of claim 13, wherein:

the composition comprises a flowable colloidal composition;

the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns; and

the microparticles are formed prior to combination of the first insulin and the microparticles in the composition

15. The method of claim 14, wherein:

the composition comprises a two phase composition comprising a dextran phase and a PEG phase;

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the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and
the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into the mammal body.

16. The method of claim 13, wherein:
the duration of efficacy of the composition when injected into a mammal is at least 24 hours; and
the duration of efficacy of the composition in the mammal is at least 100% longer than a duration of efficacy in a mammal of the same dose of the same first insulin without the microparticles.

17. The method of claim 16, wherein the duration of efficacy of the composition in the mammal is 100 to 400 % longer than a duration of efficacy in a mammal of the same dose of the same first insulin without the microparticles.

18. A method of making a dosed pharmaceutical composition, comprising:
providing crystallized dextran microparticles;
combining a therapeutically effective amount of insulin and the crystallized dextran microparticles in a solution after the microparticles have been crystallized to form a composition of insulin and crystallized dextran microparticles; and
dosing the composition for injection to a mammal.

19. The method of claim 18, wherein:
the composition comprises a flowable colloidal composition; and
the microparticles comprise crystallized dextran microparticles having an average diameter of 0.5 to 5 microns.

20. The method of claim 19, wherein:

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the composition comprises a two phase composition comprising a dextran phase and a PEG phase;

the insulin is selectively partitioned in the PEG phase and the microparticles are selectively partitioned in the dextran phase; and

the composition forms a structured implant comprising a PEG phase core and a dextran phase shell after injection into the mammal body.

21. The method of claim 18, wherein:

a duration of efficacy of the composition when injected into a mammal is at least 24 hours; and

the duration of efficacy of the composition in the mammal is at least 100% longer than a duration of efficacy in a mammal of the same dose of the same insulin without the microparticles.

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ABSTRACT

A method of lowering blood glucose in a mammal includes injecting a therapeutically effective amount of crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal. The composition may be a one phase or a structured multi-phase composition for controlled release of insulin over an extended period of time.

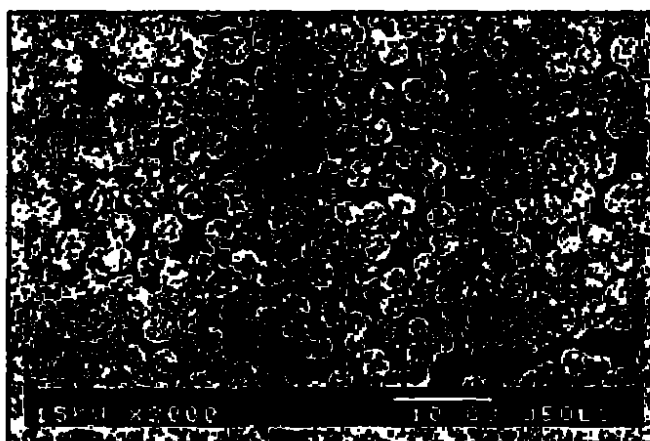


FIG. 1



FIG. 2A

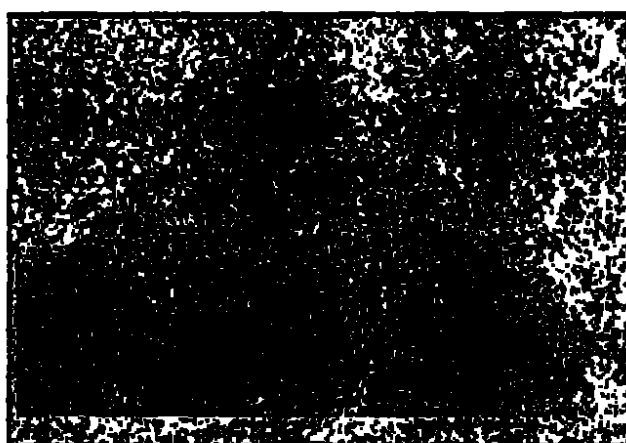


FIG. 2B



FIG. 3



FIG. 4

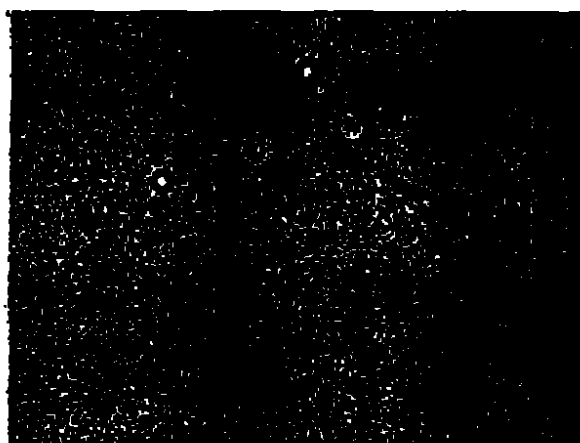


FIG. 5

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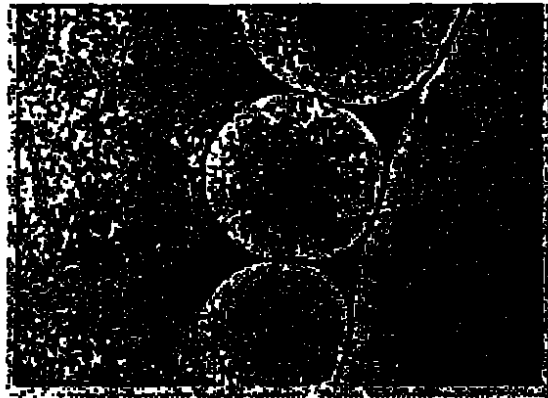


