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(54) **POLYMER-BASED SUSTAINED RELEASE DEVICE**

VORRICHTUNG AUF POLYMERBASIS MIT VERZÖGERTER FREISETZUNG

DISPOSITIF À LIBÉRATION CONTRÔLÉE À BASE DE POLYMÈRE

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Description

BACKGROUND OF THE INVENTION

[0001] Numerous proteins and peptides, collectively referred to herein as polypeptides, exhibit biological activity *in vivo* and are useful as medicaments. Many illnesses or conditions require administration of a sustained level of medicament to provide the most effective prophylactic and/or therapeutic effects. Sustained levels are often achieved by the administration of biologically active polypeptides by frequent subcutaneous injections, which often results in fluctuating levels of medicament and poor patient compliance.

[0002] As an alternative, the use of biodegradable materials, such as polymers, encapsulating the medicament can be employed as a sustained delivery system. The use of biodegradable polymers, for example, in the form of microparticles or microcarriers, can provide a sustained release of medicament, by utilizing the inherent biodegradability of the polymer to control the release of the medicament thereby providing a more consistent, sustained level of medicament and improved patient compliance.

[0003] However, these sustained release devices can often exhibit high initial bursts of medicament and minimal release thereafter, resulting in serum drug levels outside the therapeutic window and/or poor bioavailability of the medicament. In addition, the presence of polymer, physiological temperatures and body response to the sustained release composition can cause the medicament to be altered (e.g., degraded, aggregated) thereby interfering with the desired release profile for the medicament.

[0004] Further, methods used to form sustained release compositions can result in loss of activity of the medicament due to the instability of the medicament and the degradative effects of the processing steps. Degradative effects are particularly problematic when the medicament is a polypeptide. WO 03/020245 discloses a formulation with low amounts of exendin-4 and sucrose, further comprising acetate buffer.

[0005] Therefore, a need exists for a means of administering biologically active polypeptides in a sustained fashion wherein the amount of polypeptide delivered is at therapeutic levels, and retains activity and potency for the desired period of release. While much work has been developed that addresses these problems, novel solutions are required.

SUMMARY OF THE INVENTION

[0006] The invention relates to the discovery that superior release profiles (such as those characterized by a ratio of C_{max} to C_{ave} of about 3 or less) can be achieved with a formulation containing few components by optimizing the silicone oil to polymer ratio in the manufacturing process, thereby achieving a low pore volume. This invention relates to compositions for the sustained release of biologically active polypeptides. The sustained release compositions of this invention comprise a biocompatible polymer, exendin-4 as the biologically active polypeptide, and sucrose. According to claim 1 the polypeptide and sugar are dispersed in the polymer. The polypeptide and sugar can be dispersed separately or, preferably, together. The sustained release composition provides a desirable and consistent release profile,characterized as having a ratio of C_{max} to C_{ave} of about 3 or less.

[0007] The composition has a total pore volume of about 0.1 mL/g or less. The total pore volume is determined using mercury intrusion porosimetry.

[0008] The biocompatible polymer is preferably a poly lactide coglycolide polymer.

[0009] There is also described herein a method for forming compositions for the sustained release of biologically active agents, such as polypeptides, which comprises forming a mixture by combining an aqueous phase comprising water, an agent, such as a water soluble polypeptide, and a sugar with an oil phase comprising a biocompatible polymer and a solvent for the polymer; forming a water-in-oil emulsion by, for example, sonicating or homogenizing, the mixture; adding silicone oil to the mixture to form embryonic microparticles; transferring the embryonic microparticles to a quench solvent to harden the microparticles; collecting the hardened microparticles; and drying the hardened microparticles. In a particular embodiment, the silicone oil is added in an amount sufficient to achieve a silicone oil to polymer solvent ratio of about 1.5:1. Additionally or alternatively, the polymer is present in the oil phase at about 10% w/v or less.

[0010] The exendin-4, is present in the composition described herein at a concentration of 5% w/w based on the total weight of the final composition. In addition, the sucrose is present in a concentration of 2% w/w of the final weight of the composition.

[0011] The composition of this invention can be administered to a human, or other animal, by injection, implantation (e.g., subcutaneously, intramuscularly, intraperitoneally, intracranially, and intradermally), administration to mucosal membranes (e.g., intranasally, intravaginally, intrapulmonary or by means of a suppository), or *in situ* delivery (e.g., by enema or aerosol spray).

[0012] The composition is administered in a therapeutically effective amount to treat a patient suffering from diabetes mellitus, impaired glucose tolerance (IGT), obesity, cardiovascular (CV) disorder or any other disorder that can be treated by the exendin-4.

[0013] The use of a sugar in the sustained release compositions of the invention improves the bioavailability of the incorporated biologically active polypeptide, e.g, anti-diabetic or glucoregulatory peptides, and minimizes loss of activity due to instability and/or chemical interactions between the polypeptide and other components contained or used in formulating the sustained release composition, while maintaining an excellent release profile.

[0014] The advantages of the sustained release formulations as described herein include increased patient compliance and acceptance by eliminating the need for repetitive administration, increased therapeutic benefit by eliminating fluctuations in active agent concentration in blood levels by providing a desirable release profile, and a potential lowering of the total amount of biologically active polypeptide necessary to provide a therapeutic benefit by reducing these fluctuations.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015]

FIG. 1 is a graph showing the relationship between the average pore diameter and the *in vitro* release for sustained release compositions described herein (A.S. = Ammonium Sulfate).

FIG. 2 is a graph showing the effect of porosity on the *in vitro* release of exendin-4 from microparticles and the impact that the processing conditions, namely the ratio of silicone oil to methylene chloride, has on the porosity of the microparticles formed.

FIGS. 3A-3B are scans of cryogenic SEMs for selected microparticle formulations described herein.

FIG. 4A-4D are scans of cryogenic SEMs for selected microparticle formulations described herein.

FIG. 5 is a plot of % residual ethanol and methylene chloride versus Tg for microparticle formulations described herein.

FIG. 6 is a representative pharmacokinetic curve (concentration, pg/ml v. time, days with inset showing concentrations over first day) for Formulation 2-1 (3% exendin-4 and 2% sucrose), Formulation 1 (3% exendin-4 alone) and Formulation 4 (3% exendin-4 and 0.5% ammonium sulfate).

FIG. 7 is a graph of *in vivo* release profile for the three microparticle Formulations 2, 2-1 and 2-2.

FIG. 8 is a graph of the pharmacokinetic data for microparticle Formulations 5-1, 5-2 and 5-3.

FIG. 9 is a graph illustrating the relationship between process parameters and the inner emulsion size achieved by the process.

DETAILED DESCRIPTION OF THE INVENTION

[0016] The invention provides a composition for sustained release of a biologically active polypeptide, comprising a biocompatible polymer having the biologically active polypeptide dispersed therein so as to be present at 5% (w/w) of the weight of the composition, and sucrose dispersed therein so as to be present at 2% (w/w) of the weight of the composition, wherein the biologically active polypeptide is exendin-4;
wherein the total pore volume of the composition is 0.1 mL/g or less as determined using mercury intrusion porosimetry; and
wherein the composition is free from buffer and salting out salts.

[0017] The exendin-4 and sucrose are dispersed in the biocompatible polymer separately or, preferably, together. In particular, the sustained release composition is characterized by a release profile having a ratio of maximum serum concentration (C_{max}) to average serum concentration (C_{ave}) of about 3 or less. As used herein, the terms a or an refer to one or more.

The Agent

[0018] The biologically active polypeptide is the antidiabetic or glucoregulatory polypeptide exendin-4.

[0019] Exendin-4 is a 39 amino acid polypeptide. The amino acid sequence of exendin-4 can be found in U.S. Patent No. 5,424,286 issued to Eng on June 13, 1995. AC2993 and exenatide are synonymous with the term exendin-4. Exendin-4 has been shown in humans and animals to stimulate secretion of insulin in the presence of elevated blood glucose concentrations, but not during periods of low blood glucose concentrations (hypoglycemia). It has also been shown to suppress glucagon secretion, slow gastric emptying and affect food intake and body weight, as well as other actions. As such, exendin-4 and analogs and agonists thereof can be useful in the treatment of diabetes mellitus, IGT, obesity, etc.

The Sugar

[0020] The amount of sucrose present in the sustained release composition 2% (w/w) which provides excellent release profiles.

The Polymer

[0021] Polymers suitable to form the sustained release composition of this invention are biocompatible polymers which can be either biodegradable or non-biodegradable polymers or blends or copolymers thereof. A polymer is biocompatible if the polymer and any degradation products of the polymer are non-toxic to the recipient and also possess no significant deleterious or untoward effects on the recipient's body, such as a substantial immunological reaction at the injection site.

[0022] Biodegradable, as defined herein, means the composition will degrade or erode in *vivo* to form smaller units or chemical species. Degradation can result, for example, by enzymatic, chemical and physical processes. Suitable biocompatible, biodegradable polymers include, for example, poly(lactides), poly(glycolides), poly(lactide-co-glycolides), poly(lactic acid)s, poly(glycolic acid)s, polycarbonates, polyesteramides, polyanydrides, poly(amino acids), polyorthoesters, poly(dioxanone)s, poly(alkylene alkylate)s, copolymers or polyethylene glycol and polyorthoester, biodegradable polyurethane, blends thereof, and copolymers thereof.

[0023] Suitable biocompatible, non-biodegradable polymers include non-biodegradable polymers selected from the group consisting of polyacrylates, polymers of ethylene-vinyl acetates and other acyl substituted cellulose acetates, non-degradable polyurethanes, polystyrenes, polyvinylchloride, polyvinyl flouride, poly(vinyl imidazole), chlorosulphonate polyolefins, polyethylene oxide, blends thereof, and copolymers thereof.

