

Industria y Comercio

SUPERINTENDENCIA

Delegatura de propiedad Industrial

División de Nuevas Creaciones

PCT
SOLICITUD

PATENTE DE INVENCION

EXPEDIENTE No

05-100610

TÍTULO

Composición de Insulina oral y Métodos
de fabricación y uso de éstos

CLASIFICACIÓN INTERNACIONAL

AGIK 38/28, 47/36,
9/00

SOLICITANTE

The technology Development Company Ltd

DOMICILIO

Hamilton Bermuda

APODERADO

Dña Maria Rodriguez D'Alaman

BOGOTÁ, D C,

Octubre 4, 2005

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

1) SOLICITUD DE			
☒ Patente de invención		☐ Patente de Modelo de Utilidad	
☒ Capítulo I		☐ Capítulo II	
2) S LICITANTE (71)	Nombre THE TECHNOLOGY DEVELOPMENT COMPANY LTD		IDENTIFICACIÓN C C ☐ NIT ☒ C E ☐ OTRO ☐ Cual _____ NUMERO _____
	Dirección Reid House, 31 Church Street, Hamilton HM FX, Bermuda		
	Nacionalidad o domicilio Hamilton, Bermuda		
	Lugar de construcción Bermuda		
	Teléfono Fax E Mail		
3) REPRESENTANTE O APODERADO	Nombre DILIA MARÍA RODRÍGUEZ D ALEMAN		IDENTIFICACIÓN
	Dirección Carrera 11 N° 88-53 Piso 6 Bogotá D C		C C ☒ NIT ☐ C E ☐ OTRO ☐
	Teléfono 6181088 Fax 6350824 E Mail info@clarkemodet.com.co		NÚMERO 41 654 882 TP 20824 CSJ
4) INVENTOR (ES) (72)	Nombre 1 SABETSKY, Vladimir		
	Dirección Piskarevsky Prospekt 10, q 320, 105221 Saint-Petersburg, Rusia		
	Nacionalidad o Domicilio Ruso		
5) PCT (86) Solicitud Internacional No PCT/IB2004/000933 Fecha Marzo 4 2004 Publicación Internacional No WO2004/078196 A1 Fecha Sept 16, 2004			
6) Título (54) COMPOSICIÓN DE INSULINA ORAL Y MÉTODOS DE FABRICACIÓN Y USO DE ESTOS			
7) Clasificación Internacional A61K 38/28, 47/36, 9/00			
8) PRIORIDAD SI ☒ NO ☐	33) País de Origen	(32) Fecha	(31) Número de Solicitud
	Estados Unidos	Marzo 4, 2003	60/451,245
	Estados Unidos	Mayo 5, 2003	60/467,601
	Estados Unidos	Mayo 9, 2003	60/469,017
	Estados Unidos	Agosto 15, 2003	60/495,097
9) Comprobante de pago N° 05-73,189 Fecha Septiembre 21, 2005			
Instrucciones para el diligenciamiento del presente formulario Ver reverso de esta pagina			

Superintendencia de Industria y Comercio
Tramite: 1 011 PCT-PATENTE 379 FASE INCI 411 PRESENTAC
Fecha (ORD): 2005-10-04 16:38:15
Radicalizacion: 05100610 00000000
Folios: 153

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT 800176089 2

RECIBO OFICIAL DE CAJA 05 73 189
FECHA SEPTIEMBRE 21 DE 2005

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No	PAGO	FECHA PGD	Vr	PAGO
CLARKE MODET Y CO	COLONB	CONSIGNACION	BANCO POPULAR	050-00110-6	43461450	21/09/2005	3,405,000	00

***** CONCEPTO *****

CANT	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	1
		TRAMITES DE SOL DE PATENTE DE IN	443 000 00
		TOTAL	443,000 00

SOM: CUATROCIENTOS CUARENTA Y TRES MIL PESOS

RESPONSABLE _____ 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No _____

3

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 acredite 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
- ☒ Otros.
 - 1 Solicitud PCT
 - 2 Notificación de la transmisión del reporte de búsqueda
 - 3 Cesión y acuerdo DKT Nr 028093-0102
 - 4 Cesión y acuerdo DKT Nr 028093-0103
 - 5 Cesión y acuerdo DKT Nr 028093-0105

11) FIGURA CARACTERÍSTICA



12) Solicitud concesión de la patente,

NOMBRE DILIA RODRÍGUEZ D ALEMAN

FIRMA

C C 41 654 882 de Bogota TP 20824 CSJ

Instrucciones para el diligenciamiento del presente formulario. Ver reverso de esta página

(19) World Intellectual Property
Organization
International Bureau



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

PCT

(10) International Publication Number
WO 2004/078196 A1

(51) International Patent Classification⁷ A61K 38/28, 47/36 900

(21) International Application Number⁶ PCT/IB2004/000933

(22) International Filing Date 4 March 2004 (04.03.2004)

(23) Filing Language English

(26) Publication Language English

(30) Priority Data
60451,245 4 March 2003 (04.03.2003) US
60467,601 5 May 2003 (05.05.2003) US
60469,017 9 May 2003 (09.05.2003) US
60495,097 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): SANEVSKY
Vladimir [RU/RU] Piskarevsky Prospekt 10, q. 320,
105221 Saint-Petersburg (RU)

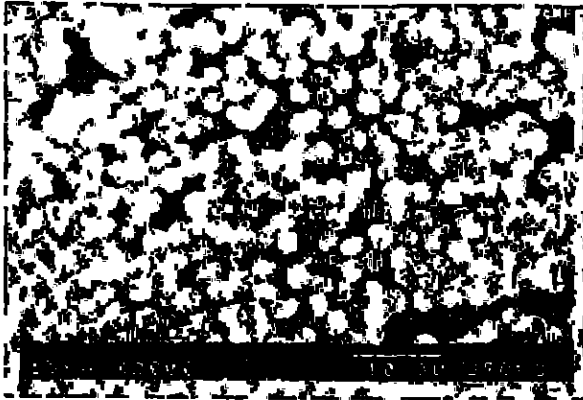
(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AN, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
FR, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SI, 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), Euro-
pean (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 "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: ORAL INSULIN COMPOSITION AND METHODS OF MAKING AND USING THEREOF



(57) Abstract. A method of low-
ering blood glucose in a mammal
includes orally administering 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.

WO 2004/078196 A1

**ORAL 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 60/489 017 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 oral 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. Furthermore, Schroder's composition is designed for injection, which is an inconvenient and painful method of administering the composition.

BRIEF SUMMARY OF THE INVENTION

[0005] A method of lowering blood glucose in a mammal includes orally administering 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.

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] Figure 10 schematically illustrates therapeutic agent delivery methods according to an embodiment of the present invention

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0018] The present inventor discovered that a composition of porous (i.e. microporous) microparticles, such as crystallized dextran microparticles may be used as a vehicle for oral delivery of insulin. The composition may be a one phase composition or a multi-phase composition which forms a structured implant in a mammal.

[0019] The first section below describes the preferred crystallized dextran microparticles, the second section describes formation of the structured implant from a multi-phase composition, and the following sections describe specific examples of oral administration of the composition into mammals and methods of making the oral insulin composition.

A Crystallized Dextran Microparticles

[0020] 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 10 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.

[0021] 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.

[0022] 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).

[0023] 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.

[0024] 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.

[0025] 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

[0026] Self assembled structures based on crystallized dextran microparticles and their aggregates may be formed based on two phase systems.

[0027] For example, in the case of oil, a special kind of 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.

[0028] 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.

[0029] 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).

[0030] 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

[0031] 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).

[0032] 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.

[0033] 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,

2

13

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.

[0034] 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.

[0035] 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.

[0036] 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 UI 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 UI of insulin.

[0037] 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 into 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 in the two phase system which may be a 40 to 500 kDa dextran solution, such as a 40 to 75 kDa solution. This is 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.

[0038] 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.

5

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.

[0039] 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.

[0040] 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 discrete 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.

[0041] 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 discrete 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 delivered into a mammal, such as an animal or human. Thus, the capsule forms a structured, three 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. It should be noted that the structure formed for orally delivered two phase systems may be generally described as a structured suspension comprising a dispersed PEG phase and a continuous dextran phase.

[0042] 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 the 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 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.

[0043] 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.

[0044] 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 Oral Insulin delivery vehicle

[0045] The present inventor discovered that a composition of porous (i.e., microporous) microparticles may be used as a vehicle for oral delivery of insulin. The porous microparticles may be any suitable porous microparticles which enable oral administration of insulin with a significant reduction in blood glucose such as at least a 5% reduction for example at least a 30% reduction within 60 minutes of oral administration. Preferably the microparticles are bioadhesive particles such as particles which adhere at least temporarily to mammal intestine walls to allow insulin deliver through the intestine wall. Most preferably the porous microparticles comprise the crystallized dextran microparticles described above.

[0046] In one preferred embodiment of the present invention shown in Figure 10, the present inventor has discovered that an aqueous suspension of crystallized dextran microparticles 12, 14 and insulin 46 orally administered to mammals 53 such as rabbits was about equally as effective in reducing blood glucose levels as an intramuscular injection of insulin alone. Figure 10 schematically illustrates the insulin 46 permeating through mammal 53 intestine 52 walls from the orally administered composition 54 comprising the microparticles. Since rabbits are a common model for humans in drug testing, the present inventor believes that a liquid or solid composition 54 comprising crystallized dextran

microparticles and insulin such as an aqueous suspension, a solution a tablet or a capsule, would also be effective in reducing blood glucose levels in human beings when orally administered

[0047] The following examples illustrate oral insulin delivery using crystallized dextran microparticles. The study involved Chinchilla rabbits (2.3 ± 0.2 kg) and the observation of their response to orally administered aqueous suspensions consisting of crystallized dextran microparticles prepared according to the method described herein and human recombinant insulin.

[0048] 3.0 g of Dextran T20 (Pharmacia, Uppsala, Sweden) was dissolved in 2.0 g of water and placed in box at temperature 60°C . Three hours later crystallized dextran microparticles were washed by centrifugation at 3,000 g with 3×5.0 ml of water. Then the crystallized dextran microparticles were suspended in 2.0 ml of water and allowed to dry at room temperature. The resulting dry powder was used to prepare an insulin containing suspension for the oral insulin delivery experiment. Insulin containing suspensions were prepared by the mixing of 250 mg of the microparticles, 0.3 ml (12 UI) or 0.6 ml (24 UI) of insulin (NovoNordisk Actrapid HM Penfill 40 UI/ml) and distilled water to reach a volume of 2.0 ml.

[0049] Samples of the suspension (2.0 ml) were introduced into the rabbits' throats with a catheter followed by the introduction of 10.0 ml of drinking water. Animals were not fed for 3 hours before the suspension's introduction. Samples of blood were taken from the rabbit's ear vein 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.

2

[0050] Examples 1 to 8 are comparative examples involving eight rabbits. Examples 9 to 14 are examples according to the present invention involving five rabbits.

[0051] In comparative examples 1 and 2 (control experiment #1 summarized in Table I) an aqueous solution of human recombinant insulin was introduced intra muscularly into two rabbits at a dose of 12 UI per animal. In comparative examples 3 and 4 (control experiment #2 summarized in Table II), the rabbits remained intact (i.e. no insulin or other injection was provided to the two rabbits). In comparative examples 5 and 6 (control experiment #3 summarized in Table III) a suspension of the crystallized dextran microparticles without insulin was provided orally to two rabbits. In comparative examples 7 and 8 (control experiment #4 summarized in Table IV), a suspension of the commercially obtained Sephadex G 150 microparticles with insulin (24 UI) was provided orally to two rabbits. According to the Amersham Biosciences website Sephadex® G-150 microparticles are beaded gel microparticles having a diameter of 20 to 150 microns, prepared by cross linking dextran with epichlorohydrin. In examples 9-14 according to a preferred embodiment of the present invention (summarized in Table V) a suspension of crystallized dextran microparticles with insulin (24 UI) was provided orally to five rabbits. The results are summarized in Tables I-V below.

Table I

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#1	12 UI i.m.	91	68	58	49	51
#2	12 UI i.m.	87	65	64	57	58

Table II

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#3	0 0	98	87	87	89	86
#4	0 0	88	90	91	94	87

Table III

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#5	0 0	92	99	84	95	92
#6	0 0	90	93	93	93	92

Table IV

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#7	24 UI per os	85	82	86	81	83
#8	24 UI per os	84	75	86	76	77

Table V

Rabbit/ Experiment #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#9	24 UI per os	94	68	59	58	57

#10	24 UI per os	93	64	52	54	52
#11	24 UI per os	78	52	51	49	48
#12	24 UI per os	92	64	52	53	47
#13	24 UI per os	89	53	48	38	49
#14	24 UI per os	97	60	38	54	52

[0052] The data in Tables I V show that average reduction of sugar (i.e., glucose) in the blood of mammals is comparable when 12 UI of insulin is administered by intramuscular injection (examples 1-2) and 24 UI of insulin is administered per os (i.e., orally) with crystallized dextran microparticles (examples 9-14). The maximum glucose reduction was about 35 to about 60 percent at 60 min after oral administration. The concentration profile of glucose is practically the same in both the injection and oral delivery modes. It should be noted that other amounts of insulin may be administered. For example, 30 UI of insulin may be administered. In general, oral administration of two to three times the insulin compared to the amount of injected insulin produces a similar drop in blood sugar for up to three hours.

[0053] It is a well known fact that insulin by itself is degraded by intestinal enzymes and is not absorbed intact across the gastrointestinal mucosa (Amidon GL, Lee HJ. Absorption of peptide and peptidomimetic drugs. Ann Rev Pharmacol Toxicol 1994; 34: 321-41). However, examples 9-14 show that crystallized dextran microparticles can be used as a vehicle for oral delivery of proteins such as insulin because the

hypoglycemia effect received was significant. Without wishing to be bound by a particular theory or mode of action, the present inventor believes that the use of the porous crystallized dextran microparticles as an insulin delivery matrix in an aqueous suspension protected the insulin from significant degradation by intestinal enzymes and allowed the insulin to be absorbed intact across the gastrointestinal mucosa. The insulin may be located in micropores in the microporous microparticles and/or in macropores between the microparticles. In contrast, the use of cross linked Sephadex G 150 dextran microparticles with insulin (Table IV examples 7-8) did not produce an appreciable reduction in blood glucose concentration.

[0054] As provided in examples 9-14, the blood glucose concentration in the mammal is lowered by at least 5 percent, preferably at least 30 percent, 60 minutes after administering the composition containing the crystallized dextran microparticles and insulin to the mammal (i.e., the blood glucose value in the mammal at 60 minutes after administration of the suspension is at least 5 percent, preferably at least 30 percent lower than that measured right before administration of the suspension). Preferably, the blood glucose concentration in the mammal is lowered by at least 5 percent, such as at least 30 percent, preferably by about 35 to about 40 percent, 30 minutes after administering the composition to the mammal. Preferably, the blood glucose concentration in the mammal is lowered by about 35 to about 60 percent, for example 35 to 45 percent, 60 minutes after administering the suspension to the mammal. More preferably, the blood glucose concentration in the mammal is lowered during the entire period ranging from 30 to 240 minutes, such as 30 to 120 minutes, after administering the composition to the mammal compared to the blood glucose concentration right before administration. For example, the blood glucose concentration in the

24

mammal is preferably lowered by at least 10 percent preferably at least 30 percent more preferably by at least 35 percent such as by 35 to 45 percent during a period ranging from 30 to 240 minutes preferably 30 to 120 minutes after administering the composition to the mammal

[0055] Thus, a preferred embodiment of the present invention provides a method of lowering blood glucose in a mammal by orally administering a therapeutically effective amount of a composition comprised of crystallized dextran microparticles and insulin. A "therapeutically effective" amount of the compositions can be determined by prevention or amelioration of adverse conditions or symptoms of diseases, injuries or disorders being treated. Preferably the composition comprises an aqueous suspension of crystallized dextran microparticles having an average diameter of about 0.5 to about 5 microns and insulin. Furthermore the microparticles are preferably porous microparticles which are crystallized prior to adding the insulin to the suspension, such that the insulin is located in contact with a surface of the microparticles and/or in pores of the microparticles.