FIG. 6

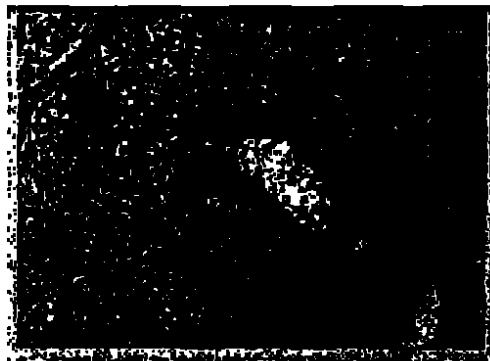


FIG. 7

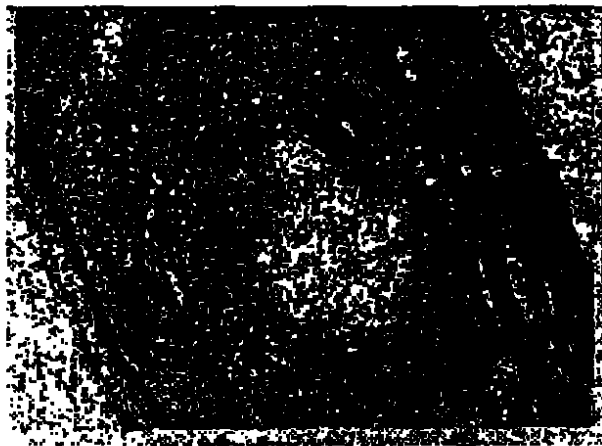
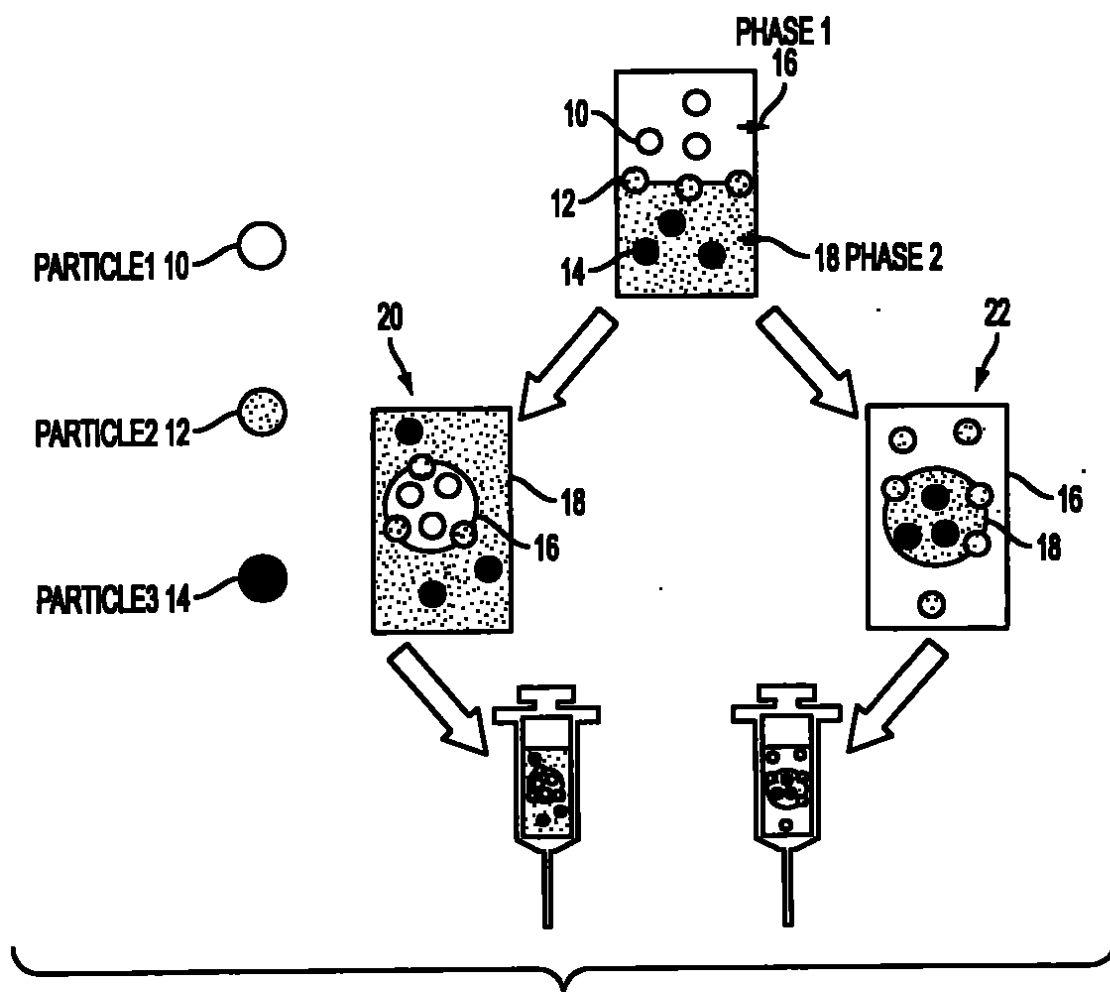


FIG. 8

37-1
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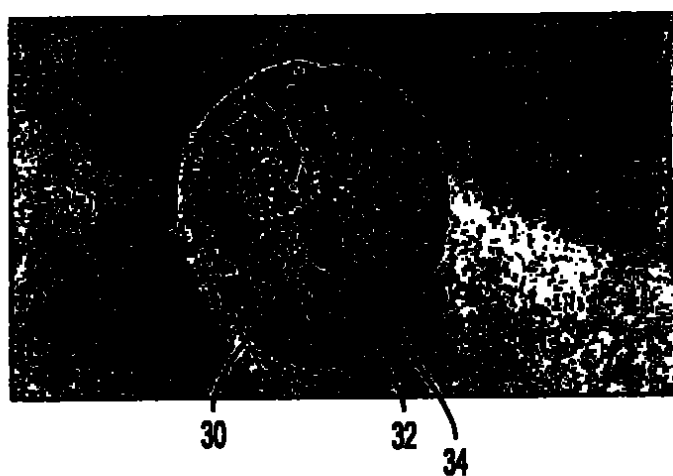