[0024] Acceptable molecular weights for polymers used in this invention can be determined by a person of ordinary skill in the art taking into consideration factors such as the desired polymer degradation rate, physical properties such as mechanical strength, end group chemistry and rate of dissolution of polymer in solvent. Typically, an acceptable range of molecular weight is of about 2,000 Daltons to about 2,000,000 Daltons. In a preferred embodiment, the polymer is biodegradable polymer or copolymer. In a more preferred embodiment, the polymer is a poly(lactide-co-glycolide) (hereinafter "PLG") with a lactide:glycolide ratio of about 1:1 and a molecular weight of about 10,000 Daltons to about 90,000 Daltons. In an even more preferred embodiment, the molecular weight of the PLG used in the present invention has a molecular weight of about 30,000 Daltons to about 70,000 Daltons such as about 50,000 to about 60,000 Daltons.

[0025] The PLGs can possess acid end groups or blocked end groups, such as can be obtained by esterifying the acid. Excellent results were obtained with a PLG with an acid end group.

[0026] Polymers can also be selected based upon the polymer's inherent viscosity. Suitable inherent viscosities include about 0.06 to 1.0 dL/g, such as about 0.2 to 0.6 dL/g, more preferably between about 0.3 to 0.5 dL/g. Preferred polymers are chosen that will degrade in 3 to 4 weeks. Suitable polymers can be purchased from Alkermes, Inc. under the tradename Medisorb®, such as those sold as 5050 DL 3A or 5050 DL 4A. Boehringer Ingelheim Resomer® PLGs may also be used, such as Resomer® RG503 and 503H.

[0027] The sustained release composition of this invention can be formed into many shapes such as a film, a pellet, a cylinder, a disc or a microparticle. A microparticle, as defined herein, comprises a polymer component having a diameter of less than about one millimeter and having biologically active polypeptide dispersed or dissolved therein. A microparticle can have a spherical, non-spherical or irregular shape. Typically, the microparticle will be of a size suitable for injection. A typical size range for microparticles is 1000 microns or less. In a particular embodiment, the microparticle ranges from about one to about 180 microns in diameter.

Additional Excipients

[0028] While it is possible that additional excipients can be added to the formulations of the claimed invention as is well known in the art, a surprising discovery of the present invention is that an excellent release profile can be achieved with the simple formulation described herein. Such additional excipients can increase or decrease the rate of release of the agent. Ingredients which can substantially increase the rate of release include pore forming agents and excipients which facilitate polymer degradation. For example, the rate of polymer hydrolysis is increased in non-neutral pH. Therefore, an acidic or a basic excipient such as an inorganic acid or inorganic base can be added to the polymer solution, used to form the microparticles, to alter the polymer erosion rate. Ingredients which can substantially decrease the rate of release include excipients that decrease the water solubility of the agent.

[0029] It has been a surprising discovery that buffering agents such as acetate, citrate, phosphate or other biologically compatible buffer was not necessary in the aqueous phase to achieve a sustained release formulation with exendin-4, with good to excellent bioavailability. It was also a surprising discovery that salting out salts were unnecessary to control burst of the exendin-4. As such, the compositions of the invention , as described herein, are characterised in the substantial (or complete) absence of buffer and/or salting out salts.

Administration

[0030] The compositions of the invention can be administered according to methods generally known in the art. The composition of this invention can be administered to a patient (e.g., a human in need of the agent) or other animal, by

injection, implantation (e.g., subcutaneously, intramuscularly, intraperitoneally, intracranially, and intradermally), administration to mucosal membranes (e.g., intranasally, intravaginally, intrapulmonary or by means of a suppository), or *in situ* delivery (e.g., by enema or aerosol spray).

[0031] The sustained release composition can be administered using any dosing schedule which achieves the desired therapeutic levels for the desired period of time. For example, the sustained release composition can be administered and the patient monitored until levels of the drug being delivered return to baseline. Following a return to baseline, the sustained release composition can be administered again. Alternatively, the subsequent administration of the sustained release composition can occur prior to achieving baseline levels in the patient.

[0032] For example, the composition is administered in a therapeutically effective amount to treat a patient suffering from diabetes mellitus, type II diabetes, IGT, obesity, cardiovascular (CV) disorder or any other disorder that can be treated by exendin-4.

[0033] The sustained release composition of the present invention can be coadministered with a corticosteroid. Coadministration of the sustained release composition of the invention with a corticosteroid can further increase the bioavailability of the biologically active polypeptide of the sustained release composition. Coadministration of a corticosteroid in combination with sustained release compositions is described in detail in U.S. Patent Application 60/419,430 entitled, "Method of Modifying the Release Profile of Sustained Release Compositions" by Dasch *et al.*

[0034] Corticosteroids, as defined herein, refers to steroidal anti-inflammatory agents also referred to as glucocorticoids.

[0035] Suitable corticosteroids include, but are not limited to, 21-Acetoxypregnenolone, Alclometasone, Algestone, Amcinonide, Beclomethasone, Betamethasone, Budesonide, Chloroprednisone, Clobetasol, Clobetasone, Clocortolone, Cloprednol, Corticosterone, Cortisone, Cortivazol, Deflazacort, Desonide, Desoximetasone, Dexamethasone, Disflorasone, Diflucortolone, Difluprednate, Enoxolone, Fluazacort, Flucloronide, Flumethasone, Flunisolide, Flucinolone Acetonide, Fluocinonide, Fluocortin Butyl, Flucortolone, Fluorometholone, Fluperolone Acetate, Fluprednidene Acetate, Fluprednisolone, Flurandrenolide, Fluticasone Propionate, Formocortal, Halcinonide, Halobetasol Propionate, Halometasone, Halopredone Acetate, Hydrocortamate, Hydrocortisone, Loteprednol Etabonate, Mazipredone, Medrysone, Meprednisone, Methylprednisolone, Mometasone Furoate, Paramethasone, Prednicarbate, Prednisolone, Prednisolone 25 - Diethylamino-acetate, Prednisolone Sodium Phosphate, Prednisone, Prednival, Prednylidene, Rimexolone, Tixocortol, Triamcinolone (all forms), for example, Triamcinolone Acetonide, Triamcinolone Acetonide 21-oic acid methyl ester, Triamcinolone Benetonide, Triamcinolone Hexacetonide, Triamcinolone Diacetate, pharmaceutically acceptable mixtures thereof and salts thereof and any other derivative and analog thereof.

[0036] The corticosteroid can be co-incorporated into the sustained release composition comprising the biocompatible polymer and the biologically active polypeptide agent incorporated therein.

[0037] The corticosteroid can be separately incorporated into a second biocompatible polymer. The second biocompatible polymer can be the same or different from the first biocompatible polymer which has the biologically active polypeptide agent incorporated therein.

[0038] The corticosteroid can be present in an unencapsulated state but commingled with the sustained release composition. For example, the corticosteroid can be solubilized in the vehicle used to deliver the sustained release composition. Alternatively, the corticosteroid can be present as a solid suspended in an appropriate vehicle. Further, the corticosteroid can be present as a powder which is commingled with the sustained release composition.

[0039] It is understood that the corticosteroid is present in an amount sufficient to increase the bioavailability of the biologically active polypeptide from the sustained release composition. Increased bioavailability refers to an increase in the bioavailability of the biologically active polypeptide from the sustained release composition when coadministered with a corticosteroid in comparison to the administration in the absence of corticosteroid over a time period beginning at two days post administration and ending at the end of the release cycle for the particular formulation.

[0040] As used herein, patient refers to a human, such as a human in need of the agent or therapy, prophylaxis or diagnostic method.

[0041] As defined herein, a sustained release of biologically active polypeptide is a release of the polypeptide from the sustained release composition of the invention which occurs over a period which is longer than that period during which a biologically significant amount of the polypeptide would be available following direct administration of a solution of the polypeptide. It is preferred that a sustained release be a release which occurs over a period of at least about one week, such as at least about two weeks, at least about three weeks or at least about four weeks. The sustained release can be a continuous or a discontinuous release, with relatively constant or varying rates of release. The continuity of release and level of release can be affected by the type of polymer composition used (e.g., monomer ratios, molecular weight, block composition, and varying combinations of polymers), polypeptide loading, and/or selection of excipients to produce the desired effect.

[0042] As used herein, a therapeutically effective amount, prophylactically effective amount or diagnostically effective amount is the amount of the sustained release composition needed to elicit the desired biological response following administration.

[0043] C_{max} as used herein is the maximum serum concentration of drug which occurs during the period of release which is monitored.

[0044] C_{ave} as used herein, is the average serum concentration of drug derived by dividing the area under the curve (AUC) of the release profile by the duration of the release.

[0045] It is preferred that the ratio of C_{max} to C_{ave} be about 3 or less. This profile is particularly desirable of anti-diabetic or glucoregulatory polypeptides, such as those described above. A ratio of about 3 or less can provide a C_{ave} in a therapeutic window while avoiding adverse drug side effects which can result from higher ratios.

[0046] Bioavailability, as that term is used herein, refers to the amount of therapeutic that reaches the circulation system. Bioavailability can be defined as the calculated Area Under the Curve (AUC) for the release profile of a particular polypeptide during the time period starting at post administration and ending at a predetermined time point. As is understood in the art, the release profile is generated by graphing the serum levels of a biologically active agent in a subject (Y-axis) at predetermined time points (X-axis). Bioavailability is often referred to in terms of % bioavailability, which is the bioavailability achieved for a particular polypeptide following administration of a sustained release composition divided by the bioavailability achieved for a particular polypeptide following intravenous administration of the same dose of drug, multiplied by 100.

[0047] A modification of the release profile can be confirmed by appropriate pharmacokinetic monitoring of the patient's serum for the presence of the biologically active polypeptide agent. For example, specific antibody-based testing (e.g., ELISA and IRMA), as is well known in the art, can be used to determine the concentration of certain biologically active polypeptide agents in the patient's serum. An example of such testing is described herein for exendin-4.

[0048] Pharmacodynamic monitoring of the patient to monitor the therapeutic effects of the agent upon the patient can be used to confirm retention of the biological activity of the released agent. Methods of monitoring pharmacodynamic effects can be selected based upon the biologically active polypeptide agent being administered using widely available techniques.