[0056] 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.

[0057] While a one phase composition 54 comprising insulin and microparticles is illustrated in Figure 10, a two phase composition, described above and illustrated in Figures 7, 8, 9A, 9B and 9C may also

be used. For example, a two phase composition comprising a dextran phase, a PEG phase, crystallized dextran microparticles and insulin may be used. In vivo, the composition has a self assembled capsule structure comprising a crystallized dextran microparticle containing wall or shell and a PEG and insulin containing core.

[0058] Preferably, the mammal receiving the oral administration of insulin comprises a human in need of lowering blood glucose, such as a human suffering from diabetes. Thus, the preferred embodiment of the present invention provides a method of treating diabetes in a human in need of the treatment by orally administering the suspension of insulin and crystallized dextran microparticles described above.

[0059] Any therapeutically effective amount of insulin may be administered to the mammal. The amount of insulin may vary based on the type of mammal (i.e., human or rabbit), the weight of the mammal, the composition of the suspension, the amount of desired reduction of blood glucose and other factors. One non-limiting example of insulin content in the suspension is about 10 UI to about 2,500 UI of human recombinant insulin per one gram of suspension, such as about 12 UI to about 30 UI, such as 24 UI of human recombinant insulin. However, this amount may vary as desired.

[0060] The present invention should not be considered limited to the preferred embodiments described above. Other matrix material may be used for oral insulin delivery, such as organic or inorganic microporous particles. Preferably, the particles are microparticles which enhance insulin penetration through gastrointestinal mucosa and/or which stabilize the composition. Furthermore, while the suspension preferably contains only water solvent, a matrix and an insulin solution or suspension, the delivery system may also contain additional materials. For example, the

composition may contain a second phase such as the PEG phase of a two phase system. Thus, another preferred aspect of the present invention includes a method of lowering blood glucose in a mammal comprising of orally administering a composition comprising a therapeutically effective amount of insulin and a matrix material to the mammal to lower blood glucose of the mammal by at least 30 percent 60 minutes after administering the suspension to the mammal. In another preferred aspect of the present invention a method of administering a suspension to a mammal comprised of orally administering an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin to the mammal.

[0061] As noted above, the crystallized dextran microparticles used as a matrix material for oral administration of insulin or other protein based drugs may be made by any suitable method (see Figure 1 for example). Preferably the microparticles are made by the process of any of the preferred embodiments described herein. Preferably but not necessarily the microparticles are formed in an aqueous solution without using an organic solvent. Thus in a preferred aspect of the present invention a therapeutically effective amount of insulin and the crystallized dextran microparticles are combined in water after the microparticles have been crystallized to form an aqueous suspension of insulin and crystallized dextran microparticles. The microparticles may be added to the water before, at the same time and/or after adding the insulin to the water. Furthermore the microparticles may be orally administered to a mammal in the solvent in which they were formed. Alternatively, they may be removed from the solvent in which they were formed and placed into water or other aqueous solutions for oral administration or dried and provided in solid form such as tablet or capsule for oral administration.

[0062] The aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of Insulin (or other suitable suspensions of Insulin and a matrix material such as a suspension of Insulin and microporous microparticles) is preferably provided as a dosed pharmaceutical composition which is dosed for oral administration to a human. In one preferred aspect of the present invention, the composition is located in a vessel in an amount dosed for a single oral administration to a human. The vessel may comprise any container which may hold a suspension, such as a plastic or glass bottle, a tube, a dropper, a spray nozzle, a pouch and/or other suitable vessels. This vessel contains an amount of suspension sufficient for a single oral dose of the composition.

[0063] In another preferred aspect of the present invention, the composition is located in any suitable vessel in an amount suitable for multiple oral administration doses. The vessel contains an instruction for oral dosage administration to a human. The instruction may be printed on the vessel, such as being printed directly on the vessel or on a label attached to the vessel, or enclosed with the vessel, such as being printed on a sheet of paper enclosed with the vessel in a cardboard box or in a pharmacy envelope. The instructions may describe the amount of the composition that should be taken with each dose, the frequency that the dose should be taken, how to measure the dose of the composition for oral administration and/or any other suitable oral drug instructions for an administering health care practitioner and/or a patient in need of the drug. Alternatively, the instructions may comprise directions for electronically or audibly accessing the dosing and administration instructions, such as a link to a website containing the instructions or a telephone number or recording where the instructions are provided audibly.

23

[0064] In another preferred aspect of the present invention the aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin located in a vessel are provided in a pharmaceutical composition kit with instructions for oral administration of the composition to a human in need thereof. The kit may comprise instructions printed on the vessel or on a label attached to the vessel or a sheet of paper enclosed with the vessel, such as in a cardboard box or pharmacy envelope including a bottle (i.e., vessel) and the sheet of instructions.

[0065] It should be noted that the composition for oral administration may be in the form of an aqueous suspension but other delivery forms may be used to lower the blood glucose in a mammal. For example, the porous crystallized dextran microparticles and the insulin may be orally administered in the form of a tablet or a capsule.

[0066] To orally administer the composition in solid form to a mammal such as a human, the solution of crystallized dextran microparticles and insulin is first dried, such as freeze dried, to form a powder. The powder may then be compressed into a tablet along with optional pharmaceutically acceptable excipients or placed into a pharmaceutically acceptable capsule.

D Materials

[0067] 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

29

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.

[0068] 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.

[0069] 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.

[0070] 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

[0071] 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 35 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.

[0072] 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

8
31

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.

[0073] 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

[0074] 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

32

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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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 a 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 orally.

[0079] Preferably, the composition which includes the microparticles and the therapeutic agent is a flowable colloidal system. Examples of flowable colloidal systems include emulsions and suspensions. In contrast some prior art compositions include a therapeutic agent in a dextran hydrogel or in a cross linked dextran matrix. A dextran hydrogel

34

and a cross-linked dextran matrix are not flowable compositions if not specifically prepared

[0080] 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.

[0081] 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.

[0082] 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.

[0083] All of the publications and patent applications and patents cited in this specification are herein incorporated in their entirety by reference.

35

I claim

1 A method of lowering blood glucose in a mammal comprising orally administering a therapeutically effective amount of a composition comprising crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal

2 The method of claim 1 wherein the composition comprises an aqueous suspension of crystallized dextran microparticles having an average diameter of about 0.5 to about 5 microns and the insulin

3 The method of claim 2, wherein the method comprises orally administering the suspension to a human in need of lowering blood glucose

4 A method of treating diabetes in a human in need thereof by orally administering the suspension of claim 2

5 The method of claim 2 wherein the microparticles are porous microparticles which are crystallized prior to adding the insulin to the suspension, such that the insulin is located in contact with a surface of the microparticles or in pores of the microparticles

6 The method of claim 2 wherein the therapeutically effective amount of insulin ranges from 10 to 2,500 UI of human recombinant insulin per 1 gram of the suspension

7 The method of claim 2, wherein the blood glucose concentration in the mammal is lowered by at least 5 percent 60 minutes after administering the suspension to the mammal

36

8 The method of claim 7 wherein the blood glucose concentration in the mammal is lowered by about 30 percent to about 60 percent 60 minutes after administering the suspension to the mammal

9 The method of claim 2 wherein the blood glucose concentration in the mammal is lowered by at least 5 percent 30 minutes after administering the suspension to the mammal

10 The method of claim 2 wherein the blood glucose concentration in the mammal is lowered by at least 10 percent during a period ranging from 30 minutes to 240 minutes after administering the suspension to the mammal

11 The method of claim 2 wherein the blood glucose concentration in the mammal is lowered during a period ranging from 30 minutes to 120 minutes after administering the suspension to the mammal

12 The method of claim 1 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 comprises a structure comprising a dispersed PEG phase and a continuous dextran phase when the composition is present in the mammal body

13 A method of lowering blood glucose in a mammal comprising orally administering a composition comprising a therapeutically effective amount of insulin and a matrix material to the mammal to lower blood glucose of the mammal by at least 5 percent 60 minutes after administering the suspension to the mammal

14 The method of claim 13 wherein the composition comprises an aqueous suspension and the matrix material comprises crystallized dextran microparticles having an average diameter of about 0.5 to about 5 microns

15 The method of claim 14 wherein the microparticles are porous microparticles which are crystallized prior to adding the insulin to the suspension such that the insulin is located in contact with a surface of the microparticles or in pores of the microparticles

16 The method of claim 13 wherein the method comprises orally administering the composition to a human in need of lowering blood glucose

17 A method of treating diabetes in a human in need thereof by orally administering the composition of claim 16

18 The method of claim 13, wherein the therapeutically effective amount of insulin ranges from 10 to 2,500 UI of human recombinant insulin per 1 gram of suspension

19 The method of claim 13 wherein the blood glucose concentration in the mammal is lowered by about 30 percent to about 60 percent 60 minutes after administering the composition to the mammal

20 The method of claim 13, wherein the blood glucose concentration in the mammal is lowered by at least 5 percent 30 minutes after administering the composition to the mammal

21 The method of claim 13, wherein the blood glucose concentration in the mammal is lowered by at least 10 percent during a period ranging from 30 minutes to 240 minutes after administering the composition to the mammal

22 The method of claim 13 wherein the blood glucose concentration in the mammal is lowered during a period ranging from 30 minutes to 120 minutes after administering the composition to the mammal

23 The method of claim 13 wherein the composition is in a form of a tablet or a capsule

24 The method of claim 13, 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 comprises a structure comprising a dispersed PEG phase and a continuous dextran phase when the composition is present in the mammal body

25 A method of administering a suspension to a mammal comprising orally administering an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin to the mammal

26 A dosed pharmaceutical composition comprising crystallized dextran microparticles and a therapeutically effective amount of insulin wherein the composition is dosed for oral administration to a human

27 The composition of claim 26, wherein
the crystallized dextran microparticles comprise dextran molecules held together by hydrogen bonds, Van Der Waals forces or ionic bonds and having substantially no covalent bonds between dextran molecules, and

the crystallized dextran microparticles are porous microparticles having an average diameter of about 0.5 to about 5 microns and such that the insulin is located in contact with a surface of the microparticles or in pores of the microparticles

28 The composition of claim 26 wherein the composition comprises an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin

29 The composition of claim 26, wherein the composition is located in a vessel in an amount dosed for a single oral administration to a human

30 The composition of claim 26, wherein the composition is located in a vessel with instruction printed on the vessel or enclosed with the vessel for oral dosage administration to a human

31 The composition of claim 26, wherein the composition comprises a tablet comprising a pharmaceutically acceptable carrier medium, the crystallized dextran microparticles and the therapeutically effective amount of insulin

32 The composition of claim 26 wherein the composition comprises a capsule comprising a pharmaceutically acceptable shell, the crystallized dextran microparticles and the therapeutically effective amount of insulin

33 The composition of claim 26 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 is adapted to form a structured suspension comprising a dispersed PEG phase and a continuous dextran phase

34 A pharmaceutical composition kit comprising
an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin located in a vessel and
instructions for oral administration of the composition to a human in need thereof

35 A pharmaceutical kit comprising
a first means for orally administering to a mammal to lower blood glucose of the mammal by at least 30 percent 60 minutes after administering the suspension to the mammal and
a storage vessel containing the first means

36 A tablet comprising a pharmaceutically acceptable carrier medium crystallized dextran microparticles and a therapeutically effective amount of insulin

37 A capsule comprising a pharmaceutically acceptable shell crystallized dextran microparticles and a therapeutically effective amount of insulin

38 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 oral administration to a mammal

**39 The method of claim 38 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**

**40 The method of claim 39 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 comprises a structure comprising a dispersed PEG
phase and a continuous dextran phase when the composition is present in
the mammal body**