FIG. 9B

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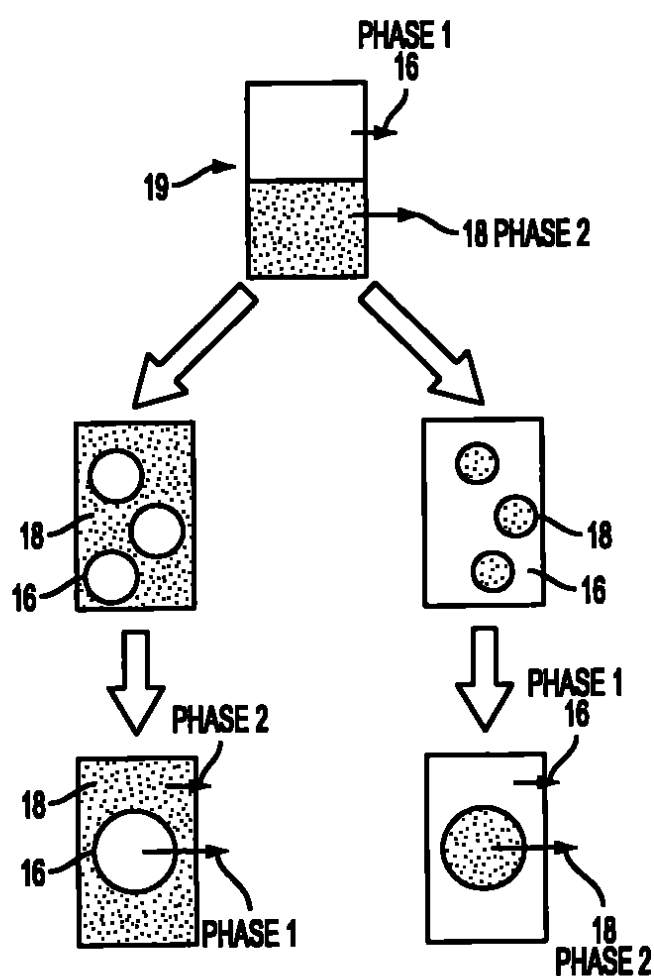


FIG. 9C

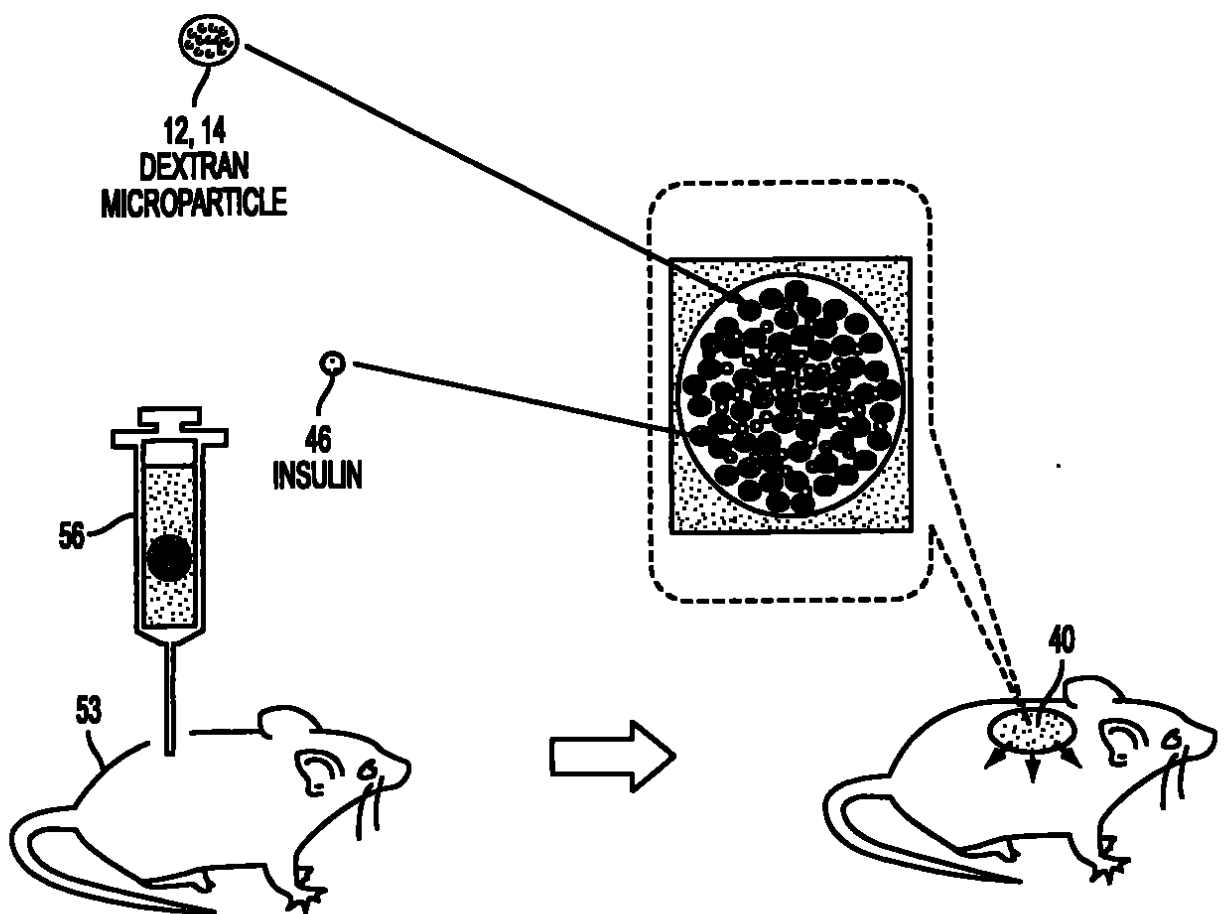
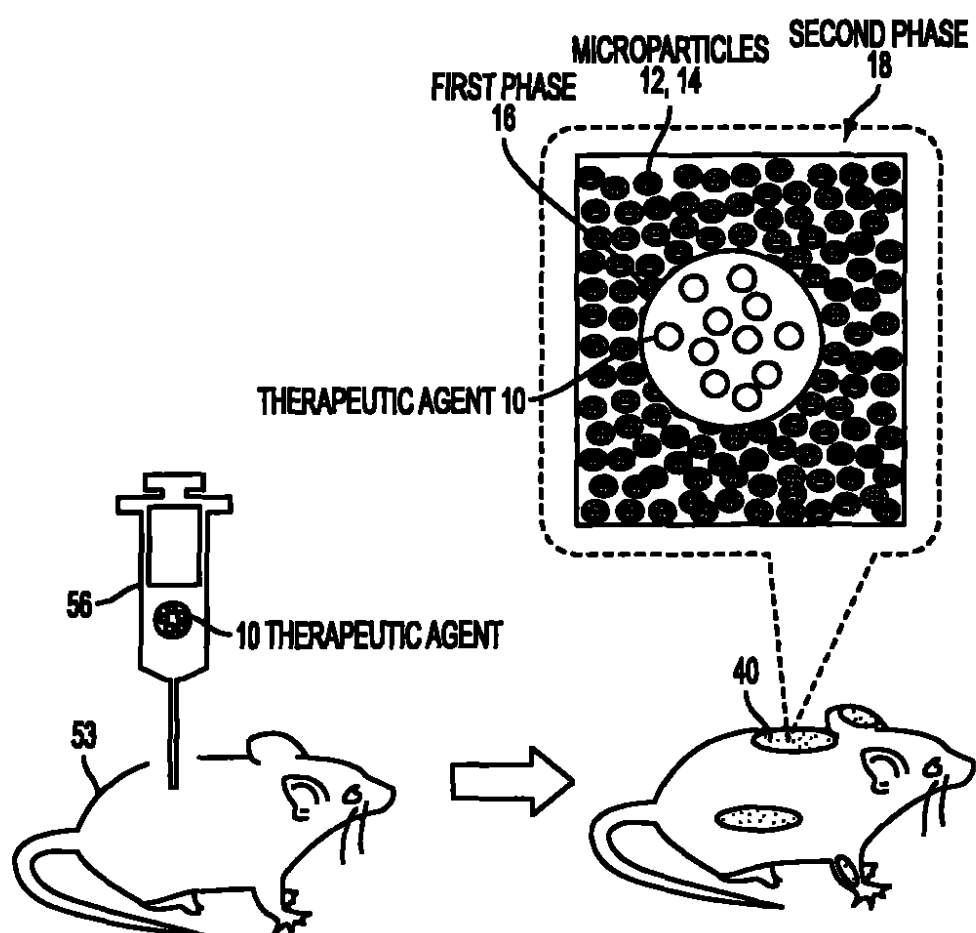