Manufacture

[0049] A number of methods are known by which sustained release compositions (polymer/biologically active polypeptide matrices) of the invention can be formed, particularly compositions having low porosity as described herein. Detailed procedures for some methods of microparticle formation are set forth in the Working Examples. In a preferred embodiment, the method of the invention for forming a composition for the sustained release of biologically active polypeptide includes forming a mixture by combining an aqueous phase comprising water, agent, such as a water soluble polypeptide, and a sugar with an oil phase comprising a biocompatible polymer and a solvent for the polymer; forming a water-in-oil emulsion; adding a coacervation agent, for example silicone oil, vegetable oil or mineral oil to the mixture to form embryonic microparticles; transferring the embryonic microparticles to a quench solvent to harden the microparticles; collecting the hardened microparticles; and drying the hardened microparticles. This process is generally referred to herein as a water-oil-oil process (W/O/O).

[0050] Preferably, the polymer can be present in the oil phase in a concentration ranging from about 3% w/w to about 25% w/w, preferably, from about 4% w/w to about 15% w/w, such as from about 5% w/w to about 10% w/w. Excellent results were obtained herein using a 6% w/w concentration of PLG in the oil phase..

[0051] The polymer is generally combined with a polymer solvent. Where the polymer is a PLG, such as those preferred herein, the polymer is added to a solvent for PLG. Such solvents are well known in the art. A preferred solvent is methylene chloride.

[0052] The agent and sugar are added in the aqueous phase, preferably in the same aqueous phase. The concentration of agent is preferably 10 to 100 mg/g, preferably between 50 to 100 mg/g. The concentration of sugar is preferably 10 to 50 mg/g and 30 to 50 mg/g.

[0053] The two phases are then mixed to form an emulsion. It is preferred that the emulsion be formed such that the inner emulsion droplet size is less than about 1 micron, preferably less than about 0.7 microns, more preferably less than about 0.5 microns, such as about 0.4 microns. Sonicators and homogenizers can be used to form such an emulsion.

[0054] A coacervation agent as used herein refers to any oil in which the polymer solution (polymer and solvent) is not readily solubilized into and thereby forms a distinct phase with the polymer solution. Suitable coacervation agents for use in the present invention include, but are not limited to, silicone oil, vegetable oil and mineral oil. In a particular embodiment, the coacervation agent is silicone oil and is added in an amount sufficient to achieve a silicone oil to polymer solvent ratio from about 0.75:1 to about 2:1. In a particular embodiment, the ratio of silicone oil to polymer is from about 1:1 to about 1.5:1. In a preferred embodiment, the ratio of silicone oil to polymer is about 1.5:1.

[0055] The resulting mixture is added to a quench, which comprises a polymer nonsolvent. Polymer non-solvents are generally well known in the art. A particularly preferred quench comprises a heptane/ethanol solvent system.

[0056] Solid drug can also be encapsulated using a modified version of the process described above. This modified process can be referred to as a solid/oil/oil (S/O/O).

[0057] For example, solid exendin-4 was suspended in methylene chloride containing 6% PLG and sonicated for about four minutes on ice. Subsequent processing was conducted in a manner analogous to the W/O/O method.

[0058] The invention will now be further and specifically described by the following examples.

EXEMPLIFICATIONS

MICROPARTICLE PREPARATION I

[0059] The sustained release compositions described herein were prepared by a phase separation process. The general process is described below for microparticles containing exendin-4 and sucrose for a 1 kg batch size.

A. Inner Water-in-Oil Emulsion Formation

[0060] A water-in-oil emulsion was created with the aid of a homogenizer. Suitable homogenizers include an in-line Megatron homogenizer MT-V 3-65 F/FF/FF, Kinematica AG, Switzerland. The water phase of the emulsion was prepared by dissolving exendin-4 and excipients such as sucrose in water. The concentration of drug in the resulting solution can be from about 50 mg/g to about 100 mg/g. For example, when the drug is exendin-4, the concentration of drug in solution can be from about 30 g to about 60 g per 600 g of water. In a particular embodiment, 50 g exendin-4 and 20 g sucrose were dissolved in 600 g water for irrigation (WFI). The specified amounts listed above represent a nominal load without adjustment to compensate for peptide content strength specific to the lot of exendin-4 used. The oil phase of the emulsion was prepared by dissolving PLGA polymer (e.g., 930 g of purified 50:50 DL4A PLGA (Alkermes, Inc.) in methylene chloride (14.6 kg or 6% w/w).

[0061] The water phase was then added to the oil phase to form a coarse emulsion with an overhead mixer for about three minutes. Then, the coarse emulsion was homogenized at approximately 10,000 rpm at ambient temperature. This resulted in an inner emulsion droplet size of less than 1 micron. It is understood that inner emulsion formation can be achieved using any suitable means. Suitable means of emulsion formation include, but are not limited to, homogenization as described above and sonication.

B. Coacervate Formation

[0062] A coacervation step was then performed by adding silicone oil (21.8 kg of Dimethicone, NF, 350 cs) over about a five minute time period to the inner emulsion. This is equivalent to a ratio of 1.5:1, silicone oil to methylene chloride. The methylene chloride from the polymer solution partitions into the silicone oil and begins to precipitate the polymer around the water phase containing exendin-4, leading to microencapsulation. The embryonic microspheres thus formed are soft and require hardening. Frequently, the embryonic microspheres are permitted to stand for a short period of time, for example, from about 1 minute to about 5 minutes prior to proceeding to the microsphere hardening step.

C. Microsphere Hardening and Rinse

[0063] The embryonic microspheres were then immediately transferred into a heptane/ethanol solvent mixture. The volume of heptane/ethanol mixture needed can be determined based on the microsphere batch size, typically a 16:1 ratio of methylene chloride to heptane/ethanol solvent. In the present example, about 210 kg heptane and 23 kg ethanol in a 3°C cooled, stirred tank were used. This solvent mixture hardened the microspheres by extracting additional methylene chloride from the microspheres. This hardening step can also be referred to as quenching. After being quenched for 1 hour at 3°C, the solvent mixture is either decanted and fresh heptane (13 Kg) is added at 3°C and held for 1 hour to rinse off residual silicone oil, ethanol and methylene chloride on the microsphere surface or pumped directly to the collection step.

D. Microsphere Drying and Collection

[0064] At the end of the quench or decant/wash step, the microspheres were transferred and collected on a 12" Sweco Pharmasep Filter/Dryer Model PH12Y6. The filter/dryer uses a 20 micron multilayered collection screen and is connected to a motor that vibrates the screen during collection and drying. A final rinse with heptane (6 Kg at 3°C) was performed to ensure maximum line transfer and to remove any excess silicone oil. The microspheres were then dried under vacuum with a constant purge of nitrogen gas at a controlled rate according to the following schedule: 6 hours at 3°C; 6 hours ramping to 41 °C; and 84 hours at 41°C.

[0065] After the completion of drying, the microspheres were discharged into a collection vessel, sieved through a 150 μm sieve, and stored at about -20 °C until filling.

[0066] For all microparticle formulations which were prepared herein the amount of polypeptide, for example, exendin-4 and excipients present in the prepared formulations is expressed as a % (w/w) based on the final weight of the sustained release composition. The % (w/w) is a nominal percentage, except where indicated.

MICROPARTICLE PREPARATION II

A. Inner Water-in-Oil Emulsion Formation

[0067] A water-in-oil emulsion was created with the aid of a sonicator. Suitable sonicators include Vibracell VCX 750 with model CV33 probe head, Sonics and Materials Inc., Newtown, CT. The water phase of the emulsion was prepared by dissolving exendin-4 and excipients such as sucrose in water. The concentration of drug in the resulting solution can be from about 50 mg/ml to about 100 mg/ml. For example, when the drug is exendin-4, the concentration of drug in solution can be from about 3.28 g to about 6.55 g per 65.5 g of water. In a particular embodiment, 5.46 g exendin-4 and 2.18 g sucrose were dissolved in 65.5 g water for irrigation or WFI. The specified amounts listed above represent a 4% overage to target load in order to compensate for losses upon filter sterilization of the components. The oil phase of the emulsion was prepared by dissolving PLGA polymer (e.g., 97.7 g of purified 50:50 DL4A PLGA (Alkermes, Inc.)) in methylene chloride (1539 g or 6% w/v).

[0068] The water phase was then added to the oil phase over about a three minute period while sonicating at 100% amplitude at ambient temperature. The water phase was pumped through a ¼" stainless steel tube with a 1" HPLC tube end (ID = 20/1000") at 5 psig, added below the sonication probe inside the sonication zone. Reactor was then stirred at 1400 to 1600 rpm, with additional sonication at 100% amplitude for 2 minutes, followed by a 30 second hold, and then 1 minute more of sonication. This resulted in an inner emulsion droplet size of less than 0.5 microns. It is understood that inner emulsion formation can be achieved using any suitable means. Suitable means of emulsion formation include, but are not limited to, sonication as described above and homogenization.

B. Coacervate Formation

[0069] A coacervation step was then performed by adding silicone oil (2294 gr of Dimethicone, NF, 350 cs) over about a three to five minute time period to the inner emulsion. This is equivalent to a ratio of 1.5:1, silicone oil to methylene chloride. The methylene chloride from the polymer solution partitions into the silicone oil and begins to precipitate the polymer around the water phase containing exendin-4, leading to microencapsulation. The embryonic microspheres thus formed are soft and require hardening. Frequently, the embryonic microspheres are permitted to stand for a short period of time, for example, from about 1 minute to about 5 minutes prior to proceeding to the microsphere hardening step.

C. Microsphere Hardening and Rinse

[0070] The embryonic microspheres were then immediately transferred into a heptane/ethanol solvent mixture. The volume of heptane/ethanol mixture needed can be determined based on the microsphere batch size. In the present example, about 22 kg heptane and 2448 g ethanol in a 3°C cooled, stirred tank (350 to 450 rpm) were used. This solvent mixture hardened the microspheres by extracting additional methylene chloride from the microspheres. This hardening step can also be referred to as quenching. After being quenched for 1 hour at 3°C, the solvent mixture was decanted and fresh heptane (13 Kg) was added at 3°C and held for 1 hour to rinse off residual silicone oil, ethanol and methylene chloride on the microsphere surface.