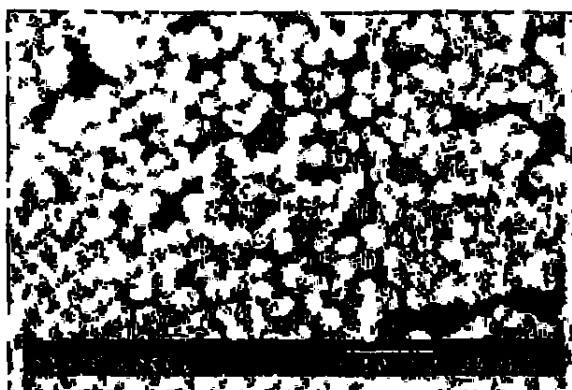


FIG 1



FIG 2A



FIG 2B

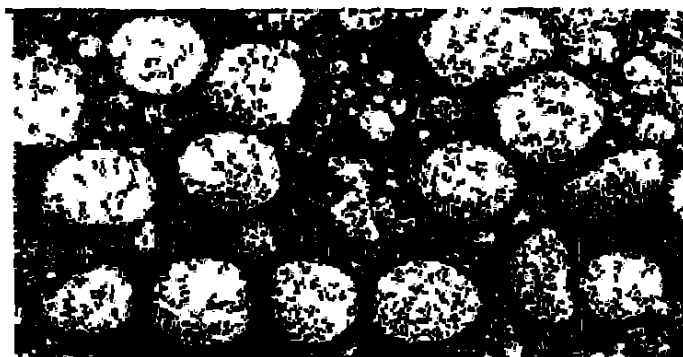


FIG 3



FIG 4



FIG 5

AS



FIG 6

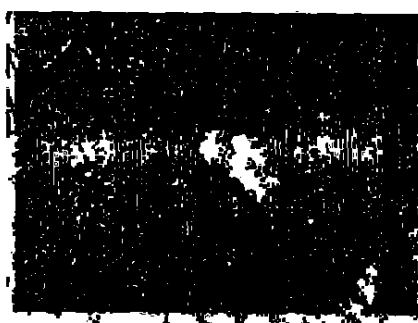


FIG 7



FIG 8

AS

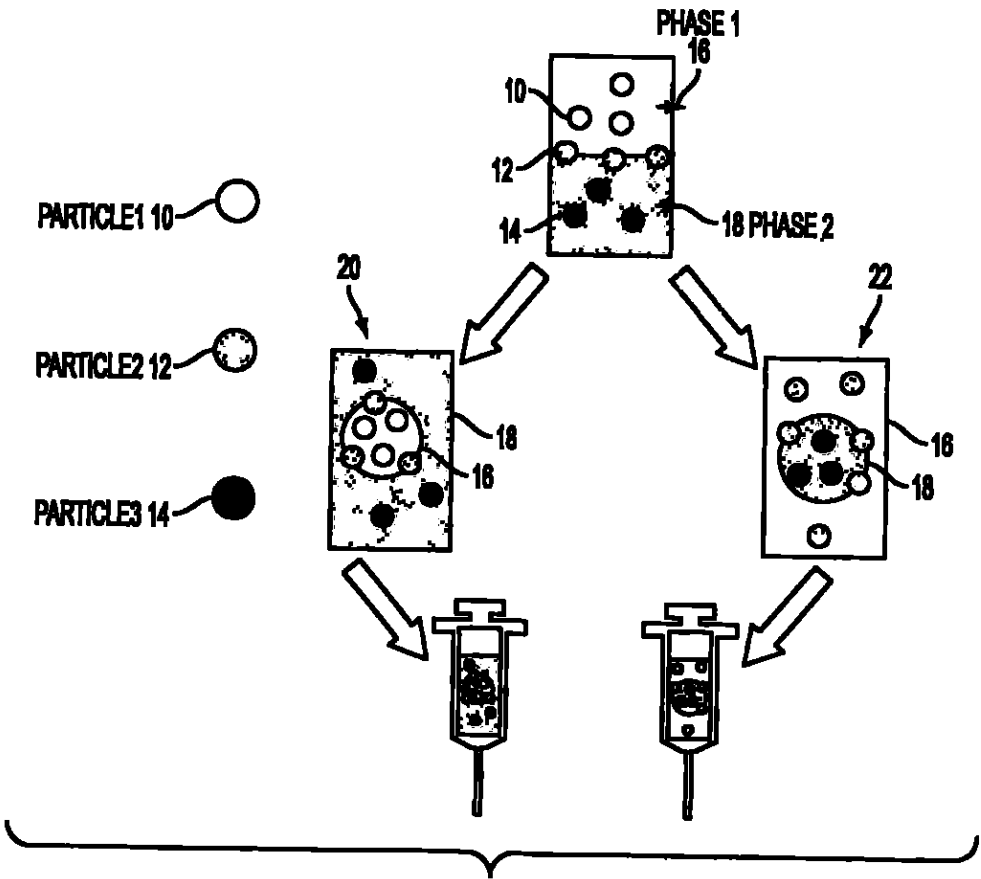


FIG 9A

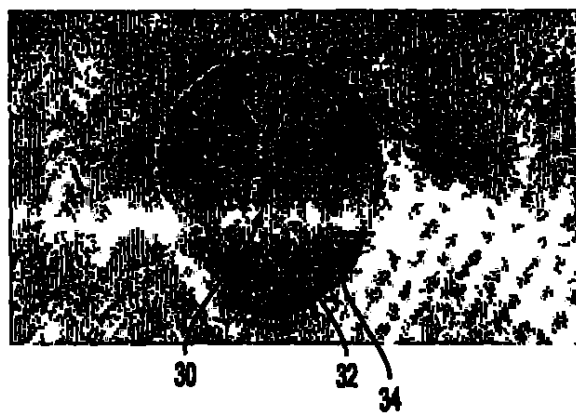


FIG 9B

27

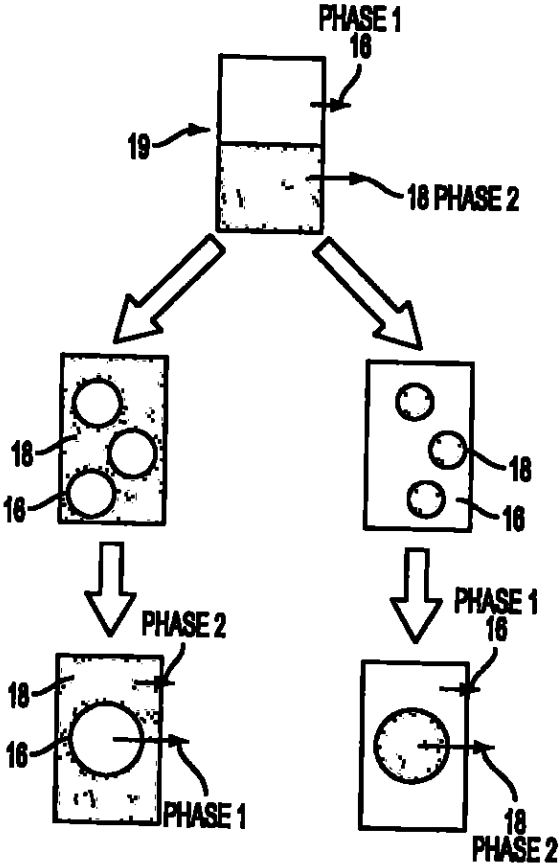
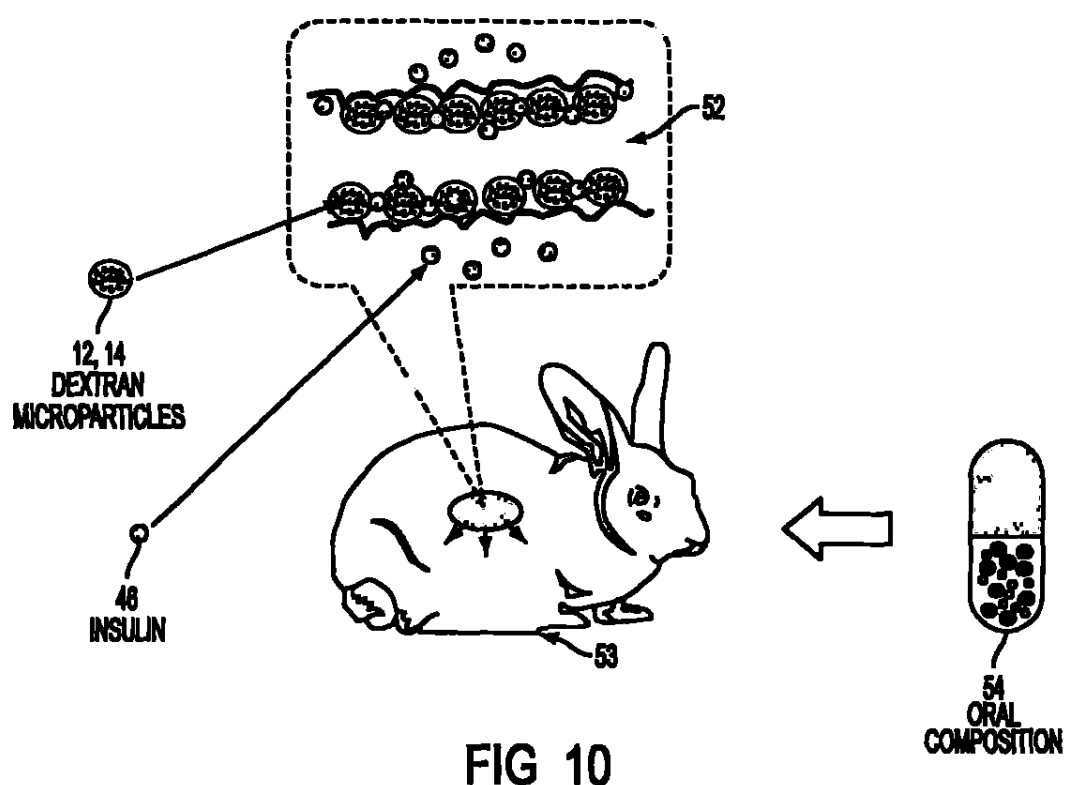


FIG 9C



COMPOSICIÓN DE INSULINA ORAL Y MÉTODOS DE FABRICACIÓN Y**USO DE ESTOS****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 aqui en su totalidad

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

ANTECEDENTE DE LA INVENCION

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

51 '11

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. Adicionalmente, la composición de Schroder se diseña para inyección, que es un método inconveniente y doloroso de administrar la composición.

BREVE RESUMEN DE LA INVENCION

[0005] Un método para reducir la glucosa sanguínea en un mamífero incluye administrar oralmente 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 multi-fase estructurada para liberación controlada de insulina

BREVE DESCRIPCION 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 MW 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

[0011] La figura 5 es una fotografía de una emulsión de solución acuosa de PEG en una solución acuosa de dextrán (MW 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 (MW 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 (MW 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 (MW 500 kDa) que contiene micropartículas de dextrán cristalizada mostrada en la figura 1

[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] La figura 10 ilustra esquemáticamente los métodos de administrar agentes terapéuticos de acuerdo con una modalidad de la presente invención

DESCRIPCIÓN DETALLADA DE LAS MODALIDADES PREFERIDAS

[0018] El presente inventor ha descubierto que una composición de micropartículas de poro (es decir micropartículas), tales como micropartículas de dextrán cristalizado que pueden ser usados como un vehículo para administración oral de insulina. La composición puede ser una composición de fase o una composición multi-fase la cual forma un implante estructurado en un mamífero

[0019] 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 administración oral de la composición en mamíferos y métodos de fabricación de la composición oral de insulina

A Micropartículas Cristalizadas de Dextrán

[0020] 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 de dextrán pueden ser usadas. Por supuesto, también las micropartículas pueden ser formadas de soluciones de 2 a 18 kDa a temperaturas por encima de la temperatura ambiente, si se desea. Las micropartículas pueden ser formadas de soluciones 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 pueden 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

[0021] 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

[0022] 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)

[0023] 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.

[0024] 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 de humedad cerca de un 5%. El polvo seco resultante consiste de partículas con un diámetro medio de cerca de 2 micrones.

[0025] La liberación lenta de macromoléculas de los implantes se ha demostrado en los experimentos en donde las macromoléculas se disolvieron en suspensiones acuosas de micropartículas de dextrán cristalizadas o sus agregados.

antes de inyecciones La figura 4 muestra un implante que contiene macromoléculas fluorescentemente etiquetadas (FITC-dextrán, PM 500 kDa) y liberación lenta de las macromoléculas desde el implante dentro del tejido de músculo de ratón en el día 14 después de la inyección intramuscular

B Sistema de dos fases

[0026] Las estructuras autoensambladas basados en micropartículas de dextrán cristalizado y sus agregados puede ser formado basado en sistemas de dos fases

[0027] Por ejemplo, en el caso del aceite, una clase especial de estructura 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, dextráneos) 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 barrera en otro

lado Adicionalmente, mientras se utiliza aceite como un ejemplo ilustrativo, el nucleo puede comprender otros materiales, tales como otros polímeros, células, etc

[0028] 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

[0029] La absorción competitiva de las dos fases de polímeros depende de la naturaleza química de los polímeros 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)

[0030] 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

[0031] 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)

[0032] 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.

[0033] 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

[0034] La emulsión puede ser preparada al mezclar el dextrán preparado y las fases PEG separadamente 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

[0035] La inyección del 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

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

[0036] 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 U1 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 U1 de insulina

[0037] 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

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.

[0038] 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

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

[0039] 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

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

[0040] 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*, 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

[0041] 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

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. Se debe notar que la estructura formada para los sistemas de administración de dos fases puede ser descrito generalmente como una suspensión que comprende una fase PEG dispersa y una fase de dextrán continua.

[0042] 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 fase PEG, una porción de la insulina puede permanecer en la fase dextrán.

[0043] 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 primera 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

[0044] 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 Oral

[0045] El presente inventor descubrió que una composición de micropartículas porosas (por ejemplo, microporos) pueden ser utilizados como un vehículo de administración oral de insulina. Las micropartículas porosas pueden ser cualquier micropartícula porosa adecuada que permita la administración oral de insulina con una reducción importante en la glucosa sanguínea, tal como al menos una reducción del 5%, por ejemplo al menos una reducción del 30% dentro de 60 minutos de administración oral. Preferiblemente, las micropartículas son partículas bioadhesivas, tales como partículas que se adhieren al menos temporalmente a las paredes del intestino del mamífero, para permitir la administración de la insulina a través de la pared del intestino. Más preferiblemente, las micropartículas de poro comprende las micropartículas de dextrán cristalizado descritas anteriormente.

[0046] En una modalidad preferida de la presente invención mostrada en la Figura 10, el presente inventor ha descubierto que una suspensión acuosa de micropartículas de dextrán cristalizado 12, 14 e insulina 46 suministrada oralmente a

mamíferos 53, tales como conejos, fue tan efectivo en la reducción de los niveles de glucosa sanguínea como en la inyección intramuscular de insulina sola. La Figura 10 ilustra esquemáticamente la insulina 46 que permea a través del intestino 52 del mamífero 53 de la composición 54 administrada oralmente que comprende las micropartículas. En razón a que los conejos son un modelo común para los humanos en el ensayo de drogas, el presente inventor cree que una composición sólida o líquida 54 que comprende micropartículas de dextrán cristalizado e insulina, tales como una suspensión acuosa, una solución, una tableta o cápsula, podría también ser efectiva en la reducción de los niveles de glucosa sanguínea en humanos cuando se administra oralmente.

[0047] Los siguientes ejemplos ilustran la liberación de insulina utilizando micropartículas de dextrán cristalizado. El estudio involucra conejos Chinchilla ($2,3 \pm 0,2$ kg) y la observación de su respuesta a la suspensión acuosa suministrada oralmente que consiste de micropartículas de dextrán cristalizado preparadas con el método descrito aquí e insulina recombinante humana.

[0048] 3,0 g de Dextrán T20 (Pharmacia, Uppsala, Sweden) fue disuelta en 2,0 g de agua y colocada en una caja a temperatura de 60° C. Tres horas después, las micropartículas

de dextrán cristalizado fueron lavadas mediante centrifugación a 3 000 g con 3 x 5,0 ml de agua. Luego las micropartículas de dextrán cristalizado fueron suspendidas en 2,0 ml de agua y se permitió secarse a temperatura ambiente. El polvo seco resultante fue utilizado para preparar una suspensión que contiene insulina para el experimento de administración de insulina oral. Las suspensiones que contienen insulina fueron preparadas al mezclar 250 mg de las micropartículas, 0,3 ml (12 UI) o 0,6 ml (24 UI) de insulina (NovoNordisk Actrapid HM Penfill, 40 UI/ml) y agua destilada para alcanzar un volumen de 2,0 ml.

[0049] Muestras de la suspensión (2,0 ml) se introdujeron en las gargantas de los conejos con un catéter seguido por la introducción de 10,0 ml de agua para beber. Los animales no fueron alimentados durante 3 horas antes de la introducción de la suspensión. Se tomaron muestras de sangre de la oreja de la vena del conejo y se analizó para concentraciones de glucosa. La glucosa sanguínea fue medida utilizando el método de oxidasa glucosa en un "analizador de glucosa de sistema de un toque" (Lifescan Johnson & Johnson, Milpitas, CA, USA) después de apropiada calibración.

[0050] Los ejemplos 1 a 8 son ejemplos comparativos que involucran ocho conejos. Los ejemplos 9 a 14 son ejemplos de acuerdo a la presente invención que involucra cinco conejos.