FIG. 10



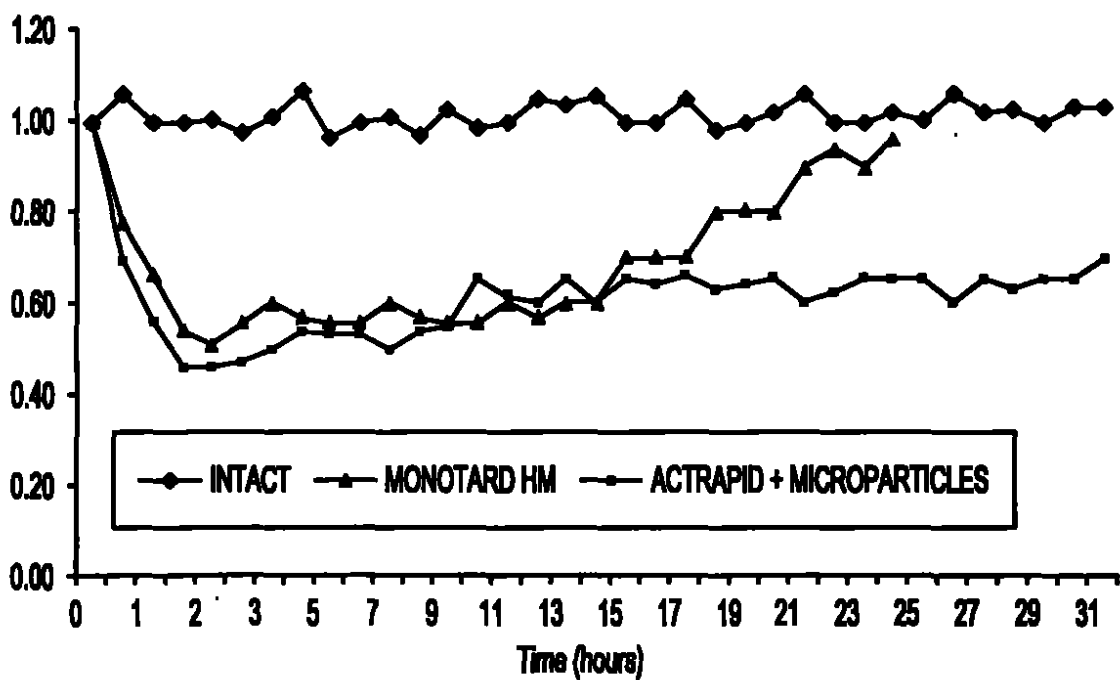


FIG. 12A

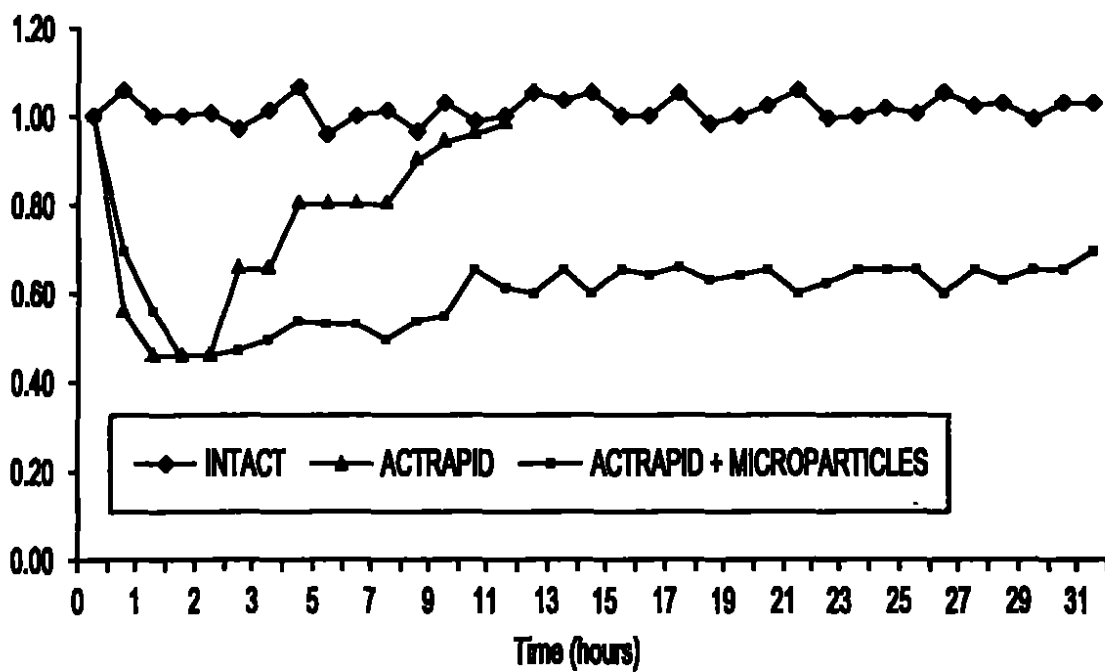


FIG. 12B

(19) World Intellectual Property
Organization
International Bureau



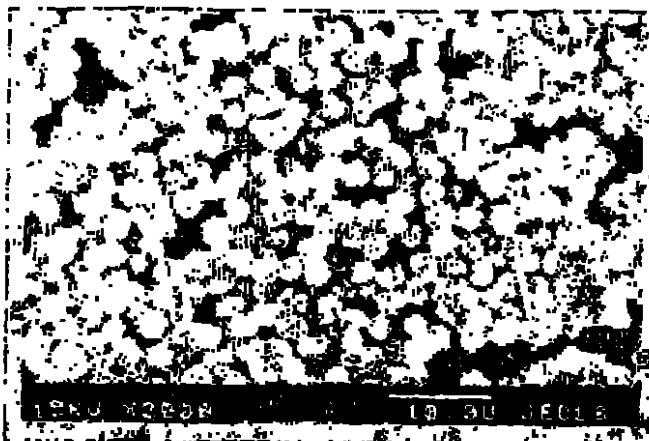
(43) International Publication Date
16 September 2004 (16.09.2004)

PCT

(10) International Publication Number
WO 2004/078198 A1

- (51) International Patent Classification⁷: A61K 38/28, 47/36, 9/00
- (21) International Application Number: PCT/IB2004/000984
- (22) International Filing Date: 4 March 2004 (04.03.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
- | | | |
|------------|-----------------------------|----|
| 60/451,245 | 4 March 2003 (04.03.2003) | US |
| 60/467,601 | 5 May 2003 (05.05.2003) | US |
| 60/469,017 | 9 May 2003 (09.05.2003) | US |
| 60/495,097 | 15 August 2003 (15.08.2003) | US |
- (71) Applicant (for all designated States except US): THE TECHNOLOGY DEVELOPMENT COMPANY LTD. [RU/—]; Reid House, 31 Church Street, Hamilton HM FX (BM).
- (72) Inventor; and
- (73) Inventor/Applicant (for US only): SABELTSKY, Vladimir [RU/RU]; Piskarevsky Prospekt 10, q. 320, St.Petersburg, 105221 (RU).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, M, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, 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, IT, LU, MC, 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
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: LONG ACTING INJECTABLE INSULIN COMPOSITION AND METHODS OF MAKING AND USING THEREOF



(57) Abstract: A method of lowering blood glucose in a mammal includes injecting a therapeutically effective amount of crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal. The composition may be a one phase or a structured multi-phase composition for controlled release of insulin over an extended period of time.

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PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

FOLEY & LARDNER LLP
Attn. Kaminski, Michael D.
Washington Harbour
3000 "K" Street, N.W., Suite 500
Washington, D.C. 20007-5101
UNITED STATES OF AMERICA

**NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION**

(PCT Rule 44.1)

Date of mailing (day/month/year) 21/07/2004	
Applicant's or agent's file reference 028093-0112	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/IB2004/000984	International filing date (day/month/year) 04/03/2004
Applicant THE TECHNOLOGY DEVELOPMENT COMPANY LTD.	

1. ☒ The applicant is hereby notified that the International search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):
When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.
Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35
For more detailed instructions, see the notes on the accompanying sheet.
2. ☐ The applicant is hereby notified that no International search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
 - ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
 - ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.
4. **Reminders**

Shortly after the expiration of 18 months from the priority date, the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the International application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

Within 18 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 18 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Sandrine Polenzani
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NOTES TO FORM PCT/ISA/220

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These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the letter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

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The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequences if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequences with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 028093-0112	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, Item 5 below.	
International application No. PCT/IB2004/000984	International filing date (day/month/year) 04/03/2004	(Earliest) Priority Date (day/month/year) 04/03/2003
Applicant THE TECHNOLOGY DEVELOPMENT COMPANY LTD.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 05 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☒ Certain claims were found unsearchable (See Box II).3. ☐ Unity of invention is lacking (see Box III).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regards to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

- b. ☐ none of the figures is to be published with the abstract.

II INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2004/000984

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K38/28 A61K47/36 A61K9/00		
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) IPC 7 A61K		
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 practical, search terms used) EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, MEDLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 713 249 A (SCHROEDER ULF) 15 December 1987 (1987-12-15) cited in the application column 6; claims 1,4,7; example 13	1-17
X	MORIYAMA K ET AL: "Regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol)" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 42, no. 3, 1 December 1996 (1996-12-01), pages 237-248, XP004069859 ISSN: 0168-3659 page 240 page 238, column 1 ----- -/--	1-21
☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "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) "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 "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 "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 "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. "G" document member of the same patent family		
Date of the actual completion of the international search 15 July 2004		Date of mailing of the international search report 21/07/2004
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Tx. 31 651 epo nl, Fac (+31-70) 340-3016		Authorized officer Bochelen, D

II INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2004/000984

5/2/04

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STENEKES R J H ET AL: "Formation of dextran hydrogels by crystallization" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 22, no. 13, July 2001 (2001-07), pages 1891-1898, XP004245950 ISSN: 0142-9612 page 1892, column 1	1-21
Y	EP 1 184 032 A (OCTOPLUS B V) 6 March 2002 (2002-03-06) page 3, paragraphs 15,16 page 4, line 46 - page 5, line 28	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2004/000984

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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search 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:
Although claims 1-5, 13-17 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the composition.
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 III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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.

II RNATI NAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB2004/000984

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4713249 A	15-12-1987	AU 9127482 A	01-06-1983
		EP 0093757 A1	16-11-1983
		WO 8301738 A1	26-05-1983
		US 4501726 A	26-02-1985
		AT 34659 T	15-06-1988
		AU 567434 B2	19-11-1987
		AU 1776083 A	08-02-1984
		DE 3376797 D1	07-07-1988
		DK 95284 A	23-02-1984
		EP 0113749 A1	25-07-1984
		FI 840923 A	07-03-1984
		NO 840676 A	22-02-1984
		SE 8204244 A	10-01-1984
		WO 8400294 A1	02-02-1984
EP 1184032 A	06-03-2002	EP 1184032 A1	06-03-2002
		AU 9437601 A	13-03-2002
		WO 0217884 A1	07-03-2002

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/B2004/000984

International filing date (day/month/year)
04.03.2004

Priority date (day/month/year)
04.03.2003

International Patent Classification (IPC) or both national classification and IPC
A61K38/28, A61K47/36, A61K9/00

Applicant
THE TECHNOLOGY DEVELOPMENT COMPANY LTD.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Bochelen, D
Telephone No. +49 89 2399-8150



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

178 \$
International application No.
PCT/IB2004/000984

Box No. 1 Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
☐ a sequence listing
☐ table(s) related to the sequence listing
 - b. format of material:
☐ in written format
☐ in computer readable form
 - c. time of filing/furnishing:
☐ contained in the international application as filed.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/IB2004/000984

1798

Box No. II Priority

1. ☒ The following document has not been furnished:

☒ copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).

☐ translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/B2004/000984

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial
applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application,
☒ claims Nos. 1-5,13-17

because:

- ☒ the said international application, or the said claims Nos. 1-5,13-17 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the whole application or for said claims Nos.
- ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:
- the written form ☐ has not been furnished
- ☐ does not comply with the standard
- the computer readable form ☐ has not been furnished
- ☐ does not comply with the standard
- ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
- ☐ See separate sheet for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

181 22
International application No.
PCT/B2004/000984

Box No. V Reasoned statement under Rule 43b/a.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1,13
	No: Claims	6,9,18
Inventive step (IS)	Yes: Claims	
	No: Claims	1-21
Industrial applicability (IA)	Yes: Claims	6-12,18-21
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 1-5 and 13-17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. Reference is made to the following documents:

- D1: US-A-4 713 249 (SCHROEDER ULF) 15 December 1987 (1987-12-15)
- D2: MORIYAMA K ET AL: "Regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol)" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 42, no. 3, 1 December 1996 (1996-12-01), pages 237-248, XP004069859 ISSN: 0168-3659
- D3: STENEKES R J H ET AL: "Formation of dextran hydrogels by crystallization" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 22, no. 13, July 2001 (2001-07), pages 1891-1898, XP004245950 ISSN: 0142-9612
- D4: EP-A-1 184 032 (OCTOPLUS B V) 6 March 2002 (2002-03-06)

If not indicated otherwise the relevant passages are those mentioned in the search report.

Document D1 discloses microspheres of crystallized dextran and insulin for prolonged release of biologically active compounds.

Document D2 discloses heterogeneous dextran hydrogels containing PEG and insulin for controlled insulin release.

Document D3 discloses a process for the preparation of crystallized dextran microspheres by physical cross-linking of aqueous dextran solutions.

Document D4 discloses a process for preparing dextran hydrogels for controlled release systems by physical crystallization of dextran.

3. Novelty (Art. 33(1)(2) PCT):

- 3.1 It is stressed that a claim directed to a product which is known is not delimited from the prior art when an alternative process is used to prepare the product. The subject-matter of claim 6 lacks thus novelty in view of documents D1 and D2.
- 3.2 The functional feature of a duration of efficacy which is 30% longer does not allow to delimit the subject-matter of said claim from the disclosure of documents D1 and D2. Claim 9 is thus not new.
- 3.3 Claim 18 lacks novelty in view of document D2.

4. Inventive step (Art. 33(1)(3) PCT):

- 4.1 It would be obvious for a skilled man to use the microspheres of crystallized dextran and insulin disclosed in D1 and D2 for lowering the blood glucose in a mammal. The subject-matter of claim 1 is thus not inventive.
- 4.2 D1 and D2 disclose the prolonged release of insulin from crystallized dextran microspheres. It would thus be obvious to use the composition of D1 and D2 for lowering blood glucose with a duration of efficacy which is 30% longer than in absence of microspheres. Claim 13 does thus not involve an inventive step.
- 4.3 Dependent claims 2-5, 7-8, 10-12, 14-17 and 19-21 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step.