D. Microsphere Drying and Collection

[0071] At the end of the rinse step, the microspheres were transferred and collected on a 6" diameter, 20 micron multilayered screen inside the cone shaped drying chamber which acted as a dead-end filter. A final rinse with heptane (6 Kg at 4°C) was performed to ensure maximum line transfer. The microspheres were then dried with a constant purge of nitrogen gas at a controlled rate according to the following schedule: 18 hours at 3°C; 24 hours at 25°C; 6 hours at 35°C; and 42 hours at 38°C.

[0072] After the completion of drying, the microspheres are discharged into a teflon/stainless steel sterilized collection vessel attached to the drying cone. The collection vessel is sealed, removed from the drying cone and stored at -20 ± 5°C until filling. Material remaining in the cone upon disassembly for cleaning is taken for drug content analysis. The yield was approximately 100 grams of microspheres.

[0073] For all microparticle formulations which were prepared herein the amount of polypeptide, for example, exendin-4 and excipients present in the prepared formulations is expressed as a % (w/w) based on the final weight of the sustained release composition. The % (w/w) is a nominal percentage, except were indicated.

POLYMER:

[0074] Examples of specific PLG polymers suitable for use are listed below. All of the polymers employed in the following examples are set forth in the list and all listed polymers were obtained from Alkermes, Inc. of Cincinnati,OH and can be described as follows:

Polymer 2A: Poly(lactide-co-glycolide); 50:50 lactide:glycolide ratio; 12.3 kD Mol. Wt.; IV=0.15 (dL/g).

Polymer 4A: Poly(lactide-co-glycolide); 50:50 lactide:glycolide ratio; Mol. Wt. 45-64 kD; IV=0.45-0.47 (dL/g).

[0075] PURIFICATION OF PLG: It is known in the art (See, for example, Peptide Acylation by Poly(α-Hydroxy Esters) by Lucke et al., Pharmaceutical Research, Vol. 19, No. 2, p. 175-181, February 2002) that proteins and peptides which are incorporated in PLG matrices can be undesirably altered (e.g., degraded or chemically modified) as a result of interaction with degradation products of the PLG or impurities remaining after preparation of the polymer. As such, the PLG polymers used in the preparation of the majority of microparticle formulations described herein were purified prior to preparation of the sustained release compositions using art recognized purification methods.

CHARACTERIZATION METHODS:

[0076] It has been determined that the following characterization methods are suitable for identifying microparticles which will provide a desirable release profile of active agent.

SEM

[0077] SEM was used to assess the particle size, shape and surface features of the microparticles. SEM imaging was performed on a Personal SEM® system (ASPEX™, LLC). All samples were deposited via spatula on standard SEM stubs covered with carbon double-sided tape. Samples were sputter coated with Au for about 90 seconds at 18 mA emission current using a Model SC 7620 "Mini" Sputter Coater (Energy Beam Sciences). All SEM imaging was performed utilizing a 20 KeV electron beam over a magnification range of approximately 250 to 2500X.

CRYOGENIC SEM

[0078] The cross-section of microparticles was studied using cryogenic SEM. The microparticle sample was mixed with HISTO PREP® Solution (Fischer) and kept in a cryostat at -20°C overnight. The hardened microparticles were mounted on a glass cover slip and then sectioned using a metal knife. The sectioned particles were mounted on aluminium stubs, sputter coated with Platinum and Palladium and observed under a Scanning Electron Microscope (Phillips 525M). Visual observation of the sections provides a method of determining the degree of porosity for the microparticles.

POROSITY MEASUREMENT-MERCURY INTRUSION

[0079] Pore volume distribution in microparticles was determined using a model SutoPor IV 9500 Moden Mercury Intrusion Porosimeter (Micromeritics, Norcross, GA). Briefly, mercury was forced into a known amount of microparticles in a penetrometer by applying pressure in a step-wise manner up to a maximum pressure of 60,000 Psia. The volume of mercury intruded into the pores at various pressures was measured. This method quantifies the pore distribution in the microparticles. That is, the size of the pores that are intruded is inversely related to the applied pressure. The equilibrium of the internal and external forces on the liquid-solid-vapor system can be described by the Washburn equation. The relationship between applied pressure and the pore size into which mercury is forced to enter is described by:

$$D=\frac{-4\gamma\cos\theta}{P}$$

Where: D = pore diameter
γ = surface tension (constant)
θ = contact angle (constant)
P= Pressure

Therefore, the size of the pore into which mercury will intrude is inversely proportional to the applied pressure. Assuming that all pores are tight cylinders, the average pore diameter (D=4V/A) can be calculated by dividing pore volume (V=πD2h/4) by the pore area (A=πDh).

RESIDUAL SOLVENTS

[0080] A single method was used for quantitation of heptane, ethanol and methylene chloride. The equipment consisted of an HP 5890 Series 2 gas chromatograph with an Rtx 1301, 30 cm x 0.53 mm column. About 130 mg microparticles were dissolved in 10 ml N,N-dimethylformamide. Propyl acetate was used as the internal standard. The sample preparation was adjusted so that concentrations of methylene chloride as low as 0.03% can be quantitated.

MICROPARTICLE PREPARATION

[0081] The microparticle batches set forth in Table 1 were prepared as described above at the 100 gram scale using the 4A polymer and a ratio of silicone oil to methylene chloride of either 1.5:1 or 1:1 and the silicone oil had a viscosity of 350 cs. The amount of exendin-4 and the excipients used in the formulation are also set forth in Table 1.

TABLE 1

Lot #	Formulation	In vitro burst (%)	Remarks
02-019-147(#1) **	0% Sucrose, 0% AS	0.40	1.5:1 Si Oil:$MeCl_2$
02-019-167(#2) **	2% Sucrose (F16)	0.40	1.5:1 Si Oil: $MeCl_2$
02-019-160(#2-1)**	2% Sucrose (F16)	0.44	1.5:1 Si Oil: $MeCl_2$
02-019-164(#2-2)**	2% Sucrose (F16)	0.45	1.5:1 Si Oil: $MeCl_2$
02-030-08(#2-3)**	2% Sucrose (F16)	0.80	1:1 Si Oil: $MeCl_2$
02-030-01(#2-4)**	2% Sucrose (F16)	1.0	1:1 Si Oil: $MeCl_2$
02-030-04(#2-5)**	2% Sucrose (F16)	1.1	1:1 Si Oil: $MeCl_2$
02-019-136(#3-1)**	2% Sucrose, 0.5% AS (F14)	1.3	50:50 Quench
02-019-115(#3-2)**	2% Sucrose, 0.5% AS (F14)	2.2	1.5:1 Si Oil: $MeCl_2$
02-019-170(#4)**	0% Sucrose, 0.5% AS	3.8	1.5:1 Si Oil: $MeCl_2$
02-019-133A(#3-3)**	2% Sucrose, 0.5% AS (F14)	12.7	100% Heptane Quench
02-019-185(#5) (5% drug load)	2% sucrose (F17)	0.5	5% drug load, 1.5:1 Si Oil: $MeCl_2$
02-019-64(#3-4)**	2% Sucrose, 0.5% AS (F14)	0.5	1.5:1 Si Oil: $MeCl_2$
02-019-10(#3-5)**	2% Sucrose, 0.5% AS (F14)	1.30	1:1 Si Oil: $MeCl_2$
02-001-196(#3-6)**	2% Sucrose, 0.5% AS (F14)	2.70	1:1 Si Oil: $MeCl_2$
02-019-24(#3-7)**	2% Sucrose, 0.5% AS (F14)	6.70	1:1 Si Oil: $MeCl_2$
*ALL FORMULATIONS HAD 3% DRUG LOAD WITH THE EXCEPTION OF #5 POROSITY **Reference Example			

[0082] The total intrusion volume obtained from the mercury intrusion porosimetry and the calculated average pore diameters are given in TABLE 2. The relationship between the average pore diameter and the in vitro release is shown in FIG. 1

TABLE 2

Lot #	Total Pore Volume (mL/g)	In vitro burst (%)	Average Pore Diameter (μm)
02-019-147(#1)*	0.033	0.40	0.0068
02-019-167(#2)*	0.035	0.40	0.0069

(continued)

Lot #	Total Pore Volume (mL/g)	In vitro burst (%)	Average Pore Diameter (μm)
02-019-160(#2-1)*	0.037	0.44	0.0070
02-019-164(#2-2)*	0.035	0.45	0.0070
02-030-08(#2-3)*	0.036	0.80	0.0070
02-030-01(#2-4)*	0.038	1.0	0.0073
02-030-04(#2-5)*	0.039	1.1	0.0074
02-019-136(#3-1)*	0.041	1.3	0.0073
02-019-115(#3-2)*	0.039	2.2	0.0078
02-019-170(#4)*	0.067	3.8	0.0125
02-019-133A(#3-3)*	0.513	12.7	0.0277
02-019-64 (#3-4)*	0.030	0.5	0.0060
02-019-10(#3-5)*	0.060	1.30	0.0090
02-001-196(#3-6)*	0.060	2.70	0.0100
02-019-24(#3-7)*	0.180	6.70	0.0170
*Reference Example			

[0083] FIG. 1 shows the effect of ammonium sulfate on the in vitro initial release. The data indicate that in vitro initial release is correlated to the microparticle pore diameter. Formulations made with ammonium sulfate showed varying levels of in vitro release and variable porosity unlike the formulations without ammonium sulfate which exhibited consistent porosity and release. During the manufacturing of microparticles the presence of ammonium sulfate in the aqueous phase can salt-out the drug substance during the preparation of the inner-emulsion. The differences in the micro-environment of the precipitates can contribute to the differences in porosity and hence the variation in the initial release. The effect was not observed in formulations prepared without ammonium sulfate. Formulations with sucrose and exendin-4 show a more desirable and consistent level of initial release as compared to formulations having exendin-4, sucrose and ammonium sulfate.