[0051] En los ejemplos comparativos 1 y 2 (control experimental # 1 resumido en la tabla I) una solución acuosa de insulina recombinante humana se introdujo intramuscularmente en dos conejos en una dosis de 12 UI por animal. En los ejemplos comparativos 3 y 4 (experimento de control # 2 resumido en la tabla II), los conejos permanecieron intactos (es decir, ninguna insulina o ninguna otra inyección se suministró a los dos conejos). En los ejemplos comparativos 5 y 6 (experimento de control # 3 resumido en la tabla III), una suspensión de micropartículas de dextrán cristalizado sin insulina se suministró oralmente a los dos conejos. En los ejemplos comparativos 7 y 8 (experimento de control # 4 resumido en la tabla IV), una suspensión de las micropartículas Sephadex G-150 obtenidas comercialmente con insulina (24UI) se suministró oralmente a dos conejos. De acuerdo con sitio web Amersham Biosciences, las micropartículas de Sephadex® G-150 son micropartículas de gel moldeadas que tienen un diámetro de 20 a 50 micrones, preparadas mediante reticulación de dextrán con epíclorohidrina. En los ejemplos 9-14 de acuerdo con una

9A

modalidad preferida de la presente invención (resumida en la tabla V), una suspensión de micropartículas de dextrán cristalizado con insulina (24 U1) se suministró oralmente a cinco conejos Los resultados se resumen en la tabla I-V adelante

Tabla I

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 1	12 U1	91	68	58	49	51
# 2	12 U1	87	65	64	57	58

Tabla II

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 3	0,0	98	87	87	89	86
# 4	0,0	88	90	91	94	87

Tabla III

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 5	0,0	92	999	94	95	92
# 6	0,0	90	93	93	93	92

Tabla IV

Conejo/ Ejemplo #	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 7	24 U1 Por os	85	82	86	81	83
# 8	24 U1 Por os	84	75	86	76	77

Tabla V

Conejo/ Ejemplo	Dosis de insulina	0 min Mg/dl	30 min Mg/dl	60 min Mg/dl	90 min Mg/dl	120 min Mg/dl
# 9	24 U1 Por os	94	68	59	58	57

9/6

# 10	24 U1 Por os	93	64	52	54	52
# 11	24 U1 Por os	78	52	51	49	48
# 12	24 U1 Por os	92	64	52	53	47
# 13	24 U1 Por os	89	53	48	38	49
# 14	24 U1 Por os	97	60	38	54	52

[0052] Los datos en las tablas I-V romanos muestran que el promedio de reducción de azúcar (es decir, glucosa) en la sangre de los mamíferos es comparable cuando 12 U1 de insulina es administrada por inyección intramuscular (ejemplos 1-2) y 24 U1 de insulina es administrada por os (es decir, oralmente) con micropartículas de dextrán cristalizado (ejemplos 9-14) El máximo de reducción de glucosa fue cerca de 35 a 60 por ciento en 60 minutos después de la administración oral El perfil de concentración de la glucosa es prácticamente el mismo tanto en ambos la inyección y el modo de administración oral Se debe notar que otras cantidades de insulina pueden ser administradas Por ejemplo, 30 U1 puede ser administrado En general, la administración

37

oral de dos o tres veces la insulina comparada a la cantidad inyectada de insulina produce una gota similar en el azúcar sanguíneo hasta las tres horas

[0053] Es bien conocido el hecho de que la insulina por sí misma se degrada mediante las enzimas intestinales y no se absorbe intacta a través de la mucosa gastrointestinal (Amidon GL, Lee HJ, Absorption of peptide and peptidomimetic drugs, Ann Rev Pharmacol Toxicol 1994, 34 321-41) Sin embargo, los ejemplos 9-14 muestran que las micropartículas de dextrán cristalizado pueden ser utilizados como un vehículo de administración oral de proteínas, tales como insulina porque el efecto de hipoglicemia recibido fue significativo Sin desear unirse a una teoría particular o modo de acción, el presente inventor cree que el uso de las micropartículas de dextrán cristalizado, porosas como la matriz de administración de insulina en una suspensión acuosa protege la insulina de la degradación importante por las enzimas gastrointestinales y permite a la insulina ser absorbida intacta a través de la mucosa gastrointestinal La insulina puede ser ubicada en microporos en las micropartículas de microporo y/o en los macroporos entre las micropartículas Por el contrario, el uso de micropartículas de dextrán de Sephadex G-150 reticuladas con insulina (tabla

78

IV, ejemplos 7-8) no producen una reducción apreciable en la concentración de glucosa sanguínea

[0054] Como se suministra en los ejemplos 9-14, la concentración de glucosa en la sangre en el mamífero es más reducida al menos al 5 por ciento, preferible al menos al 30 por ciento, 60 minutos después de administrar la composición que contiene micropartículas de dextrán cristalizado e insulina al mamífero (es decir, el nivel de glucosa sanguínea en el mamífero a 60 minutos después de la suspensión es al menos 5 por ciento, preferiblemente al menos 30 por ciento menos que la medición antes de la administración de la suspensión) Preferiblemente, la concentración de glucosa en el mamífero se reduce al menos 5 por ciento, tal como al menos 30 por ciento, preferiblemente cerca de 35 a cerca de 40 por ciento 30 minutos después de administrar la composición al mamífero Preferiblemente, la concentración de glucosa en el mamífero se reduce cerca de 35 a cerca de 60 por ciento, por ejemplo 35 a 45 por ciento 60 minutos después de la administración de la suspensión al mamífero Más preferiblemente, la concentración de glucosa sanguínea en el mamífero se reduce durante el periodo entero variando de 30 a 240 minutos, tal como 30 a 120 minutos, después de administrar la composición al mamífero comparado con la concentración de glucosa sanguínea después de la

79

administración Por ejemplo la concentración de glucosa sanguínea en el mamífero se reduce preferiblemente el 10 por ciento, preferiblemente al menos 30 por ciento y mas preferiblemente al menos el 35 por ciento, tal como 35 a 45 por ciento durante un periodo que varía de 30 a 240 minutos, preferiblemente como 30 a 120 minutos, después de administrar la composición al mamífero

[0055] Así, una modalidad preferida de la presente invención suministra un método para reducir la glucosa sanguínea en un mamífero mediante la administración oral de una cantidad terapéuticamente efectiva de una composición comprendida de micropartículas de dextrán cristalizadas e insulina Una cantidad 'terapéuticamente efectiva de las composiciones puede ser determinada mediante la prevención o el mejoramiento de condiciones adversas o síntomas de enfermedades, heridas o desordenes que son tratados Preferiblemente la composición comprende una suspensión acuosa de micropartículas de dextrán cristalizadas que tienen un diámetro promedio de 0,5 a 5 micrones e insulina Adicionalmente, las micropartículas son preferiblemente micropartículas de poro que son cristalizadas antes de agregar la insulina a la suspensión, tal que la insulina se ubica en contacto con la superficie de las micropartículas y/o en los poros de las micropartículas

[0056] Las micropartículas cristalizadas preferiblemente están comprendidas de moléculas de dextrán (es decir, moléculas de polímero) que están mantenidas juntas mediante una pluralidad de enlaces de hidrógeno, las fuerzas de Van Der Waals y/o enlaces iónicos y que no tienen sustancialmente enlaces covalentes entre las moléculas de dextrán. Así, las moléculas entre las micropartículas no están preferiblemente e intencionalmente reticuladas (es decir, una etapa de reticulación no se lleva a cabo) y las micropartículas no contienen enlaces covalentes entre las moléculas o menos del 10% de enlaces covalentes entre moléculas.

[0057] Mientras una composición de fase 54 que comprende insulina y micropartículas se ilustra en la Figura 10, una composición de dos fases, descrita anteriormente e ilustrada en las figuras 7, 8, 9A, 9B y 9C pueden también ser usadas. Por ejemplo, una composición de dos fases que comprende una fase dextrán, una fase PEG, micropartículas de dextrán cristalizadas e insulina pueden ser utilizadas. In vivo, la composición tiene una estructura de cápsula autoensamblada que comprende una micropartículas de dextrán cristalizada que contiene una pared o concha y un núcleo que contiene PEG e insulina.

[0058] Preferiblemente, el mamífero que recibe la administración oral de la insulina comprende un humano en necesidad de reducir la necesidad de glucosa sanguínea, tal como un humano que sufre de diabetes Así, la modalidad preferida de la presente invención suministra un método para tratar diabetes en un humano en necesidad de tratamiento mediante administración oral de la suspensión de insulina y de micropartículas de dextrán cristalizadas descritas anteriormente

[0059] Cualquier cantidad efectiva terapéuticamente de insulina puede ser administrada al mamífero La cantidad de insulina puede variar basado en el tipo de animal (es decir, humano o conejo), el peso del mamífero, la composición de la suspensión, la cantidad de reducción deseada de glucosa sanguínea y otros factores Un ejemplo no limitativo del contenido de insulina en la suspensión es cerca de 10 UI a cerca de 2 500 UI de insulina humana recombinante por un gramo de suspensión, tal como cerca de 12 UI a cerca de 30 UI, tal como 24 UI de insulina recombinante humana Sin embargo, esta cantidad puede variar según se desee

[0060] La presente invención no debe ser considerada limitada a las modalidades descritas anteriormente Otro material de matriz puede ser utilizado para administración de

insulina oral, tal como partículas de microporo orgánico e inorgánico Preferiblemente, las micropartículas son micropartículas que mejoran la penetración de la glucosa a través de la mucosa gastrointestinal y/o estabiliza la composición Adicionalmente, mientras la suspensión solo contiene preferiblemente solvente agua una matriz y una solución de insulina o suspensión, el sistema de administración también puede contener materiales adicionales Por ejemplo, la composición puede contener una segunda fase tal como una segunda fase PEG de un sistema de dos fases Así, otro aspecto preferido de la presente invención incluye un método para reducir el nivel de glucosa sanguíneo en un mamífero que comprende de administrar oralmente una composición que comprende una cantidad de insulina terapéuticamente efectiva y un material de matriz para reducir el nivel de glucosa en el mamífero por al menos 30 por ciento en 60 minutos después de administrar la suspensión al mamífero En otro aspecto preferido de la presente invención un método para administrar una suspensión a un mamífero comprende administrar oralmente una suspensión acuosa de micropartículas de dextrán cristalizadas y una cantidad de insulina terapéuticamente efectiva al mamífero

[0061] Como se notó anteriormente, las micropartículas de dextrán cristalizadas utilizadas como un material de matriz

para administración oral de insulina u otras drogas basadas en proteína puede ser hecha mediante cualquier método adecuado (ver Figura 1, por ejemplo) Preferiblemente, las micropartículas son hechas mediante los procesos de cualquiera de las modalidades preferidas descritas aquí Preferiblemente, pero no necesariamente, las micropartículas se forman en una solución acuosa sin utilizar un solvente orgánico Así, en un aspecto preferido de la presente invención, una cantidad terapéuticamente efectiva de la insulina y de las micropartículas de dextrán cristalizado se combinan en agua después de que las micropartículas han sido cristalizadas para formar una suspensión acuosa de insulina y micropartículas de dextrán cristalizado Las micropartículas pueden ser agregados antes al agua, al mismo tiempo y/o después de agregar la insulina al agua Adicionalmente, las micropartículas pueden ser administradas oralmente a un mamífero en el solvente en el que ellas se formaron Alternativamente, ellas pueden ser removidas del solvente en que ellas se formaron y colocadas en agua u otras soluciones acuosas para administración oral o secadas y administradas en forma sólida, tal como tabletas o cápsulas, para administración oral

[0062] La suspensión acuosa de micropartículas de dextrán cristalizadas y una cantidad terapéuticamente efectiva de

48

insulina (u otras suspensiones de insulina adecuadas y un material matriz, tal como una suspensión de insulina y micropartículas de microporos) es preferiblemente suministrada como una composición farmacéutica dosificada la cual se dosifica por administración oral a un humano. En un aspecto preferido de la presente invención, la composición se localiza en un frasco en una cantidad dosificada para una administración oral única a un humano. El frasco puede comprender cualquier contenedor que pueda mantener una suspensión, tal como una botella plástica o de vidrio, un tubo, un gotero, una boquilla de rociado, una bolsa y/u otros frascos adecuados. Este frasco contiene una cantidad de suspensión suficiente para una dosificación oral única de la composición.

[0063] En otro aspecto preferido de la presente invención, la composición es ubicada en cualquier frasco adecuado en una cantidad adecuada para dosis de administración oral múltiple. El frasco contiene una instrucción para administración oral a un humano. La instrucción puede ser impresa en el frasco, tal como impresas directamente en el frasco o en una etiqueta adherida al frasco, o incluida con el frasco, tal como impresa en una hoja de papel incluida con el frasco en una caja de cartón o sobre de farmacia. Las instrucciones pueden describir la cantidad de la composición que debe ser tomada.

con cada dosis, la frecuencia que la dosis debe ser tomada, como medir la dosis de la composición de la administración oral y/o cualquier otra instrucción de droga oral adecuada para una administración a un médico y/o a un paciente en necesidad de la droga Alternativamente, las instrucciones pueden comprender indicaciones para acceder electrónicamente o audiblemente las instrucciones de dosificación, tal como un enlace a un sitio web que contiene las instrucciones o a un número de teléfono o grabación en donde las instrucciones se suministran en forma audible

[0064] En otro aspecto preferido de la presente invención, la suspensión acuosa de micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina ubicada en un frasco se suministra en un kit de composición farmacéutica con instrucciones para administración oral de la composición de un humano en necesidad de este El kit puede comprender instrucciones impresas en el frasco o en una etiqueta adherida al frasco, o una hoja de papel incluida con el frasco, tal como en una caja de cartón o sobre de farmacia que incluye una botella (es decir, un frasco) y la hoja de instrucciones

[0065] Se debe notar que la composición para la administración oral puede estar en la forma de una suspensión

acuosa, pero otras formas de administración pueden ser utilizadas para reducir la glucosa sanguínea en un mamífero. Por ejemplo, las micropartículas de dextrán cristalizado porosas y la insulina pueden ser administrados oralmente en la forma de una tableta o una cápsula.

[0066] Para administrar oralmente la composición en forma sólida a un mamífero, tal como un humano, la solución de las micropartículas de dextrán cristalizado y la insulina es primero secado, tal como una boquilla de secado, para formar un polvo. El polvo puede ser luego comprimido en una tableta, junto con los excipientes aceptables farmacéuticamente opcionales y colocados en una cápsula farmacéuticamente aceptable.

D Materiales

[0067] 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 término "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.

[0068] El término "análogo de insulina", "insulina monomérica y los similares son usados intercambiabilmente aquí y se pretende que abarque alguna forma de "insulina" 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

88

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.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] Adicionalmente, mientras las micropartículas de dextrán no reticuladas porosas, tales como micropartículas cristalizadas, son preferiblemente usadas con el agente terapéutico, otras micropartículas orgánicas e inorgánicas adecuadas pueden ser utilizadas en cambio, tales como otras micropartículas de polímero que incluye polisacáridos, PLA, PLGA, PMMA, poliimidas, poliésteres, acrilatos, acrilamidas, acetato de 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 administración del agente terapéutico, el ambiente en donde el agente terapéutico será liberado y otros factores.

[0073] 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.

E Métodos de Fabricación

[0074] 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

[0075] Preferiblemente, el agente terapéutico se suministra en los poros de micropartículas porosas después de que las micropartículas son formadas. Así, las micropartículas porosas son primero formadas y luego el agente terapéutico se suministra dentro de una solución que contiene las micropartículas para permitir al agente 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.

[0076] 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

43

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 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.

[0077] 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.

[0078] 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 oralmente.

[0079] Preferiblemente, la composición que incluye las micropartículas y el agente terapéutico es un sistema coloidal fluible. Ejemplos de sistemas coloides fluibles incluyen emulsiones y suspensiones. Por el contrario, algunas composiciones de la técnica anterior incluyen 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

[0080] 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 descritas anteriormente. Esto mejora además la liberación efectiva del agente terapéutico.

[0081] 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.