5. Industrial applicability (Art. 33(1)(4) PCT):

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/IB2004/000984

For the assessment of the present claims 1-5 and 13-17 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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ASSIGNMENT AND AGREEMENT

WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221, Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled BIOCOMPATIBLE IMPLANT AND METHOD FOR MANUFACTURE THEREOF (Atty. Dkt. No. 028093-0102) for which a provisional application for United States Letters Patent was filed on March 4, 2003 as Application No. 60/451,245; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

NOW THEREFORE, in consideration of One Dollar (\$1.00) and other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, ASSIGNOR has sold, assigned, and transferred, and by these presents hereby sells, assigns, and transfers, unto ASSIGNEE, its successors and assigns, the full and exclusive right, title, and interest in and to (a) the above-identified invention or inventions and all improvements and modifications thereof, (b) the above-identified application and all other applications for Letters Patent of the United States and countries foreign thereto for the above-identified invention or inventions and all improvements and modifications thereof, (c) all Letters Patent which may issue from said applications in the United States and countries foreign thereto, (d) all divisions, continuations, reissues, and extensions of said applications and Letters Patent, and (e) the right to claim for any of said applications the full benefits and priority rights under the International Convention and any other international agreement to which the United States adheres; such right, title, and interest to be held and enjoyed by ASSIGNEE, its successors and assigns, to the full end of the term or terms for which any and all such Letters Patent may be granted as fully and entirely as would have been held and enjoyed by ASSIGNOR had this Assignment not been made.

ASSIGNOR HEREBY AUTHORIZES AND REQUESTS the Commissioner of Patents and Trademarks to issue said Letters Patent to ASSIGNEE as assignee of the entire interest, for the sole use and benefit of ASSIGNEE, its successors and assigns.

ASSIGNOR HEREBY AGREES (a) to communicate to ASSIGNEE, its successors and assigns, or their representatives or agents, all facts and information known or available to ASSIGNOR respecting said invention or inventions, improvements, and modifications including evidence for interference, reexamination, reissue, opposition, revocation, extension, or infringement purposes or other legal, judicial, or administrative proceedings, whenever requested by ASSIGNEE; (b) to testify in person or by affidavit as required by ASSIGNEE, its successors and assigns, in any such proceeding in the United States or a country foreign thereto; (c) to execute and deliver, upon request by ASSIGNEE, all lawful papers including, but not limited to, original, divisional, continuation, and reissue applications, renewals, assignments, powers of attorney, oaths, affidavits, declarations, depositions; and (d) to provide all reasonable assistance to ASSIGNEE, its successors and assigns, in obtaining and enforcing proper title in and protection for said invention or inventions, improvements, and modifications under the intellectual property laws of the United States and countries foreign thereto.

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ASSIGNOR HEREBY REPRESENTS AND WARRANTS that ASSIGNOR has the full and unencumbered right to sell, assign, and transfer the interests sold, assigned, and transferred herein, and that ASSIGNOR has not executed and will not execute any document or instrument in conflict herewith.

ASSIGNOR HEREBY GRANTS to the law firm of Foley & Lardner the power and authority to insert in this Assignment any further identification which may be necessary or desirable to comply with the rules of the U.S. Patent and Trademark Office for recordation of this Assignment.

ASSIGNOR UNDERSTANDS AND AGREES that the attorneys and agents of the law firm of Foley & Lardner do not personally represent ASSIGNOR or ASSIGNOR's legal interests, but instead represent the interests of ASSIGNEE; since said attorneys and agents cannot provide legal advice to ASSIGNOR with respect to this Assignment, ASSIGNOR acknowledges its right to seek its own independent legal counsel.

Executed this 19 day of February, 2004



VLADIMIR SABETSKY

State of DISTRICT OF COLUMBIA
County of _____

On this 19th day of FEBRUARY, 2004, before me, a notary public in and for said county, appeared Vladimir SABETSKY, who is personally known to me to be the same person whose name is subscribed to the foregoing instrument, and he/she acknowledged that he/she signed, sealed, and delivered the said instrument as his/her free and voluntary act for the uses and purposes therein set forth.



Notary Public Charles H. Hahn

(Seal)

My Commission Expires: _____

My Commission Expires
August 14, 2008

ASSIGNMENT AND AGREEMENT

WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221 Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled **NOVEL DELIVERY SYSTEM FOR DRUG AND CELL THERAPY** (Atty. Dkt. No. 028093-0103) for which a provisional application for United States Letters Patent was filed on May 5, 2003 as Application No. 60/467,601; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

NOW THEREFORE, in consideration of One Dollar (\$1.00) and other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, ASSIGNOR has sold, assigned, and transferred, and by these presents hereby sells, assigns, and transfers, unto ASSIGNEE, its successors and assigns, the full and exclusive right, title, and interest in and to (a) the above-identified invention or inventions and all improvements and modifications thereof, (b) the above-identified application and all other applications for Letters Patent of the United States and countries foreign thereto for the above-identified invention or inventions and all improvements and modifications thereof, (c) all Letters Patent which may issue from said applications in the United States and countries foreign thereto, (d) all divisions, continuations, reissues, and extensions of said applications and Letters Patent, and (e) the right to claim for any of said applications the full benefits and priority rights under the International Convention and any other international agreement to which the United States adheres; such right, title, and interest to be held and enjoyed by ASSIGNEE, its successors and assigns, to the full end of the term or terms for which any and all such Letters Patent may be granted as fully and entirely as would have been held and enjoyed by ASSIGNOR had this Assignment not been made.

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100-801

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Executed this 19 day of February, 2004



VLADIMIR SABETSKY

State of DISTRICT OF COLUMBIA
County of _____)ss.

On this 19th day of FEBRUARY, 2004, before me, a notary public in and for said county, appeared Vladimir SABETSKY, who is personally known to me to be the same person whose name is subscribed to the foregoing instrument, and he/she acknowledged that he/she signed, sealed, and delivered the said instrument as his/her free and voluntary act for the uses and purposes therein set forth.