[0084] FIG. 2 further demonstrates the effect of porosity on the in vitro release and the impact that the processing conditions, namely the ratio of silicone oil to methylene chloride, has on the porosity of the microparticles formed. Briefly, microparticle formulations prepared using a silicone oil-to-methylene chloride ratio of 1:1 (Formulations 2-4 and 2-5 of Table 1) have a higher initial release than the same formulations prepared using a silicone-to-methylene chloride ratio of 1.5:1 (Formulations 2, 2-1 and 2-2 of Table 1). FIG. 2 suggests that a higher ratio of silicone oil-to-methylene chloride results in a lower porosity which results in a lower initial release.

CRYOGENIC SEM

[0085] Cryogenic SEM analysis was conducted as described above on Formulations of the Types 2, 3 and 5 of Table 1. FIGS. 3A-3B are scans of micrographs for selected formulations of Type 2 (Formulation 2-2, FIG. 3A) and of Type 5 (5% exendin-4, 2% sucrose, FIG. 3B). FIGS. 4A-D are scans of micrographs for Formulations 3-4, 3-5, 3-6 and 3-7, respectively of Table 1. Again the variation in porosity exhibited with the use of ammonium sulfate which can contribute to the variability in initial release, can be seen in the cryogenic SEM cross sections of FIGS. 4A-D.

RESIDUAL SOLVENT LEVELS

[0086] The level of residual solvents in a given formulation can impact the Tg of the formulation. Residual solvent levels were determined for microparticle formulations of Types 2 and 5 of Table 1. A single method was used for quantitation of heptane, ethanol and methylene chloride. The equipment consisted of an HP 5890 Series 2 gas chromatograph with an Rtx 1301, 30 m x 0.53 mm column. About 130 mg microparticles were dissolved in 10 ml N,N-dimethylformamide. Propyl acetate was used as the internal standard. The sample preparation was adjusted so that concentrations of methylene chloride as low as 0.03% can be quantitated.

[0087] FIG. 5 is a plot of % residual ethanol and methylene chloride for formulations of Types 2 and 5 of Table 1 (3

or 5% exendin-4, 2% sucrose). FIG. 5 shows that the Tg decreases as the amount of residual solvent increases.

PREPARATION OF MICROPARTICLES HAVING 3% EXENDIN-4 AND 2% SUCROSE

[0088] In view of the variation in porosity introduced by the presence of ammoniun sulfate in the microparticle formulations and the identification of porosity as a characteristic which significantly impacts initial release, ammonium sulfate was not pursued in further discovery.

IMPACT OF INNER EMULSION DROPLET SIZE

[0089] The following study was done to determine the impact of process parameters on forming the inner emulsion as well as stability of the resulting emulsion and resulting 24 hour in vitro release of microspheres produced using the different process parameters. Inner emulsions of the water phase and solvent phase were formed by either sonication as described above for the 100 gr scale or homogenization using an MT5000 homogenizer with a 36/4 generator (Kinematica AG, Switzerland) at either a low speed (10,800 rpm) or high speed (21,300 rpm). Following inner emulsion formation by the different techniques, the emulsions were held in the reactor with gentle agitation with an overhead stirrer for 5, 15 or 60 minutes prior to an aliquot being removed. Following the designated hold times, the inner emulsion was further processed as described above into microparticles and then the 24 hour in vitro release determined for each batch as described below.

Inner emulsion droplet size characterization can be determined using the Horiba particle size analyzer

[0090] An aliquot of the inner emulsion was withdrawn from the reactor using a glass pipet. Using a transfer pipet, ~30 drops of the inner emulsion was added to ~10 ml of 6% Medisorb® 50:50 4A PLG polymer solution in a 20 cc screw-cap scintillation vial followed by mixing. The 6% Medisorb® 50:50 4A PLG polymer solution also served as the reference blank solution. About 9 ml of this diluted emulsion sample was then transferred into a clean 10 ml Horiba sample holder. A cover was placed on the sample holder to prevent rapid evaporation of the polymer solvent. The prepared sample was within the acceptable % transmission reading range of 0.65% - 0.90% per the blue bar (Lamp). A relative refractive index setting of 0.94-0.00i was selected in the program setup. The sample was then measured by a Horiba particle size analyzer such as model LA 910 for droplet size. The data correlating the process parameters and the achieved inner emulsion size over the 5, 15 and 60 minute hold times as well as the resulting 24 hour in vitro release results (in parenthesis) are shown in Figure 9.

MICROSPHERE CHARACTERIZATION

[0091] Exendin-4 microspheres were routinely characterized with respect to drug content, particle size, residual solvents, initial in vitro release, and PK characteristics in rats. Drug was extracted to obtain a preliminary assessment of exendin-4 purity post-encapsulation in selected batches.

IN VITRO INITIAL RELEASE

[0092] The initial release of exendin-4 was determined by measuring the concentration of exendin-4 after 1 hour in release buffer (10 mM HEPES, 100 mM NaCl, pH 7.4). 150± 5 mg of microspheres were placed in 5.0 mL of 10mM HEPES, 100mM NaCl, pH 7.4 buffer at room temperature, vortexed for about 30 seconds to suspend the solution and then placed in a 37 °C air chamber for 1 hour. After 1 hour, the samples were removed from the chamber and inverted several times to mix, followed by centrifuging at 3500 rpm for 10 minutes. The supernatant was removed and analyzed immediately by HPLC using the following conditions: Column: TSK-GEL®, 7.8 mm x 30 cm, 5 m (TSOH BIOSEP PART #08540); Column Oven Temperature: Ambient; Autosampler Temperature: 6 °C; Flow Rate: 0.8 mL/minute; Detection: 280 nm; Injection Volume: 10 L; Mobile Phase: 35% Acetonitrile/65% Water with 0.1 % TFA/liter (v/v); Run Time: Approximately 20 minutes. Exendin-4 bulk drug substance, 0.2 mg/mL prepared in 30 mM Acetate Buffer, pH 4.5, was used as a standard.

ANIMAL STUDIES

[0093] All pharmacokinetic (PK) studies described herein were conducted in adult male Sprague-Dawley rats weighing approximately 500±50 g.

[0094] For PK characterization of the microparticle formulations, each animal received a subcutaneous injection of microparticles suspended in diluent (3% carboxymethylcellulose, 0.9% NaCl, 0.1 % Tween 20) to the inter-scapular

region. Generally, the dose was approximately 1.0 mg exendin-4 per rat in an injection volume of 0.75 mL. Blood samples were collected via lateral tail vein at 0.5, 2, 4, 6, 10, 24 hours, and 2, 4, 7, 10, 14, 17, 21, 24 and 28 days post-dose. Blood samples were immediately placed in MICROTAINER® tubes containing EDTA and centrifuged at about 14,000 X g for about two minutes. Plasma was then transferred to MICROTAINER® tubes without additive and stored at - 70°C until time of assay. IRMA was used to determine plasma exendin concentrations.

IN VIVO RELEASE-IRMA

[0095] The method for quantifying exendin-4 in plasma is a sandwich immunoassay, with the analyte captured by a solid phase monoclonal antibody EXE4:2-8.4 and detected by the radioiodinated monoclonal antibody GLP-1:3-3. Counts bound are quantitated from a standard calibration curve. This assay is specific for full length or intact exendin-4 and does not detect exendin-4 (3-39). A typical standard curve range is 30 pg/mL to 2000 pg/mL depending on the age of the tracer antibody.

IN VITRO AND IN VIVO RELEASE

[0096] Formulations 2, 2-1 and 2-2 (3% exendin-4 and 2% sucrose) were tested for initial release in vitro as described above. The in vitro release was 0.4%, 0.4% and 0.5%, respectively. All three batches also had a relatively low in vivo initial release in the range of 1154 to 1555 pg/mL for C_{max} 0-1 day. FIG. 6 is a representative pharmacokinetic curve for the formulations having 3% exendin-4 and 2% sucrose _(2-1) and also for 3% exendin-4 alone (1) and 3% exendin-4 and 0.5% ammonium sulfate (4). A ratio of silicone oil-to-methylene chloride of 1.5:1 was used and the viscosity of the silicone oil was 350 cs.

[0097] From FIG. 6 it can be seen that the formulations not containing ammonium sulfate exhibit a lower initial release. Although the formulation having exendin-4 alone showed a suitable initial release the post encapsulation purity of the drug was decreased as compared to the formulation having the exendin-4 in combination with the sucrose. The addition of sugar in the formulations decreases degradation of the agent.

[0098] The *in vivo* release profile for the three formulations 2, 2-1 and 2-2 compared above, are shown in FIG. 7. All three batches exhibited a relatively low initial release followed by a "trough" (low serum levels between about day 4 to day 17), followed by a sustained release over about day 21 to day 28. The low initial release and the shape of the release profile were consistent for the three formulations.

FORMULATION USING A 1:1 RATIO OF SILICONE OIL TO METHYLENE CHLORIDE

[0099] Formulations 2-3, 2-4 and 2-5 from Table 1 (3% exendin-4, 2% sucrose) were prepared using a 1:1 ratio of silicone oil to methylene chloride. The initial release was higher for these formulations than for formulations 2, 2-1 and 2-2 of Table 1 (3% exendin-4, 2% sucrose with a 1.5:1 silicone to methylene chloride ratio). Specifically the 1.5:1 ratio formulations provided an average initial release about 0.4%, whereas the 1:1 ratio formulations provided an average initial release about 1.0%. The same trend was observed in vivo with C_{max} 0-1 day in rats was 2288 $\pm$520pg/mL for a 1:1 ratio, whereas the C_{max} 0-1 day in rats was 1300$\pm$221 pg./mL for the 1.5:1 ratio.

INCREASED DRUG LOADING

[0100] Increasing the exendin-4 load to 4% while maintaining the sucrose at 2% resulted in an initial release *in vitro* and *in vivo* in the same range as for the 3% loading.