[0082] 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

[0083] Todas las publicaciones y solicitudes de patentes citadas en esta especificación se incorporan aquí en su totalidad para referencia

REIVINDICACIONES

- 1 Un método para reducir la glucosa sanguínea en un mamífero, que comprende administrar oralmente una cantidad terapéuticamente efectiva de una composición de micropartículas de dextrán cristalizado e insulina al mamífero para reducir el nivel de glucosa sanguínea en el mamífero
- 2 El método de la reivindicación 1, en donde la composición comprende una suspensión acuosa de micropartículas de dextrán cristalizado que tiene un diámetro promedio de 0,5 a 5 micrones y la insulina
- 3 El método de la reivindicación 2, en donde el método comprende administrar oralmente la suspensión a un humano en necesidad de reducir la glucosa sanguínea
- 4 Un método para tratar diabetes en un humano en necesidad de este mediante la administración oral de la suspensión de la reivindicación 2
- 5 El método de la reivindicación 2, en donde las micropartículas son micropartículas porosas las

cuales son cristalizadas antes de agregar la insulina a la suspensión, de tal forma que la insulina se ponga en contacto con la superficie de la micropartículas o en los poros de las micropartículas

- 6 El método de la reivindicación 2, en donde la cantidad terapéuticamente efectiva de insulina varía de 10 a 2 500 UL de insulina recombinante humana para 1 gramo de la suspensión
- 7 El método de la reivindicación 2, en donde la concentración de glucosa sanguínea se reduce al menos al 5 por ciento 60 minutos después de administrar la suspensión al mamífero
- 8 El método de la reivindicación 7, en donde la concentración de glucosa en el mamífero se reduce del 30 por ciento al 60 por ciento 60 minutos después de la administración de la suspensión al mamífero
- 9 El método de la reivindicación 2, en donde la concentración de la glucosa en el mamífero se reduce el 5 por ciento 30 minutos después de administrar la suspensión al mamífero

10 El método de la reivindicación 2, en donde la concentración de glucosa en el mamífero se reduce el 10 por ciento durante un periodo de 30 a 240 minutos después de la administración de la suspensión al mamífero

11 El método de la reivindicación 2, en donde la concentración de glucosa en el mamífero se reduce durante un periodo entre 30 minutos a 120 minutos después de la administración de la suspensión al mamífero

12 El método de la reivindicación 1, 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 son particionadas selectivamente en la fase dextrán, y

La composición comprende una estructura que comprende una fase dispersada de PEG y una fase dextrán

100

continua cuando la composición está presente en el cuerpo del mamífero

- 13 El método para reducir la glucosa sanguínea en el mamífero, comprende una administración oral de una composición que comprende una cantidad de insulina terapéuticamente efectiva y un material matriz al mamífero para reducir el nivel de glucosa sanguínea en el mamífero por lo menos 5 por ciento 60 minutos después de administrar la suspensión al mamífero
- 14 El método de la reivindicación 13, donde la composición comprende una suspensión acuosa y el material matriz comprende micropartículas de dextrán cristalizadas que tienen un diámetro promedio de cerca de 0,5 a 5 micrones
- 15 El método de la reivindicación 14, en donde las micropartículas son micropartículas porosas las cuales son cristalizadas antes de agregar la insulina a la suspensión, de tal forma que la insulina se ponga en contacto con la superficie de la micropartículas o en los poros de las micropartículas

101

- 16 El método de la reivindicación 13, en donde el método comprende suministrar oralmente la composición a un humano que en necesidad de reducir la glucosa sanguínea
- 17 Un método para tratar diabetes en un humano que lo necesita mediante administración oral de la composición de reivindicación 16
- 18 El método de la reivindicación 13, en donde la cantidad terapéuticamente efectiva de insulina varía de 10 a 2 500 UL de insulina recombinante humana por 1 gramo de la suspensión
- 19 El método de la reivindicación 13, en donde la concentración de glucosa en el mamífero se reduce del 30 por ciento al 60 por ciento 60 minutos después de la administración de la suspensión al mamífero
- 20 El método de la reivindicación 13, en donde la concentración de la glucosa en el mamífero se reduce el 5 por ciento 30 minutos después de administrar la suspensión al mamífero

21 El método de la reivindicación 13, en donde la concentración de glucosa en el mamífero se reduce el 10 por ciento durante un periodo de 30 a 240 minutos después de la administración de la suspensión al mamífero

22 El método de la reivindicación 13, en donde la concentración de glucosa en el mamífero se reduce durante un periodo entre 30 minutos a 120 minutos después de la administración de la suspensión al mamífero

23 El método de la reivindicación 13, en donde la composición está en forma de una tableta o cápsula

24 El método de la reivindicación 13, en donde

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

25 Un método de administrar una suspensión a un mamífero, comprende administrar oralmente una suspensión acuosa de micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina al mamífero

26 Una composición farmacéuticamente dosificada, comprende

micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina en donde la composición es dosificada por administración oral al humano

27 La composición de la reivindicación 26, en donde

Las micropartículas de dextrán cristalizado comprenden moléculas de dextrán unidas por enlaces de hidrógeno, las fuerzas de Van Der Waals y/o

104

enlaces iónicos y que no tienen sustancialmente enlaces covalentes entre las moléculas de dextrán y

Las micropartículas de dextrán cristalizado son micropartículas porosas que tienen un diámetro promedio de 0,5 a 5 micrones y, de modo que la insulina es puesta en contacto con la superficie de las Las micropartículas o en los poros de las Las micropartículas

- 28 La composición de la reivindicación 26, en donde la composición comprende una suspensión acuosa de micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina
- 29 La composición de la reivindicación 26, en donde la composición es envasada en una cantidad dosificada para una simple administración oral a un humano
- 30 La composición de la reivindicación 26, en donde la composición es ubicada en un frasco con instrucciones impresas en el frasco o incluidas con el frasco para una administración de dosis oral en humano

31 La composición de la reivindicación 26, en donde la composición comprende una tableta que contiene un medio transportador farmacéuticamente aceptable, las micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina

32 La composición de la reivindicación 26, en donde la composición comprende una cápsula que contiene una concha farmacéuticamente aceptable, las micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina

33 La composición de la reivindicación 26, en donde

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

La composición es adaptada para formar una suspensión estructurada que comprende una fase dispersa PEG y una fase continua de dextrán

34 Un kit de composición farmacéutica, comprende

Una suspensión acuosa de micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina ubicada en un frasco y

Instrucciones para administración oral de la composición a un humano en necesidad de este

35 Un kit de composición farmacéutica, comprende

Unos primeros medios para administrar oralmente a un mamífero para reducir la glucosa sanguínea del mamífero por lo menos 30 por ciento 60 minutos después de la administración de la suspensión al mamífero, y

Un frasco de almacenamiento que contiene los primeros medios

36 Una tableta que comprende un medio portador farmacéuticamente aceptable, micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina

37 Una cápsula que contiene una concha farmacéuticamente aceptable, micropartículas de dextrán cristalizado y una cantidad terapéuticamente efectiva de insulina

38 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

39 El método de la reivindicación 38, 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

40 El método de la reivindicación 39, en donde

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 comprenden una estructura que comprende una fase PEG dispersante y una fase continua de dextrán cuando la composición está presente el cuerpo del mamífero

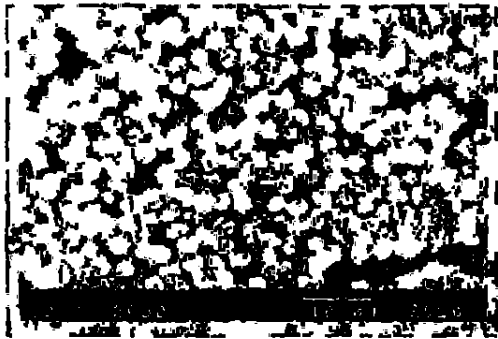


FIG 1

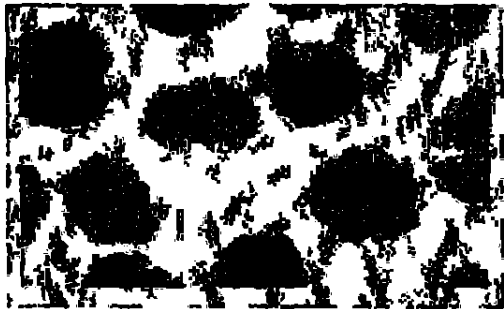


FIG 2A



FIG 2B

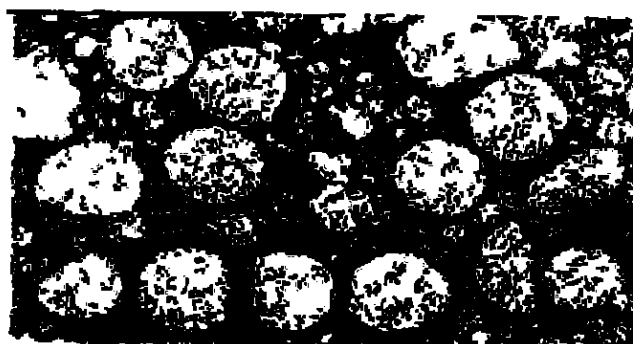


FIG 3



FIG 4



FIG 5



FIG 6

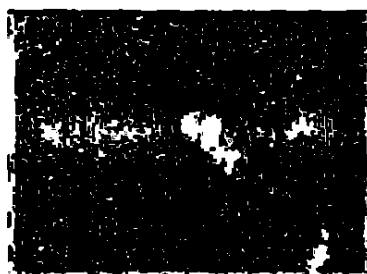


FIG 7

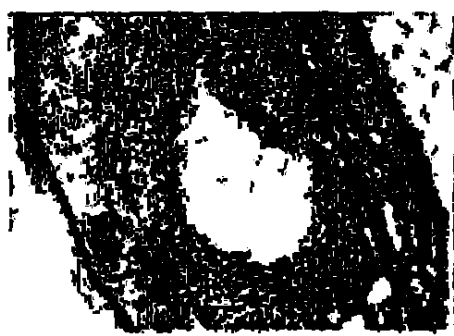


FIG 8

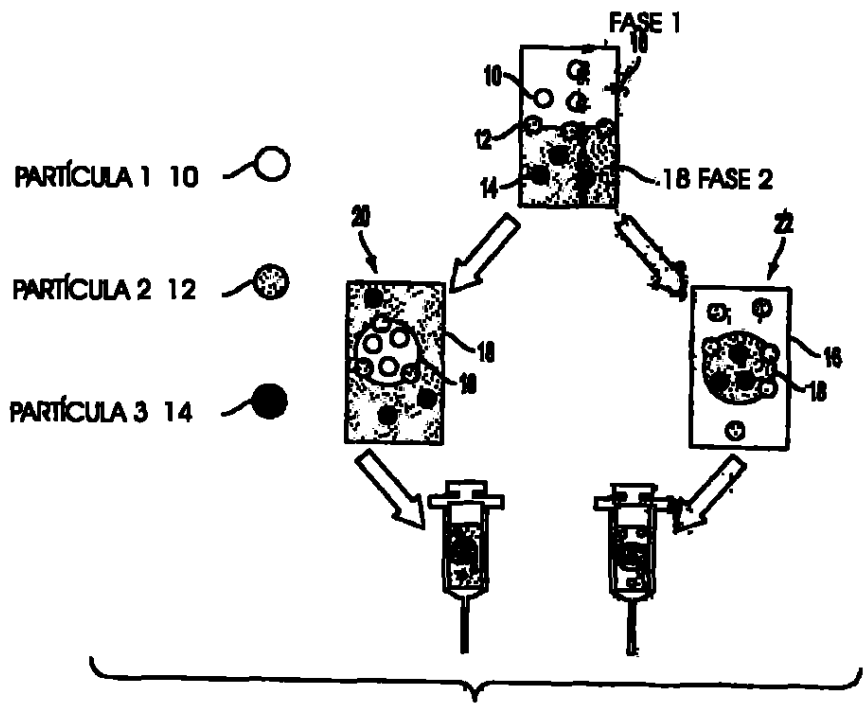


FIG 9A

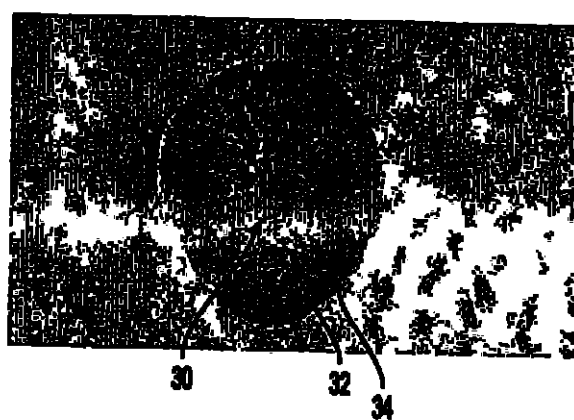


FIG 9B

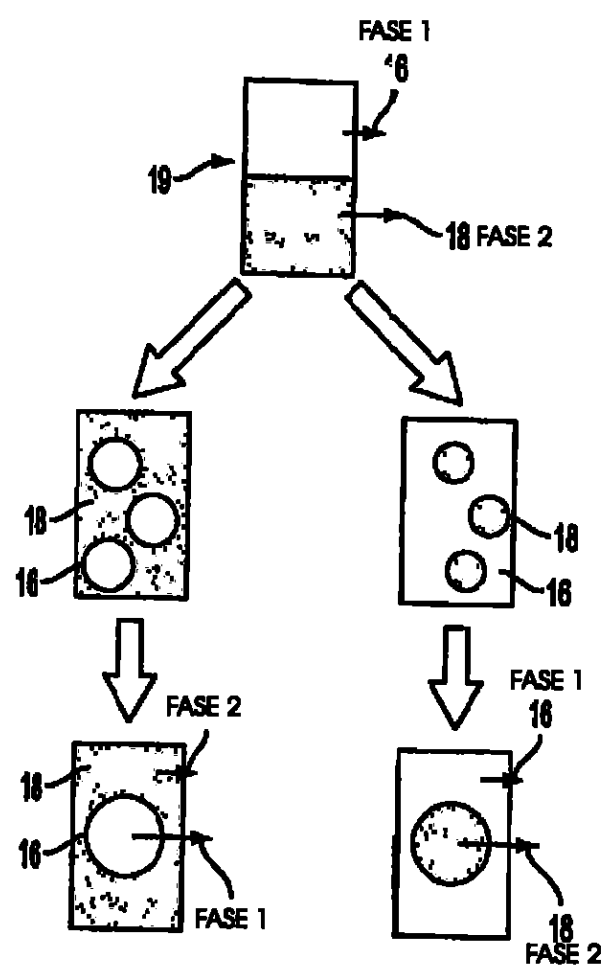
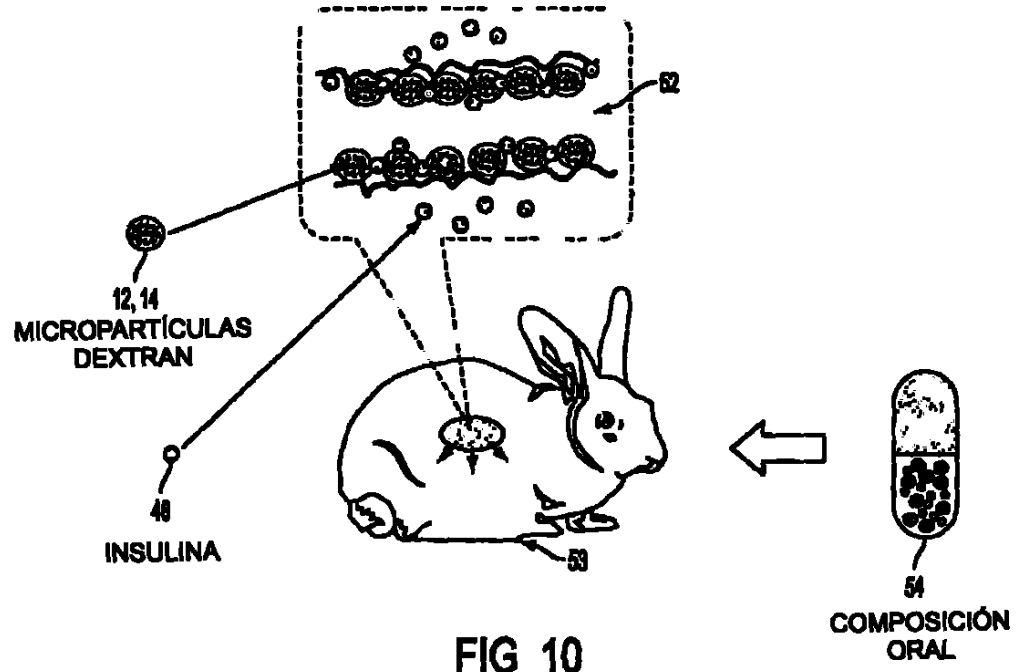


FIG 9C



116

PCT/IB2004/000933

IPC 7 A61K38/28 A61K47/36 A61K9/00

B. FIELDS SEARCHED

IPC 7 A61K

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

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	26-32 34, 38, 39
Y	column 6 claims 1, 4, 7 example 13 — —/—	1-25, 35-37

X Patient family members are listed in annex.

- 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

"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.