Notary Public Wendy Huber

My Commission Expires: _____

(Seal)

My Commission Expires
My Commission Expires
August 14, 2008

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2007

ASSIGNMENT AND AGREEMENT

WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221 Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled **NOVEL DELIVERY SYSTEM FOR DRUG AND CELL THERAPY** (Atty. Dkt. No. 028093-0105) for which a provisional application for United States Letters Patent was filed on May 9, 2003 as Application No. 60/469,017; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

NOW THEREFORE, in consideration of One Dollar (\$1.00) and other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, ASSIGNOR has sold, assigned, and transferred, and by these presents hereby sells, assigns, and transfers, unto ASSIGNEE, its successors and assigns, the full and exclusive right, title, and interest in and to (a) the above-identified invention or inventions and all improvements and modifications thereof, (b) the above-identified application and all other applications for Letters Patent of the United States and countries foreign thereto for the above-identified invention or inventions and all improvements and modifications thereof, (c) all Letters Patent which may issue from said applications in the United States and countries foreign thereto, (d) all divisions, continuations, reissues, and extensions of said applications and Letters Patent, and (e) the right to claim for any of said applications the full benefits and priority rights under the International Convention and any other international agreement to which the United States adheres; such right, title, and interest to be held and enjoyed by ASSIGNEE, its successors and assigns, to the full end of the term or terms for which any and all such Letters Patent may be granted as fully and entirely as would have been held and enjoyed by ASSIGNOR had this Assignment not been made.

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VLADIMIR SABETSKY

State of DISTRICT OF COLUMBIA
County of _____

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Notary Public WENOT HUBER

My Commission Expires: _____

(Seal)

My Commission Expires
August 14, 2008

2/2/01

ASSIGNMENT AND AGREEMENT

WHEREAS, Vladimir SABETSKY of Piskarevsky Prospekt 10, q.320, 105221 Saint-Petersburg, Russia; (hereinafter referred to singly and collectively as "ASSIGNOR") have invented a certain invention entitled **NOVEL DELIVERY SYSTEM F R DRUG AND CELL THERAPY** (Atty. Dkt. No. 028093-0106) for which a provisional application for United States Letters Patent was filed on August 15, 2003 as Application No. 60/495,097; and

WHEREAS, THE TECHNOLOGY DEVELOPMENT COMPANY Ltd., a Bermuda corporation (hereinafter referred to as "ASSIGNEE") is desirous of acquiring the entire interest therein;

NOW THEREFORE, in consideration of One Dollar (\$1.00) and other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, ASSIGNOR has sold, assigned, and transferred, and by these presents hereby sells, assigns, and transfers, unto ASSIGNEE, its successors and assigns, the full and exclusive right, title, and interest in and to (a) the above-identified invention or inventions and all improvements and modifications thereof, (b) the above-identified application and all other applications for Letters Patent of the United States and countries foreign thereto for the above-identified invention or inventions and all improvements and modifications thereof, (c) all Letters Patent which may issue from said applications in the United States and countries foreign thereto, (d) all divisions, continuations, reissues, and extensions of said applications and Letters Patent, and (e) the right to claim for any of said applications the full benefits and priority rights under the International Convention and any other international agreement to which the United States adheres; such right, title, and interest to be held and enjoyed by ASSIGNEE, its successors and assigns, to the full end of the term or terms for which any and all such Letters Patent may be granted as fully and entirely as would have been held and enjoyed by ASSIGNOR had this Assignment not been made.

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Executed this 19 day of February, 2004



VLADIMIR SABETSKY

State of DISTRICT OF COLUMBIA
County of _____)ss.

On this 19th day of FEBRUARY, 2004, before me, a notary public in and for said county, appeared Vladimir SABETSKY, who is personally known to me to be the same person whose name is subscribed to the foregoing instrument, and he/she acknowledged that he/she signed, sealed, and delivered the said instrument as his/her free and voluntary act for the uses and purposes therein set forth.



Notary Public WENDY HUSE

My Commission Expires: _____

(Seal)

**My Commission Expires
August 14, 2008**



Expediente No		05100609	No de Folio
Item		No de unidades	✓
1	CD		
2	DVD		
3	REVISTA		
4	LIBRO		
5	PERIODICO		
6	DISQUETES		
7	SOBRE DE MANILA	1	
8	SOBRE BLANCO TAMAÑO CARTA		
9	SOBRE BLANCO TAMAÑO OFICIO		
10	OTROS:		
Observaciones: Acta Final			

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REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION <i>901</i>	()	()
MODELO DE UTILIDAD	()	()
- Indicación de que se solicita la concesión de una patente.	()	(X)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	()	(X)
- Descripción de la invención.	()	(X)
- Dibujos de ser estos pertinentes.	()	(X)
- Comprobante de Pago de las tasas establecidas.	()	()
COMPLETA <i>94</i>	INCOMPLETA ()	()
DISEÑO INDUSTRIAL ()		
- Indicación de que se solicita el registro de Diseño Industrial	()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.	(X)	()
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	()	()
- Comprobante de pago de las tasas establecidas	()	()
COMPLETA ()	INCOMPLETA ()	()
ESQUEMA DE TRAZADO ()		
- Indicación de que solicita el registro de un Esquema de trazado.	()	()
- Datos de identificación del solicitante o de la persona que presenta La solicitud.	()	()
- Representación gráfica del esquema de trazado	()	()
- Comprobante de pago de las tasas establecidas.	()	()
COMPLETA ()	INCOMPLETA ()	()

Firma Responsable:

Fecha:

195-146

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y -COMERCIO

Bogotá,
2020

Doctor(a)
RODRIGUEZ D'ALEMAN DILIA MARIA

REFERENCIA Radicación No. 5 100609
Solicitud internacional IB2004/000984
Fecha inicio fase nacional 04/10/2005
Trámite 11
Evento 379
Actuación 430
Oficio No. 13506

P686

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. El solicitante deberá presentar el documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante, debidamente autenticada y apostillada.
2. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.


CLARA DE JARAMA
Jefe de la división de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista de fecha 23 DIC 2020
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