[0101] Three formulations of Type 5 from Table 1 were prepared (5% drug load, 2% sucrose, 1.5:1 silicone oil-to-methylene chloride ratio). The three batches, 5-1, 5-2 and 5-3 all exhibited a low *in vitro* initial release ranging from 0.2 to 0.5%. Similarly, the *in vivo* C_{max} of the formulations was consistently low ranging from 467 pg/mL to 1267 pg/mL. FIG. 8 shows a graph of the pharmacokinetic data for the three batches tested. Compared to the behavior of the 3% exendin-4 formulation having 2% sucrose, the 5% formulations exhibited higher serum levels of drug over about day 1 and day 2. The remainder of the profile for the 5% formulations was similar to the 3% formulations having a trough followed by release of drug primarily over day 21 to day 28.

Claims

1. A composition for sustained release of a biologically active polypeptide, comprising a biocompatible polymer having the biologically active polypeptide dispersed therein so as to be present at 5% (w/w) of the weight of the composition, and sucrose dispersed therein so as to be present at 2% (w/w) of the weight of the composition, wherein the

biologically active polypeptide is exendin-4;
wherein the total pore volume of the composition is 0.1 mL/g or less as determined using mercury intrusion porosimetry; and
wherein the composition is free from buffer and salting out salts.

2. The sustained release composition of claim 1, wherein the biocompatible polymer is selected from the group consisting of poly(lactides), poly(glycolides), poly(lactide-co-glycolides), poly(lactic acid)s, poly(glycolic acid)s, polycarbonates, polyesteramides, polyanhydrides, poly(amino acids), polyorthoesters, poly (dioxanones), biodegradable polyurethanes, blends thereof and copolymers thereof.

3. The sustained release composition of claim 1, wherein the biocompatible polymer is selected from poly(lactides), poly(glycolides), poly(lactide-co-glycolides), poly(lactic acid)s, poly(glycolic acid)s, and blends and copolymers thereof.

4. The sustained release composition of claim 1, wherein the biocompatible polymer comprises poly(lactide-co-glycolide).

5. The sustained release composition of claim 4, wherein the biocompatible polymer is purified 50:50 poly(lactide-co-glycolide).

6. The sustained release composition of any one of claims 1 to 5, which is in the form of microparticles.

7. A composition as claimed in any one of claims 1 to 6 for use in treating type 2 diabetes.

Patentansprüche

1. Zusammensetzung zur anhaltenden Freisetzung eines biologisch aktiven Polypeptids, umfassend ein biokompatibles Polymer mit dem darin so dispergierten biologisch aktiven Polypeptid, dass es bei 5% (Gew./Gew.) des Gewichts der Zusammensetzung vorhanden ist, und darin so dispergierter Saccharose, dass sie bei 2% (Gew./Gew.) des Gewichts der Zusammensetzung vorhanden ist, wobei das biologisch aktive Polypeptid Exendin-4 ist;
wobei das gesamte Porenvolumen der Zusammensetzung 0,1 ml/g oder weniger ist, wie unter Verwendung von Quecksilber-Intrusionsporosimetrie bestimmt; und
wobei die Zusammensetzung frei von Puffer und aussalzenden Salzen ist.

2. Zusammensetzung zur anhaltenden Freisetzung nach Anspruch 1, worin das biokompatible Polymer ausgewählt ist aus der Gruppe bestehend aus Poly(lactiden), Poly(glykoliden), Poly(lactid-coglykoliden), Poly(milchsäure)n, Poly(glycolsäure)n, Polycarbonaten, Polyesteramiden, Polyanhydriden, Poly(aminosäuren), Polyorthoestern, Poly (dioxanonen), biologisch abbaubaren Polyurethanen, Mischungen daraus und Copolymeren daraus.

3. Zusammensetzung zur anhaltenden Freisetzung nach Anspruch 1, worin das biokompatible Polymer ausgewählt ist aus Poly(lactiden), Poly(glykoliden), Poly(lactid-co-glykoliden), Poly(milchsäure)n, Poly(glycolsäure)n und Mischungen und Copolymeren daraus.

4. Zusammensetzung zur anhaltenden Freisetzung nach Anspruch 1, worin das biokompatible Polymer Poly(lactid-co-glykolid) umfasst.

5. Zusammensetzung zur anhaltenden Freisetzung nach Anspruch 4, worin das biokompatible Polymer gereinigtes 50:50 Poly(lactid-co-glykolid) ist.

6. Zusammensetzung zur anhaltenden Freisetzung nach einem von Ansprüchen 1 bis 5, welche die Form von Mikropartikeln hat.

7. Zusammensetzung wie in einem von Ansprüchen 1 bis 6 beansprucht zur Verwendung beim Behandeln von Typ-2-Diabetes.

Revendications

1. Composition pour une libération prolongée d'un polypeptide biologiquement actif, comprenant un polymère biocompatible dans lequel sont dispersés un polypeptide biologiquement actif de façon à ce qu'il soit présent à raison de 5 % (en poids) du poids de la composition, et du saccharose de façon à ce qu'il soit présent à raison de 2 % (en poids) du poids de la composition, dans laquelle le polypeptide biologiquement actif est l'exendine-4 ;
dans laquelle le volume de pores total de la composition est de 0,1 ml/g ou moins, déterminée par porosimétrie par intrusion de mercure ; et
dans laquelle la composition est dépourvue de tampon et de sels relargants.

2. Composition à libération prolongée selon la revendication 1, dans laquelle le polymère biocompatible est choisi dans le groupe constitué par les poly(lactides), les poly(glycolides), les poly(lactide-co-glycolides), les poly(acides lactiques), les poly(acides glycoliques), les polycarbonates, les polyesteramides, les polyanhydrides, les poly(acides aminés), les polyorthoesters, les poly(dioxanones), les polyuréthanes biodégradables, les mélanges de ceux-ci et les copolymères de ceux-ci.

3. Composition à libération prolongée selon la revendication 1, dans laquelle le polymère biocompatible est choisi parmi les poly(lactides), les poly(glycolides), les poly(lactide-co-glycolides), les poly(acides lactiques), les poly(acides glycoliques) et les mélanges et les copolymères de ceux-ci.

4. Composition à libération prolongée selon la revendication 1, dans laquelle le polymère biocompatible comprend du poly(lactide-co-glycolide).

5. Composition à libération prolongée selon la revendication 4, dans laquelle le polymère biocompatible est du poly (lactide-co-glycolide) 50:50 purifié.

6. Composition à libération prolongée selon l'une quelconque des revendications 1 à 5, qui est sous la forme de microparticules.

7. Composition selon l'une quelconque des revendications 1 à 6, destinée à une utilisation dans le traitement du diabète de type 2.

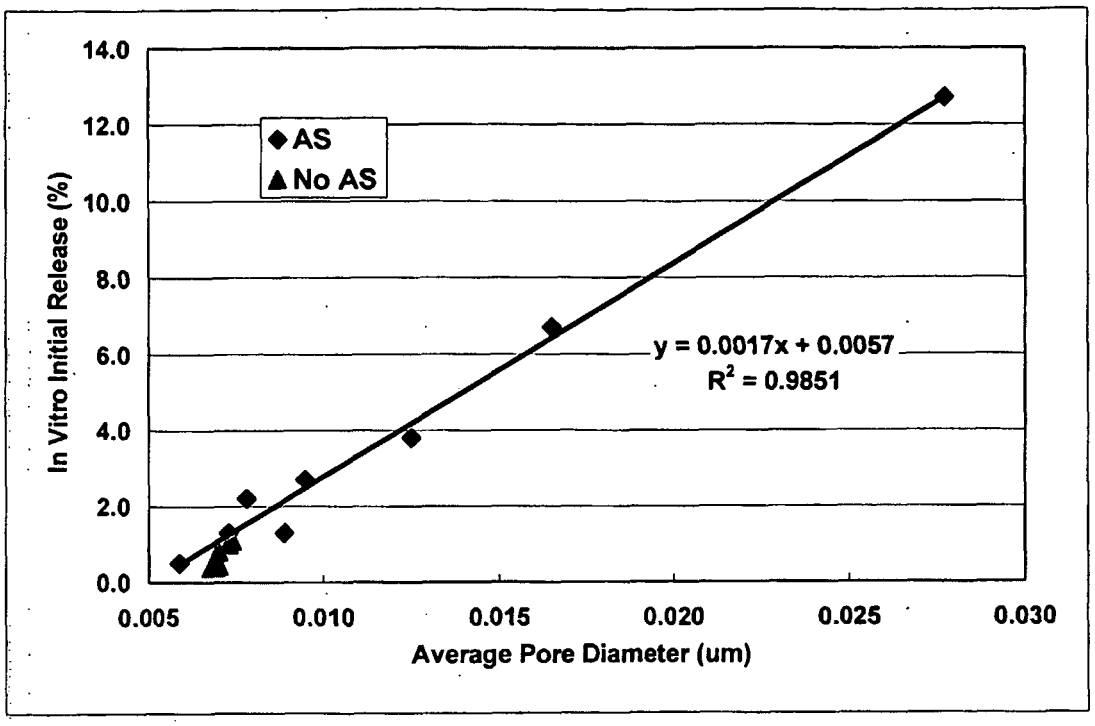
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
In Vitro Initial Release (%)
AS
No AS
y = 0.0017x + 0.0057
R2 = 0.9851
0.005
0.010
0.015
0.020
0.025
0.030
Average Pore Diameter (um)