'8' document number of the same serial family

Date of mailing of the international search report

21/07/2004

Authorized officer

Bochalen, D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2004/000933

G.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages /	Relevant to claim No.
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	26-34
Y	page 238, column 1	1-34, 36-40
X	US 4 963 526 A (ECANOW BERNARD) 16 October 1990 (1990-10-16) figure 2, example 1	35
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	26-40
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	26-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2004/000933

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 1-25 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/IB2004/000933

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
US 4963526	A	16-10-1990	US 4849405 A	18-07-1989
			EP 0179904 A1	07-05-1986
			IL 75263 A	05-11-1990
			US 4914084 A	03-04-1990
			WO 8505029 A1	21-11-1985
			US 4963367 A	16-10-1990
EP 1184032	A	06-03-2002	EP 1184032 A1	06-03-2002
			AU 9437601 A	13-03-2002
			WO 0217884 A1	07-03-2002

PCT
REQUEST

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

Sheet No. 1

297571

120

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)

028093-0111

Box No. I TITLE OF INVENTION **ORAL 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.)

SABETSKY Vladimir
Pialonrevsky Prospekt 10 q.320
105221 Saint-Petersburg
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.)

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

Telephone No.
(202) 672-5490

Facsimile No.
(202) 672-5399

Teleprinter No.

Agent's registration No. with the Office
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(1) to (4) 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:

- (1) 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;
 - (2) 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;
 - (3) 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;
 - (4) 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;
 - (5) 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(c)).

Continuation of Box IV

PAVAN K. AGARWAL
 GEORGE C. BECK
 STEPHEN A. BENT
 BETH A. BURROUS
 ALAN I. CANTOR
 WILLIAM T. ELLIS
 JOHN J. FELDHAUS
 MARY MICHELLE KILE
 KENNETH E. KROSIN
 GLENN LAW
 PETER G. MACK
 STEPHEN B. MARBIUS
 BRIAN J. MC NAMARA
 RICHARD C. PEET
 GEORGE E. QUILLIN
 ANDREW E. RAWLINS
 RICHARD L. SCHWAAB
 MICHELE M. SIMKIN
 HAROLD C. WEGNER

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 ceasing 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 Organization for which that earlier application was filed (Rule 4.10(a)(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

123

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)				

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 description (excluding sequence listings and/or tables related thereto) 30 claims: 7 abstract 1 drawings 7 Sub-total number of sheets 50 Sequence listings tables related thereto (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) (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 (Not to each document. Indicate the name of the person signing and the capacity in which the person signs of each capacity is not obvious from reading the request.)  Michael Omschinski, 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

For International Bureau use only
Date of receipt of the record copy by the International Bureau:

ORAL 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 oral 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)

[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. Furthermore, Schroder's composition is designed for injection, which is an inconvenient and painful method of administering the composition.

BRIEF SUMMARY OF THE INVENTION

[0005] A method of lowering blood glucose in a mammal includes orally administering 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.

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 intramuscular 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] Figure 10 schematically illustrates therapeutic agent delivery methods according to an embodiment of the present invention

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0018] The present inventor discovered that a composition of porous (i.e., microporous) microparticles such as crystallized dextran microparticles may be used as a vehicle for oral delivery of insulin. The composition may be a one phase composition or a multi-phase composition which forms a structured implant in a mammal.

[0019] The first section below describes the preferred crystallized dextran microparticles, the second section describes formation of the structured implant from a multi-phase composition, and the following sections describe specific examples of oral administration of the composition into mammals and methods of making the oral insulin composition.

A Crystallized Dextran Microparticles

[0020] 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 – 85 % W/W) with molecular weights ranging from 10 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.

[0021] 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.

[0022] 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).

[0023] 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.

[0024] 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.

[0025] 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

[0026] Self-assembled structures based on crystallized dextran microparticles and their aggregates may be formed based on two-phase systems.

[0027] For example, in the case of oil, a special kind of 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

[0028] 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

[0029] 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)

[0030] 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

[0031] 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)

[0032] 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

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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 UI 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). 10 g of the suspension contains 8 UI of insulin.

[0037] 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 into 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 in the two phase system, which may be a 40 to 500 kDa dextran solution, such as a 40 to 75 kDa solution. This is 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.

[0038] 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.

[0039] 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.

[0040] 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

[0041] 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 discrete 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 delivered into a mammal such as an animal or human. Thus the capsule forms a structured, three 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. It should be noted that the structure formed for orally delivered two phase systems may be generally described as a structured suspension comprising a dispersed PEG phase and a continuous dextran phase.

[0042] 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.

[0043] 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.

[0044] 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 Oral Insulin delivery vehicle

[0045] The present inventor discovered that a composition of porous (i.e., microporous) microparticles may be used as a vehicle for oral delivery of insulin. The porous microparticles may be any suitable porous microparticles which enable oral administration of insulin with a significant reduction in blood glucose, such as at least a 5% reduction, for example at least a 30% reduction within 60 minutes of oral administration. Preferably the microparticles are bioadhesive particles such as particles which adhere at least temporarily to mammal intestine walls to allow insulin deliver through the intestine wall. Most preferably the porous microparticles comprise the crystallized dextran microparticles described above.

[0046] In one preferred embodiment of the present invention shown in Figure 10, the present inventor has discovered that an aqueous suspension of crystallized dextran microparticles 12, 14 and insulin 46 orally administered to mammals 53 such as rabbits was about equally as effective in reducing blood glucose levels as an intramuscular injection of insulin alone. Figure 10 schematically illustrates the insulin 46 permeating through mammal 53 intestine 52 walls from the orally administered composition 54 comprising the microparticles. Since rabbits are a common model for humans in drug testing, the present inventor believes that a liquid or solid composition 54 comprising crystallized dextran

microparticles and insulin such as an aqueous suspension a solution a tablet or a capsule, would also be effective in reducing blood glucose levels in human beings when orally administered

[0047] The following examples illustrate oral insulin delivery using crystallized dextran microparticles. The study involved Chinchilla rabbits (2.3 ± 0.2 kg) and the observation of their response to orally administered aqueous suspensions consisting of crystallized dextran microparticles prepared according to the method described herein and human recombinant insulin.

[0048] 3.0 g of Dextran T20 (Pharmacia Uppsala Sweden) was dissolved in 2.0 g of water and placed in box at temperature 60°C . Three hours later crystallized dextran microparticles were washed by centrifugation at 3000 g with 3×5.0 ml of water. Then the crystallized dextran microparticles were suspended in 2.0 ml of water and allowed to dry at room temperature. The resulting dry powder was used to prepare an insulin containing suspension for the oral insulin delivery experiment. Insulin containing suspensions were prepared by the mixing of 250 mg of the microparticles 0.3 ml (12 UI) or 0.6 ml (24 UI) of insulin (NovoNordisk Actrapid HM Penfill 40 UI/ml) and distilled water to reach a volume of 2.0 ml.

[0049] Samples of the suspension (2.0 ml) were introduced into the rabbits' throats with a catheter followed by the introduction of 10.0 ml of drinking water. Animals were not fed for 3 hours before the suspension's introduction. Samples of blood were taken from the rabbit's ear vein 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.

[0050] Examples 1 to 8 are comparative examples involving eight rabbits. Examples 9 to 14 are examples according to the present invention involving five rabbits.

[0051] In comparative examples 1 and 2 (control experiment #1 summarized in Table I) an aqueous solution of human recombinant insulin was introduced intra muscularly into two rabbits at a dose of 12 UI per animal. In comparative examples 3 and 4 (control experiment #2 summarized in Table II) the rabbits remained intact (i.e., no insulin or other injection was provided to the two rabbits). In comparative examples 5 and 6 (control experiment #3 summarized in Table III) a suspension of the crystallized dextran microparticles without insulin was provided orally to two rabbits. In comparative examples 7 and 8 (control experiment #4 summarized in Table IV) a suspension of the commercially obtained Sephadex G 150 microparticles with insulin (24 UI) was provided orally to two rabbits. According to the Amersham Biosciences website, Sephadex® G-150 microparticles are beaded gel microparticles having a diameter of 20 to 150 microns prepared by cross linking dextran with epichlorohydrin. In examples 9-14 according to a preferred embodiment of the present invention (summarized in Table V), a suspension of crystallized dextran microparticles with insulin (24 UI) was provided orally to five rabbits. The results are summarized in Tables I-V below.

Table I

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#1	12 UI i.m.	91	68	58	49	51
#2	12 UI i.m.	87	65	64	57	58

Table II

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#3	0 0	98	87	87	89	86
#4	0 0	88	90	91	94	87

Table III

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#5	0 0	92	99	94	95	92
#6	0 0	90	93	93	93	92

Table IV

Rabbit/ Example #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#7	24 UI per os	85	82	86	81	83
#8	24 UI per os	84	75	86	76	77

Table V

Rabbit/ Experiment #	Insulin dose	0 min mg/dl	30 min mg/dl	60 min mg/dl	90 min mg/dl	120 min mg/dl
#9	24 UI per os	94	68	59	58	57

#10	24 UI per os	93	64	52	54	52
#11	24 UI per os	78	52	51	49	48
#12	24 UI per os	92	64	52	53	47
#13	24 UI per os	89	53	48	38	49
#14	24 UI per os	97	60	38	54	52

[0052] The data in Tables I-V show that average reduction of sugar (i.e. glucose) in the blood of mammals is comparable when 12 UI of insulin is administered by intramuscular injection (examples 1-2) and 24 UI of insulin is administered per os (i.e. orally) with crystallized dextran microparticles (examples 9-14). The maximum glucose reduction was about 35 to about 60 percent at 60 min after oral administration. The concentration profile of glucose is practically the same in both the injection and oral delivery modes. It should be noted that other amounts of insulin may be administered. For example, 30 UI of insulin may be administered. In general, oral administration of two to three times the insulin compared to the amount of injected insulin produces a similar drop in blood sugar for up to three hours.

[0053] It is a well known fact that insulin by itself is degraded by intestinal enzymes and is not absorbed intact across the gastrointestinal mucosa (Amidon GL, Lee HJ. Absorption of peptide and peptidomimetic drugs. Ann Rev Pharmacol Toxicol 1994, 34: 321-41). However, examples 9-14 show that crystallized dextran microparticles can be used as a vehicle for oral delivery of proteins such as insulin because the

hypoglycemia effect received was significant. Without wishing to be bound by a particular theory or mode of action, the present inventor believes that the use of the porous crystallized dextran microparticles as an insulin delivery matrix in an aqueous suspension protected the insulin from significant degradation by intestinal enzymes and allowed the insulin to be absorbed intact across the gastrointestinal mucosa. The insulin may be located in micropores in the microporous microparticles and/or in macropores between the microparticles. In contrast, the use of cross-linked Sephadex G 150 dextran microparticles with insulin (Table IV, examples 7-8) did not produce an appreciable reduction in blood glucose concentration.

[0054] As provided in examples 9-14, the blood glucose concentration in the mammal is lowered by at least 5 percent, preferably at least 30 percent, 60 minutes after administering the composition containing the crystallized dextran microparticles and insulin to the mammal (i.e., the blood glucose value in the mammal at 60 minutes after administration of the suspension is at least 5 percent, preferably at least 30 percent lower than that measured right before administration of the suspension). Preferably, the blood glucose concentration in the mammal is lowered by at least 5 percent, such as at least 30 percent, preferably by about 35 to about 40 percent, 30 minutes after administering the composition to the mammal. Preferably, the blood glucose concentration in the mammal is lowered by about 35 to about 60 percent, for example 35 to 45 percent, 60 minutes after administering the suspension to the mammal. More preferably, the blood glucose concentration in the mammal is lowered during the entire period ranging from 30 to 240 minutes, such as 30 to 120 minutes, after administering the composition to the mammal compared to the blood glucose concentration right before administration. For example, the blood glucose concentration in the

mammal is preferably lowered by at least 10 percent preferably at least 30 percent more preferably by at least 35 percent such as by 35 to 45 percent during a period ranging from 30 to 240 minutes, preferably 30 to 120 minutes after administering the composition to the mammal

[0055] Thus a preferred embodiment of the present invention provides a method of lowering blood glucose in a mammal by orally administering a therapeutically effective amount of a composition comprised of crystallized dextran microparticles and insulin A "therapeutically effective" amount of the compositions can be determined by prevention or amelioration of adverse conditions or symptoms of diseases, injuries or disorders being treated Preferably, the composition comprises an aqueous suspension of crystallized dextran microparticles having an average diameter of about 0.5 to about 5 microns and insulin Furthermore the microparticles are preferably porous microparticles which are crystallized prior to adding the insulin to the suspension, such that the insulin is located in contact with a surface of the microparticles and/or in pores of the microparticles

[0056] 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

[0057] While a one phase composition 54 comprising insulin and microparticles is illustrated in Figure 10 a two phase composition described above and illustrated in Figures 7, 8, 9A, 9B and 9C may also

be used. For example, a two phase composition comprising a dextran phase and a PEG phase, crystallized dextran microparticles and insulin may be used. In vivo, the composition has a self assembled capsule structure comprising a crystallized dextran microparticle containing wall or shell and a PEG and insulin containing core.

[0058] Preferably, the mammal receiving the oral administration of insulin comprises a human in need of lowering blood glucose, such as a human suffering from diabetes. Thus, the preferred embodiment of the present invention provides a method of treating diabetes in a human in need of the treatment by orally administering the suspension of insulin and crystallized dextran microparticles described above.

[0059] Any therapeutically effective amount of insulin may be administered to the mammal. The amount of insulin may vary based on the type of mammal (i.e., human or rabbit), the weight of the mammal, the composition of the suspension, the amount of desired reduction of blood glucose and other factors. One non-limiting example of insulin content in the suspension is about 10 UI to about 2,500 UI of human recombinant insulin per one gram of suspension, such as about 12 UI to about 30 UI, such as 24 UI of human recombinant insulin. However, this amount may vary as desired.

[0060] The present invention should not be considered limited to the preferred embodiments described above. Other matrix material may be used for oral insulin delivery, such as organic or inorganic microporous particles. Preferably, the particles are microparticles which enhance insulin penetration through gastrointestinal mucosa and/or which stabilize the composition. Furthermore, while the suspension preferably contains only water solvent, a matrix and an insulin solution or suspension, the delivery system may also contain additional materials. For example, the

composition may contain a second phase such as the PEG phase of a two phase system. Thus another preferred aspect of the present invention includes a method of lowering blood glucose in a mammal comprising of orally administering a composition comprising a therapeutically effective amount of insulin and a matrix material to the mammal to lower blood glucose of the mammal by at least 30 percent 60 minutes after administering the suspension to the mammal. In another preferred aspect of the present invention, a method of administering a suspension to a mammal comprised of orally administering an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin to the mammal.

[0061] As noted above the crystallized dextran microparticles used as a matrix material for oral administration of insulin or other protein based drugs may be made by any suitable method (see Figure 1 for example). Preferably the microparticles are made by the process of any of the preferred embodiments described herein. Preferably but not necessarily the microparticles are formed in an aqueous solution without using an organic solvent. Thus, in a preferred aspect of the present invention a therapeutically effective amount of insulin and the crystallized dextran microparticles are combined in water after the microparticles have been crystallized to form an aqueous suspension of insulin and crystallized dextran microparticles. The microparticles may be added to the water before, at the same time and/or after adding the insulin to the water. Furthermore, the microparticles may be orally administered to a mammal in the solvent in which they were formed. Alternatively they may be removed from the solvent in which they were formed and placed into water or other aqueous solutions for oral administration or dried and provided in solid form such as tablet or capsule for oral administration.

[0062] The aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin (or other suitable suspensions of insulin and a matrix material such as a suspension of insulin and microporous microparticles) is preferably provided as a dosed pharmaceutical composition which is dosed for oral administration to a human. In one preferred aspect of the present invention, the composition is located in a vessel in an amount dosed for a single oral administration to a human. The vessel may comprise any container which may hold a suspension such as a plastic or glass bottle, a tube, a dropper, a spray nozzle, a pouch and/or other suitable vessels. This vessel contains an amount of suspension sufficient for a single oral dose of the composition.

[0063] In another preferred aspect of the present invention, the composition is located in any suitable vessel in an amount suitable for multiple oral administration doses. The vessel contains an instruction for oral dosage administration to a human. The instruction may be printed on the vessel such as being as printed directly on the vessel or on a label attached to the vessel or enclosed with the vessel such as being printed on a sheet of paper enclosed with the vessel in a cardboard box or in a pharmacy envelope. The instructions may describe the amount of the composition that should be taken with each dose, the frequency that the dose should be taken, how to measure the dose of the composition for oral administration and/or any other suitable oral drug instructions for an administering health care practitioner and/or a patient in need of the drug. Alternatively, the instructions may comprise directions for electronically or audibly accessing the dosing and administration instructions, such as a link to a website containing the instructions or a telephone number or recording where the instructions are provided audibly.

[0064] In another preferred aspect of the present invention the aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin located in a vessel are provided in a pharmaceutical composition kit with instructions for oral administration of the composition to a human in need thereof. The kit may comprise instructions printed on the vessel or on a label attached to the vessel or a sheet of paper enclosed with the vessel such as in a cardboard box or pharmacy envelope including a bottle (i.e. vessel) and the sheet of instructions.

[0065] It should be noted that the composition for oral administration may be in the form of an aqueous suspension but other delivery forms may be used to lower the blood glucose in a mammal. For example, the porous crystallized dextran microparticles and the insulin may be orally administered in the form of a tablet or a capsule.

[0066] To orally administer the composition in solid form to a mammal such as a human, the solution of crystallized dextran microparticles and insulin is first dried such as freeze dried to form a powder. The powder may then be compressed into a tablet, along with optional pharmaceutically acceptable excipients or placed into a pharmaceutically acceptable capsule.

D Materials

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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

[0071] 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.

[0072] 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

151

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.

[0073] 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

[0074] 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

[0075] 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

[0076] 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.

[0077] 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.

[0078] 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 a 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 orally.

[0079] Preferably the composition which includes the microparticles and the therapeutic agent is a flowable colloidal system. Examples of flowable colloidal systems include emulsions and suspensions. 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

[0080] 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.

[0081] 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.

[0082] 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.

[0083] All of the publications and patent applications and patents cited in this specification are herein incorporated in their entirety by reference.

I claim

- 1 A method of lowering blood glucose in a mammal comprising orally administering a therapeutically effective amount of a composition comprising crystallized dextran microparticles and insulin to the mammal to lower blood glucose of the mammal**
- 2 The method of claim 1 wherein the composition comprises an aqueous suspension of crystallized dextran microparticles having an average diameter of about 0.5 to about 5 microns and the insulin**
- 3 The method of claim 2 wherein the method comprises orally administering the suspension to a human in need of lowering blood glucose**
- 4 A method of treating diabetes in a human in need thereof by orally administering the suspension of claim 2**
- 5 The method of claim 2 wherein the microparticles are porous microparticles which are crystallized prior to adding the insulin to the suspension, such that the insulin is located in contact with a surface of the microparticles or in pores of the microparticles**
- 6 The method of claim 2, wherein the therapeutically effective amount of insulin ranges from 10 to 2,500 UI of human recombinant insulin per 1 gram of the suspension**
- 7 The method of claim 2, wherein the blood glucose concentration in the mammal is lowered by at least 5 percent 60 minutes after administering the suspension to the mammal**

8 The method of claim 7, wherein the blood glucose concentration in the mammal is lowered by about 30 percent to about 60 percent 60 minutes after administering the suspension to the mammal

9 The method of claim 2 wherein the blood glucose concentration in the mammal is lowered by at least 5 percent 30 minutes after administering the suspension to the mammal

10 The method of claim 2 wherein the blood glucose concentration in the mammal is lowered by at least 10 percent during a period ranging from 30 minutes to 240 minutes after administering the suspension to the mammal

11 The method of claim 2 wherein the blood glucose concentration in the mammal is lowered during a period ranging from 30 minutes to 120 minutes after administering the suspension to the mammal

**12 The method of claim 1 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 comprises a structure comprising a dispersed PEG phase and a continuous dextran phase when the composition is present in the mammal body**

13 A method of lowering blood glucose in a mammal comprising orally administering a composition comprising a therapeutically effective amount of insulin and a matrix material to the mammal to lower blood glucose of the mammal by at least 5 percent 60 minutes after administering the suspension to the mammal

14 The method of claim 13, wherein the composition comprises an aqueous suspension and the matrix material comprises crystallized dextran microparticles having an average diameter of about 0.5 to about 5 microns

15 The method of claim 14, wherein the microparticles are porous microparticles which are crystallized prior to adding the insulin to the suspension such that the insulin is located in contact with a surface of the microparticles or in pores of the microparticles

16 The method of claim 13 wherein the method comprises orally administering the composition to a human in need of lowering blood glucose

17 A method of treating diabetes in a human in need thereof by orally administering the composition of claim 16

18 The method of claim 13 wherein the therapeutically effective amount of insulin ranges from 10 to 2,500 UI of human recombinant insulin per 1 gram of suspension

19 The method of claim 13 wherein the blood glucose concentration in the mammal is lowered by about 30 percent to about 60 percent 60 minutes after administering the composition to the mammal

20 The method of claim 13 wherein the blood glucose concentration in the mammal is lowered by at least 5 percent 30 minutes after administering the composition to the mammal

21 The method of claim 13 wherein the blood glucose concentration in the mammal is lowered by at least 10 percent during a period ranging from 30 minutes to 240 minutes after administering the composition to the mammal

22 The method of claim 13 wherein the blood glucose concentration in the mammal is lowered during a period ranging from 30 minutes to 120 minutes after administering the composition to the mammal

23 The method of claim 13 wherein the composition is in a form of a tablet or a capsule

**24 The method of claim 13 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 comprises a structure comprising a dispersed PEG phase and a continuous dextran phase when the composition is present in the mammal body**

25 A method of administering a suspension to a mammal comprising orally administering an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin to the mammal

26 A dosed pharmaceutical composition comprising crystallized dextran microparticles and a therapeutically effective amount of insulin wherein the composition is dosed for oral administration to a human

**27 The composition of claim 26 wherein
the crystallized dextran microparticles comprise dextran molecules held together by hydrogen bonds Van Der Waals forces or ionic bonds and having substantially no covalent bonds between dextran molecules and**

159

the crystallized dextran microparticles are porous microparticles having an average diameter of about 0.5 to about 5 microns and such that the insulin is located in contact with a surface of the microparticles or in pores of the microparticles

28 The composition of claim 26 wherein the composition comprises an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin

29 The composition of claim 26, wherein the composition is located in a vessel in an amount dosed for a single oral administration to a human

30 The composition of claim 26 wherein the composition is located in a vessel with instruction printed on the vessel or enclosed with the vessel for oral dosage administration to a human

31 The composition of claim 26 wherein the composition comprises a tablet comprising a pharmaceutically acceptable carrier medium the crystallized dextran microparticles and the therapeutically effective amount of insulin

32 The composition of claim 26, wherein the composition comprises a capsule comprising a pharmaceutically acceptable shell the crystallized dextran microparticles and the therapeutically effective amount of insulin

33 The composition of claim 26 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 is adapted to form a structured suspension comprising a dispersed PEG phase and a continuous dextran phase

34 A pharmaceutical composition kit comprising an aqueous suspension of crystallized dextran microparticles and a therapeutically effective amount of insulin located in a vessel and instructions for oral administration of the composition to a human in need thereof

35 A pharmaceutical kit, comprising a first means for orally administering to a mammal to lower blood glucose of the mammal by at least 30 percent 60 minutes after administering the suspension to the mammal and a storage vessel containing the first means

36 A tablet comprising a pharmaceutically acceptable carrier medium crystallized dextran microparticles and a therapeutically effective amount of insulin

37 A capsule comprising a pharmaceutically acceptable shell, crystallized dextran microparticles and a therapeutically effective amount of insulin

38 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 oral administration to a mammal

**39 The method of claim 38, 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**

**40 The method of claim 39 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 comprises a structure comprising a dispersed PEG
phase and a continuous dextran phase when the composition is present in
the mammal body**

ABSTRACT

A method of lowering blood glucose in a mammal includes orally administering 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.