FIG. 1

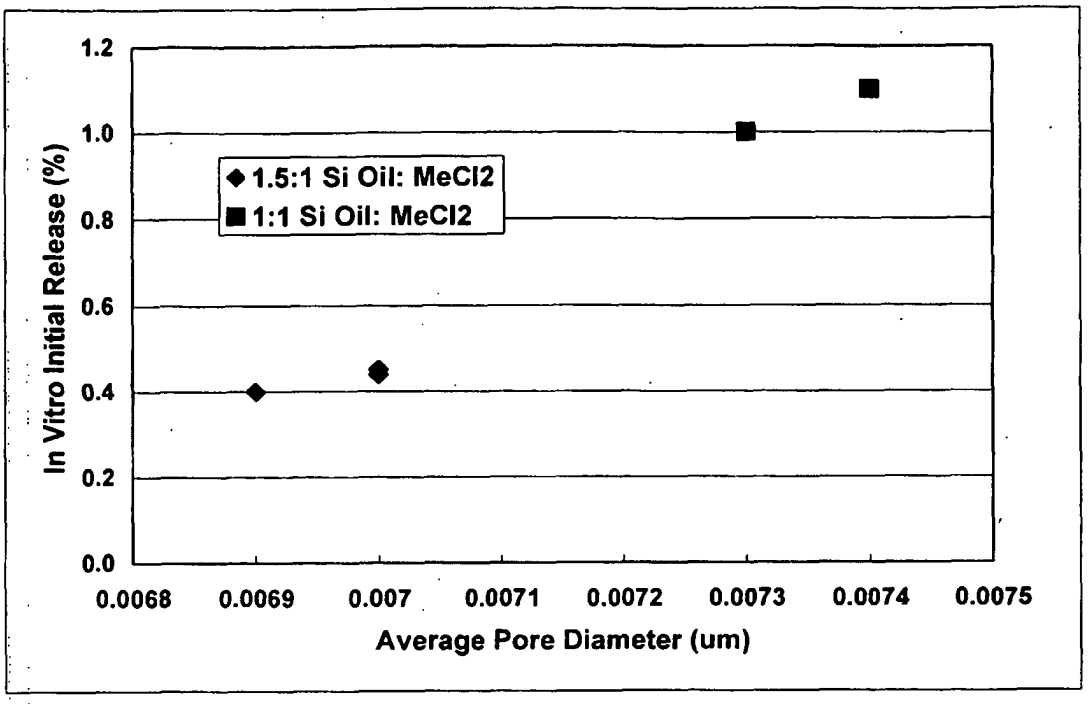
1.2
1.0
0.8
0.6
0.4
0.2
0.0
In Vitro Initial Release (%)
1.5:1 Si Oil: MeCl2
1:1 Si Oil: MeCl2
0.0068
0.0069
0.007
0.0071
0.0072
0.0073
0.0074
0.0075
Average Pore Diameter (um)

FIG. 2

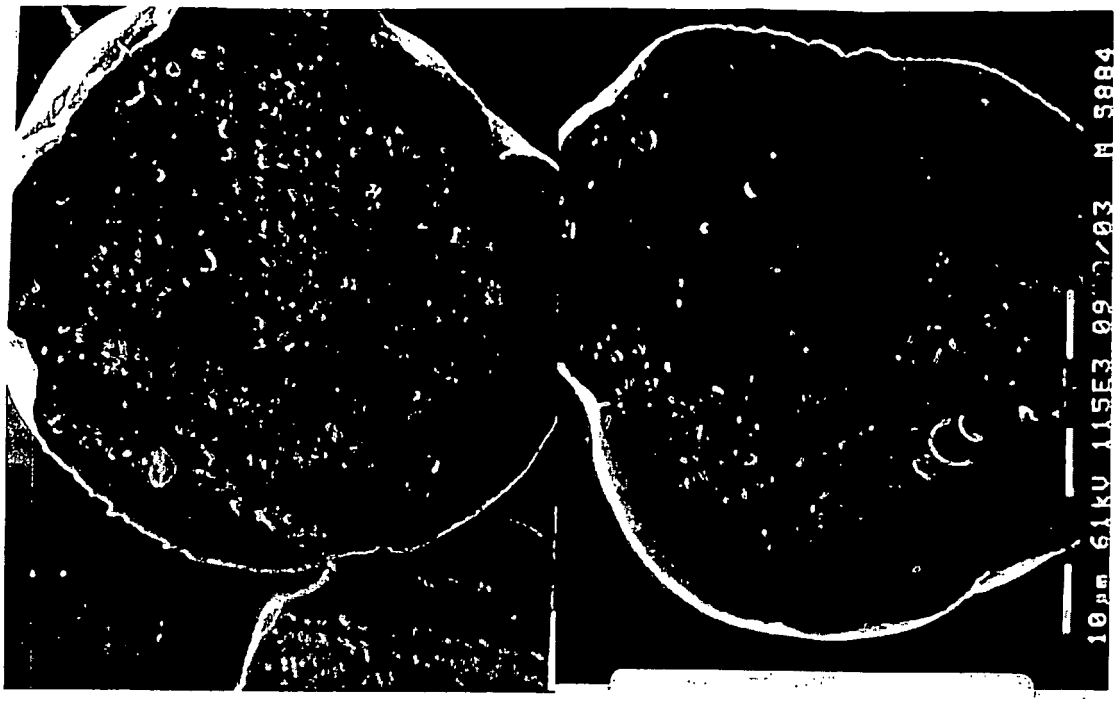

FIG. 3A

FIG. 3B

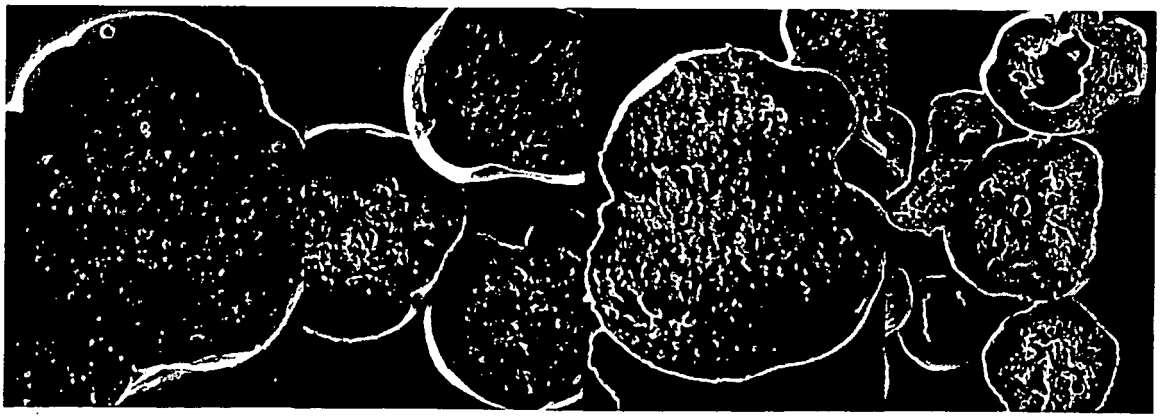

FIG. 4A FIG. 4B FIG. 4C FIG. 4D

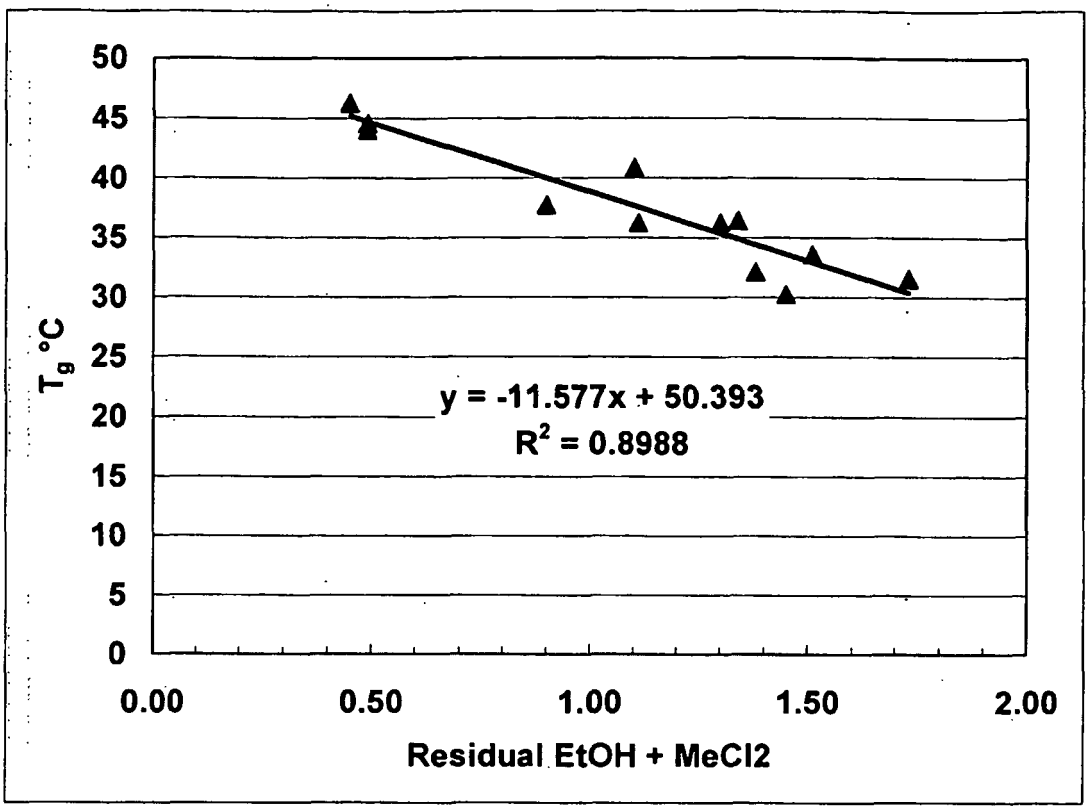
50
45
40
35
30
25
20
15
10
5
0
T_g °C
y = -11.577x + 50.393
R^2 = 0.8988
0.00
0.50
1.00
1.50
2.00
Residual EtOH + MeCl2