4

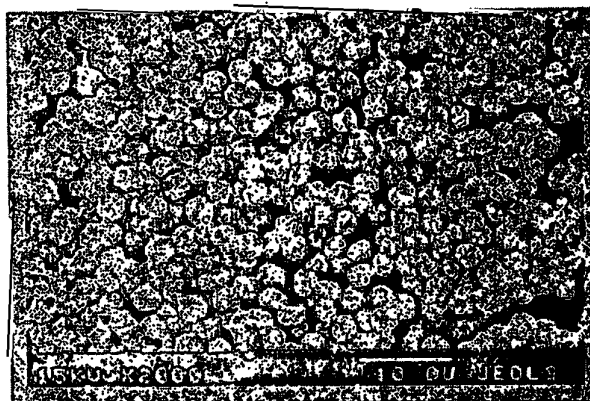


FIG. 1



FIG. 2A

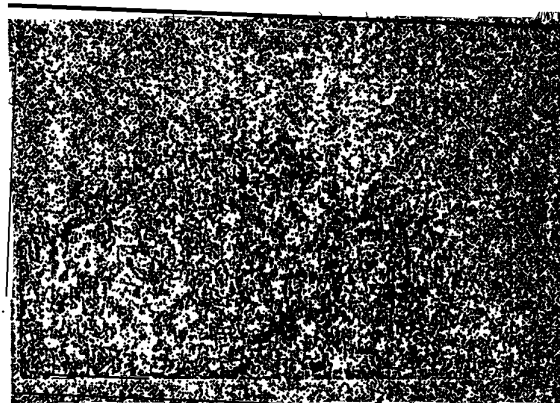


FIG. 2B

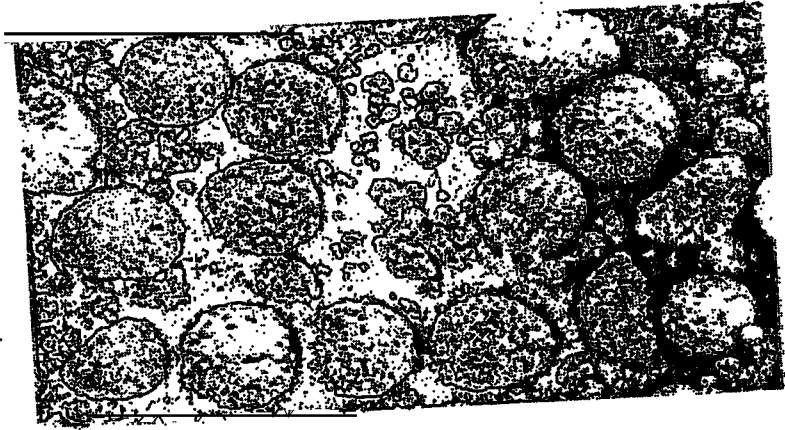


FIG. 3

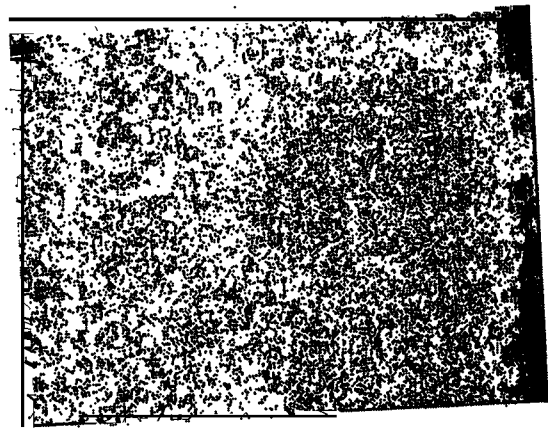


FIG. 4

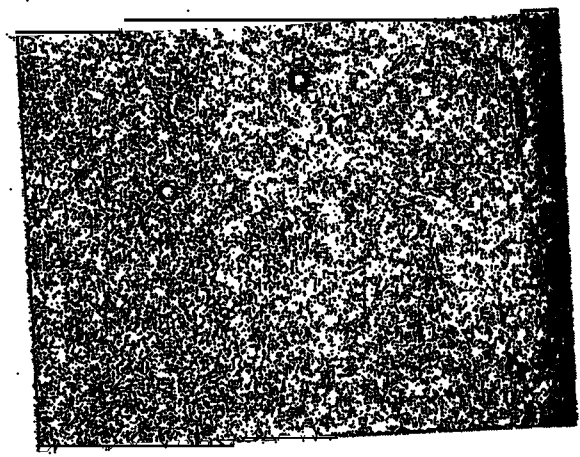


FIG. 5

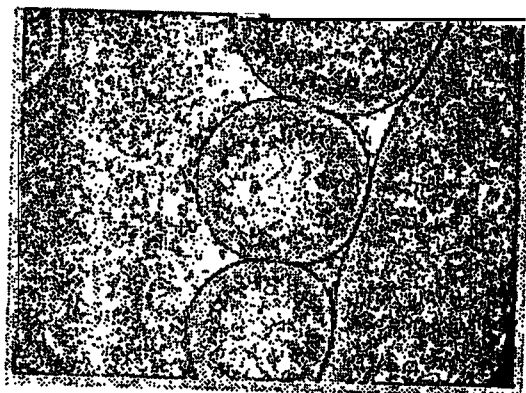


FIG. 6



FIG. 7



FIG. 8

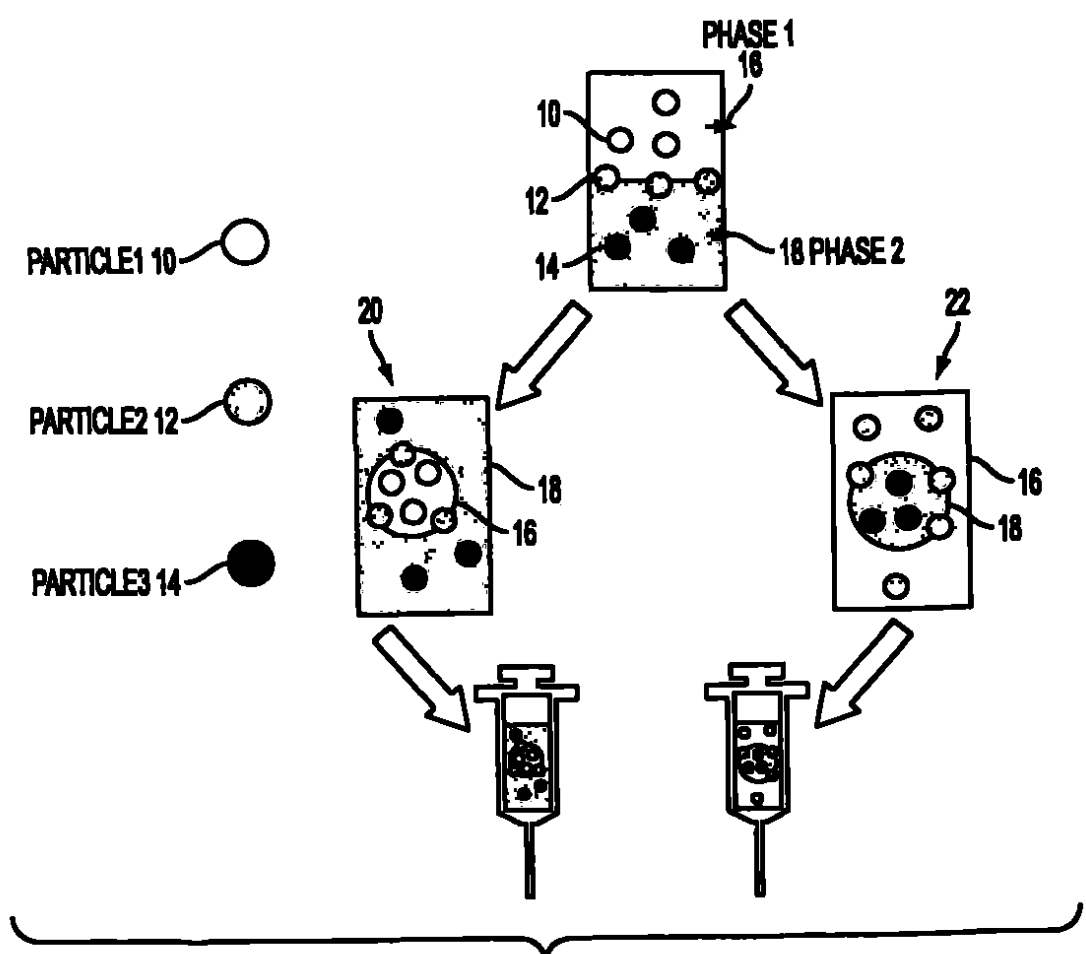


FIG 9A

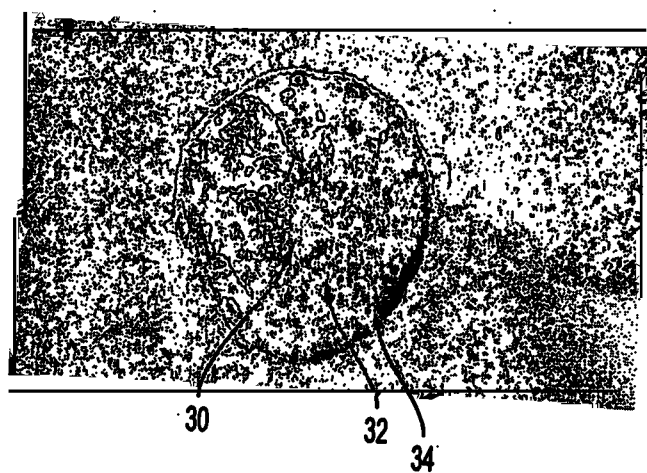


FIG. 9B

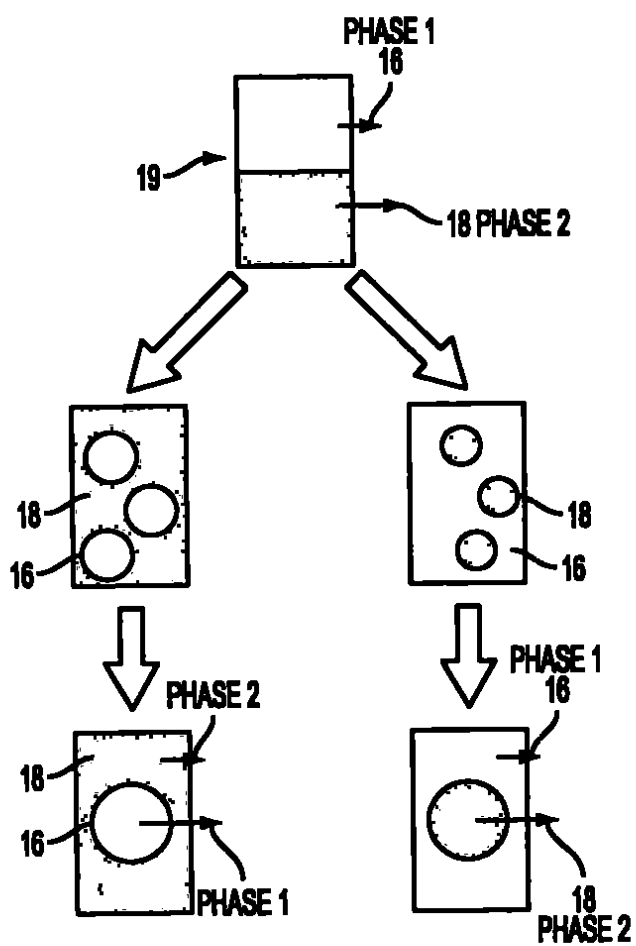


FIG 9C

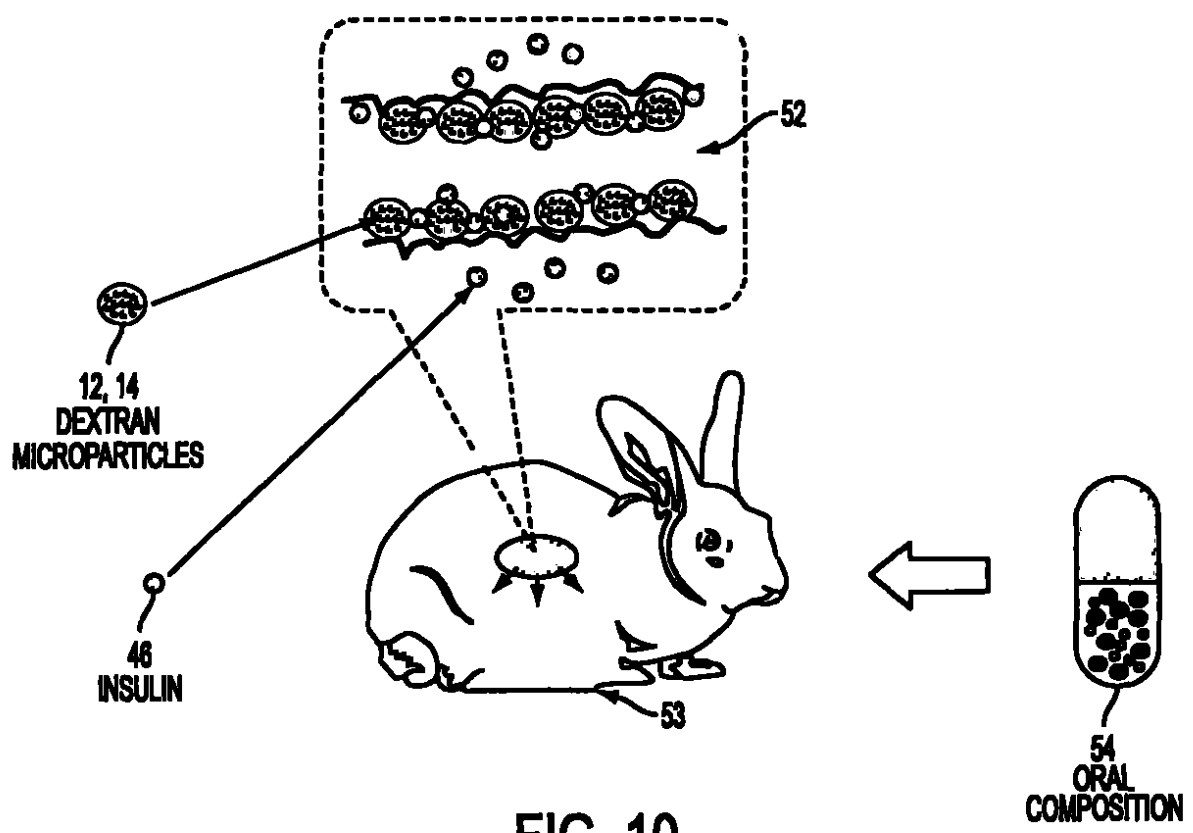


FIG 10

(19) World Intellectual Property
Organization
International Bureau



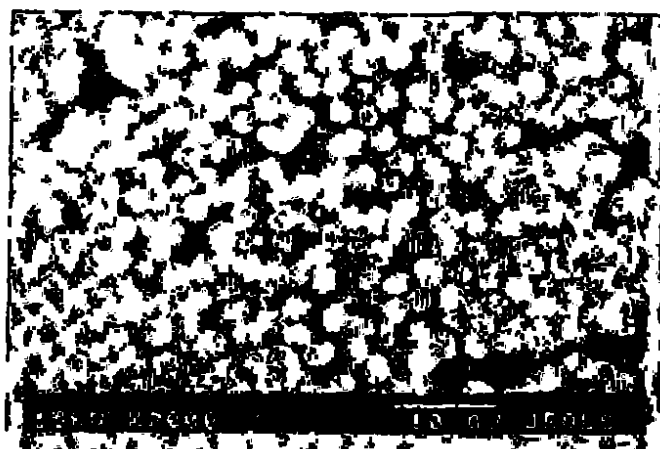
(43) International Publication Date
16 September 2004 (16.09.2004)

PCT

(10) International Publication Number
WO 2004/078196 A1

- (51) International Patent Classification⁷ A61K 38/28, (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, GR, 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, OM, 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
- (21) International Application Number: PCT/IB2004/000933
- (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
- (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)
- (71) Applicant (for all designated States except US): THE TECHNOLOGY DEVELOPMENT COMPANY LTD. [RU/—] Reid House, 31 Church Street, Hamilton HM FX (BM)
- 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
- (72) Inventor; and
(75) Inventor/Applicant (for US only): SIBETSKY, Vladimir [RU/RU] Plakarevsky Prospekt 10, q 320, 105221 Saint Petersburg (RU)
- 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: ORAL INSULIN COMPOSITION AND METHODS OF MAKING AND USING THEREOF



(57) Abstract. A method of lowering blood glucose in a mammal includes orally administering 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.

WO 2004/078196 A1

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

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

To:

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

(PCT Rule 44.1)

Date of mailing
(day/month/year)

21/07/2004

Applicant's or agent's file reference
028093-0111

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/IB2004/000933

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 18:

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) 340-3018

Authorized officer

Sandrine Polenzani

NOTES TO FORM PCT/ISA/220

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 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.

123

NOTES TO FORM PCT/ISA/220 (continued)

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 48.4)

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.

Consequence 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).

Consequence 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.

137

PATENT COOPERATION TREATY
PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 028093-0111	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, Item 5 below	
International application No. PCT/IB2004/000933	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.

INTERNATIONAL SEARCH REPORT

national Application No

PCT/IB2004/000933

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	26-32, 34, 38, 39
Y	column 6, claims 1 4, 7, example 13 — -/-	1-25, 35-37

☒ 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 relating 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
- Z 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. 6618 Patentplan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Bochelen D

INTERNATIONAL SEARCH REPORT

national Application No
PCT/IB2004/000933

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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	26-34
Y	page 238, column 1	1-34, 36-40
X	US 4 963 526 A (ECANOW BERNARD) 16 October 1990 (1990-10-16) figure 2, example 1	35
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	26-40
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	26-40

17711

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2004/000933

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 1-25 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.

INTERNATIONAL SEARCH REPORT

Information on patent family members

National Application No

PCT/IB2004/000933

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
US 4963526	A	16-10-1990	US 4849405 A	18-07-1989
			EP 0179904 A1	07-05-1986
			IL 75263 A	05-11-1990
			US 4914084 A	03-04-1990
			WO 8505029 A1	21-11-1985
			US 4963367 A	16-10-1990
EP 1184032	A	06-03-2002	EP 1184032 A1	06-03-2002
			AU 9437601 A	13-03-2002
			WO 0217884 A1	07-03-2002

179

PCT

see form PCT/ISA/220

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

Date of mailing
(day/month/year) see form PCTASA210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA220

FOR FURTHER ACTION
See paragraph 2 below

International application No
PCT/IB2004/000933

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, A61K47/96, 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

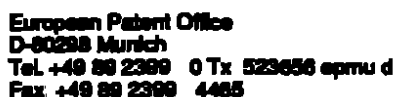
If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 68 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/ISA220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/SA220

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

Name and mailing address of the ISA



Authorized Officer

Bochelen, D

Telephone No. +49 89 2389-8150



180

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No
PCT/IB2004/000933

Box No. I 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/IB2004/000933

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/IB2004/000933

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. 1-25

because

☒ the said international application, or the said claims Nos. 1-25 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**

International application No
PCT/IB2004/000933

**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 3 17,25,36-38
	No	Claims	26 34-35
Inventive step (IS)	Yes	Claims	
	No	Claims	1-40
Industrial applicability (IA)	Yes	Claims	26-40
	No	Claims	

2 Citations and explanations

see separate sheet

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

International application No

PCT/IB2004/000933

Re Item III

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

- 1 Claims 1-25 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 Prior art

The following documents are referred to in this communication. The numbering will be adhered to in the rest of the procedure.

- D1 US 4 713 249 A (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 US 4 963 526 A (ECANOW BERNARD) 16 October 1990 (1990-10-16)
- D4 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
- D5 EP 1 184 032 A (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 and a process for preparing

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

International application No

PCT/IB2004/000933

the microspheres

Document D2 discloses heterogenous dextran hydrogels containing PEG and Insulin for controlled Insulin release

Document D3 discloses an insulin composition for oral administration comprising a polymeric carrier

Document D4 discloses a process for the preparation of crystallized dextran microspheres by physical cross-linking of aqueous dextran solutions

Document D5 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 The subject matter of claims 26 is anticipated by documents D1 and D2 which disclose microspheres of crystallized dextran and insulin which appear to be suitable for oral administration
- 3 2 The instructions provided with the product of claim 34 are not a characterizing feature. The subject matter of claim 34 is thus anticipated by D1 D2 which disclose compositions comprising crystallized dextran microspheres and insulin
- 3 3 The subject-matter of claim 35 is anticipated by document D3 which discloses oral compositions comprising insulin

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

- 4 1 Document D1-D2 discloses microspheres of crystallized dextran and insulin for prolonged release. The use of such compositions for lowering blood glucose (claims 1 and 13), treating diabetes (claim 17) or for orally administering Insulin (claim 25) would be obvious for a skilled person. Said claims do thus not involve an inventive step.
The dependent claims 2-12 14-16 and 18 24 do not contain a feature which taken into combination with the claims to which they refer would be inventive

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

International application No.

PCT/IB2004/000933

- 4 2 It would be obvious for a skilled person to produce a tablet or a capsule with the microspheres disclosed in D1 and D2. Claims 36 and 37 are not inventive
- 4 3 The process of claim 38 is an obvious alternative to the process disclosed in D1 and D2
- 5 Industrial applicability (Art. 33(1)(4) PCT)

For the assessment of the present claims 1-25 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.

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

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 William H. Hume

My Commission Expires _____

(Seal)

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

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

190

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 Wendy H. Hagan

My Commission Expires _____

(Seal)

My Commission Expires
My Commission Expires
August 14, 2008

191

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

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

192

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

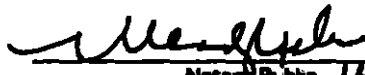
Executed this 19 day of February 2004



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 WENDY HUBER

My Commission Expires _____

(Seal)

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 028093-0106) 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

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

12A

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 WENDY HOWE

My Commission Expires _____

(Seal)

My Commission Expires
August 14, 2008



F RMATO DE DIGITALIZACI N
RELACION DE ANEXOS



Royal
Technologies S.A.
Bogotá, Colombia - República de Colombia

195

Expediente No		05100610	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

Sobre en ante Gratia

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL
TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION <i>PCT</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 *(X)* 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 fotografica 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 grafica del esquema de trazado	()	()
Comprobante de pago de las tasas establecidas	()	()

COMPLETA () INCOMPLETA () ()

Firma Responsable

Fecha

1193

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá
2020

Doctor(a)
RODRIGUEZ D ALEMAN DILIA MARIA

50600

REFERENCIA	Radicación No	5 100610
	Solicitud internacional	IB2004/000933
	Fecha inicio fase nacional	04/10/2005
	Trámite	11
	Evento	379
	Actuación	430
	Oficio No	13599

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 JAIMES
Jefe de la división de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista de fecha 19 DIC. 2005
adpall