FIG. 5

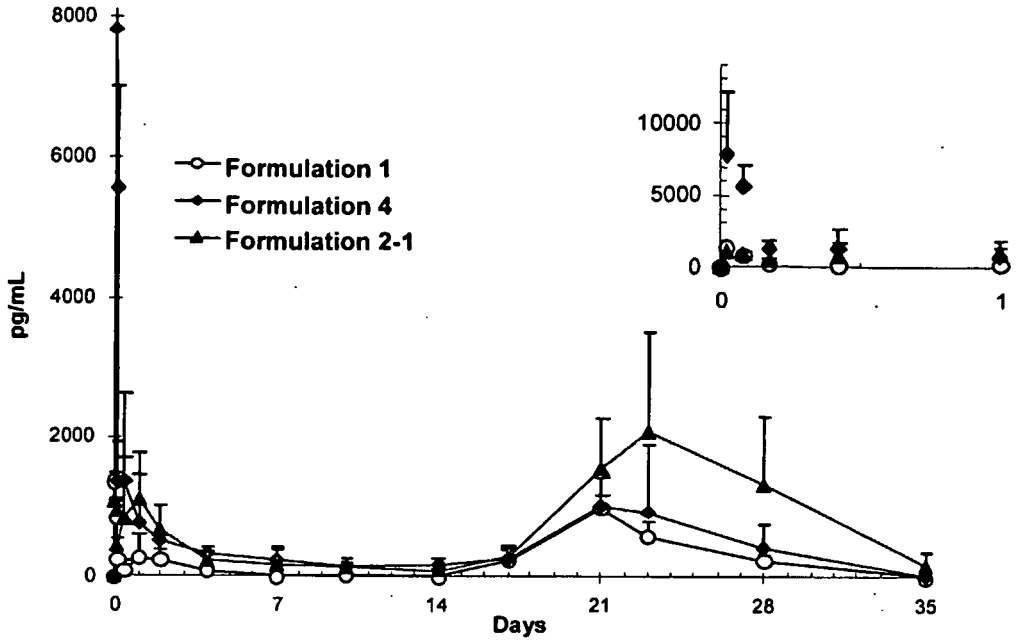
8000
6000
4000
2000
0
pg/mL
Formulation 1
Formulation 4
Formulation 2-1
0
7
14
21
28
35
Days
10000
5000
0
0
1

FIG. 6

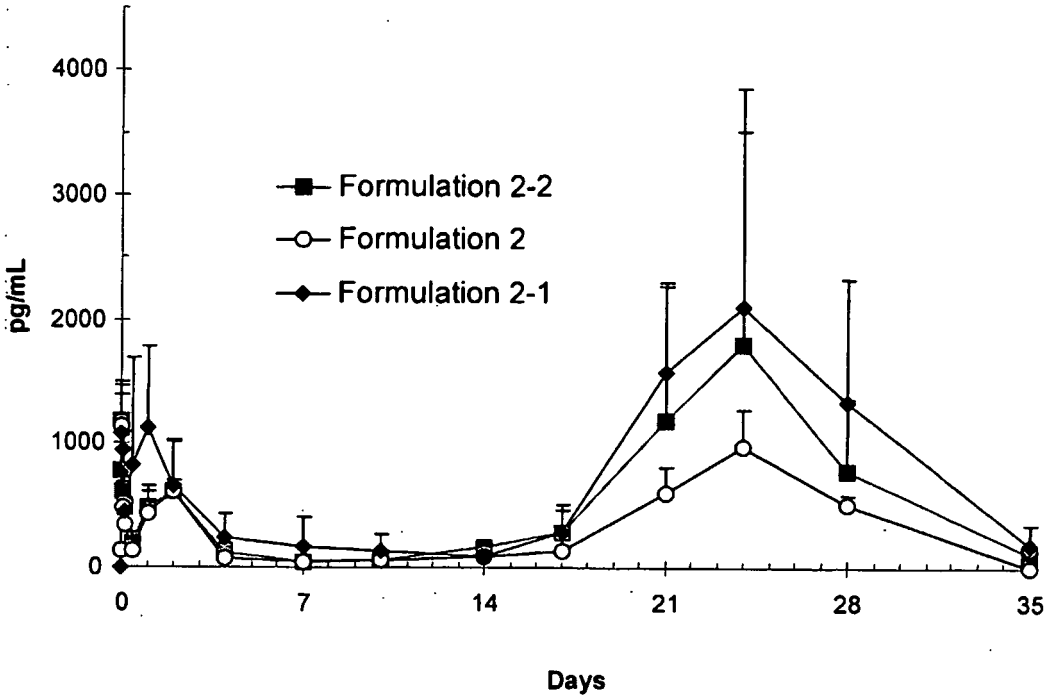
4000
3000
2000
1000
0
pg/mL
Formulation 2-2
Formulation 2
Formulation 2-1
0
7
14
21
28
35
Days

FIG. 7

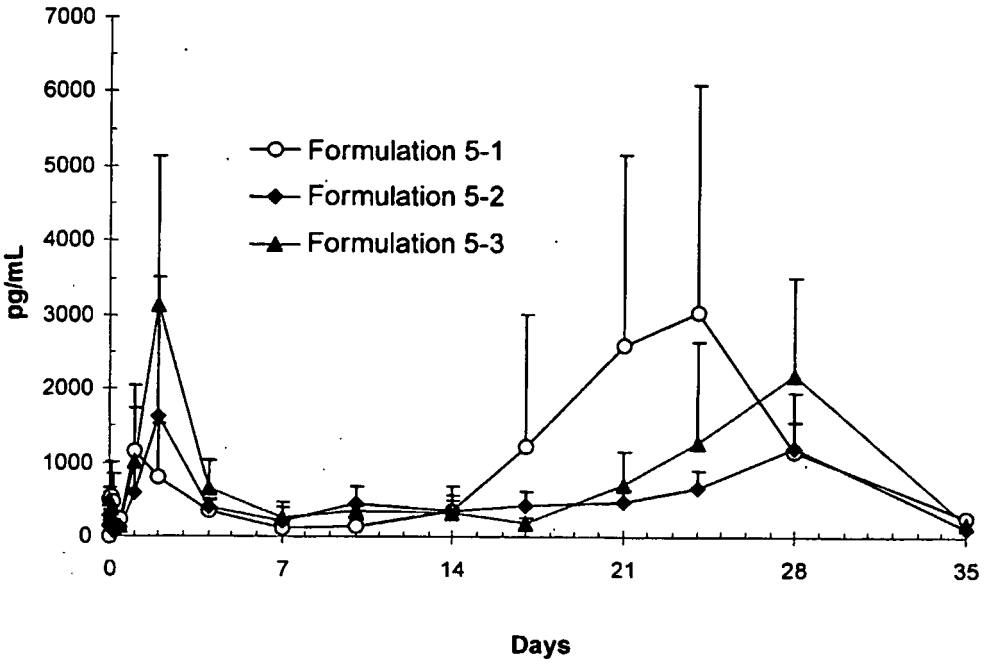
7000
6000
5000
4000
3000
2000
1000
0
pg/mL
Formulation 5-1
Formulation 5-2
Formulation 5-3
0
7
14
21
28
35
Days

FIG. 8

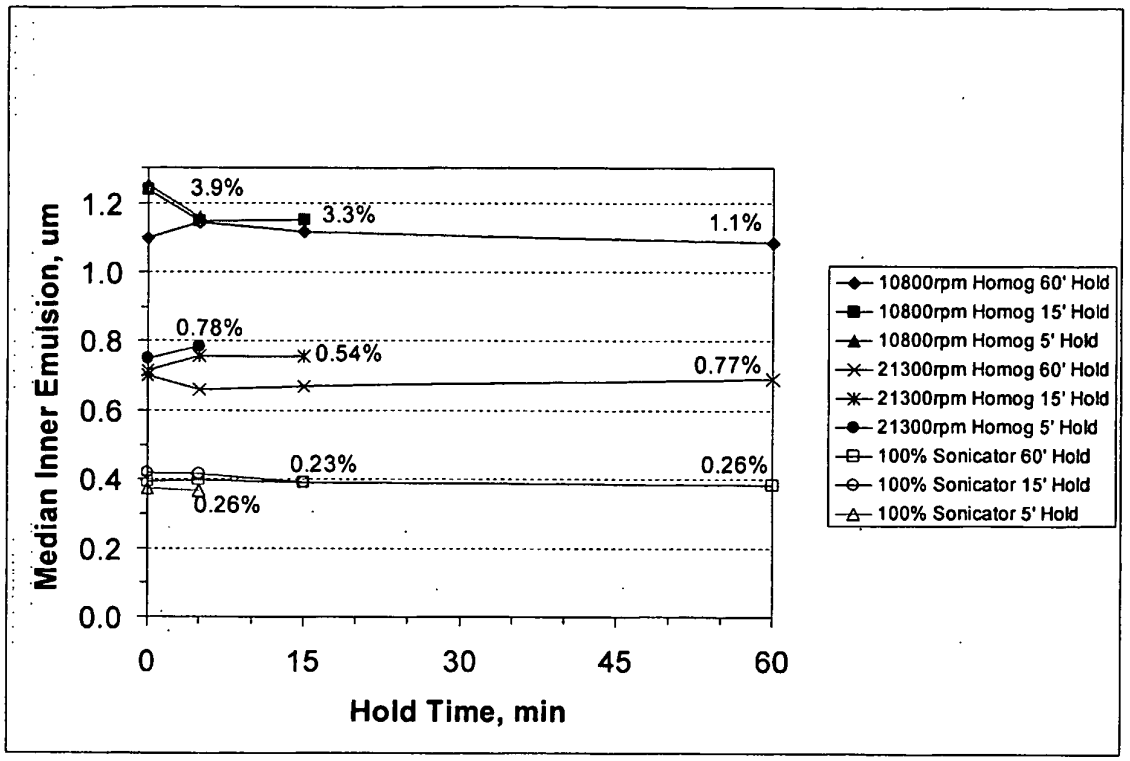
Median Inner Emulsion, um
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
15
30
45
60
Hold Time, min
3.9%
3.3%
1.1%
0.78%
0.54%
0.77%
0.23%
0.26%
0.26%
10800rpm Homog 60' Hold
10800rpm Homog 15' Hold
10800rpm Homog 5' Hold
21300rpm Homog 60' Hold
21300rpm Homog 15' Hold
21300rpm Homog 5' Hold
100% Sonicator 60' Hold
100% Sonicator 15' Hold
100% Sonicator 5' Hold

FIG. 9

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 03020245 A **[0004]**
- US 5424286 A, Eng **[0019]**
- US 419430 P **[0033]**

Non-patent literature cited in the description

- **Lucke et al.** Peptide Acylation by Poly(α-Hydroxy Esters). *Pharmaceutical Research,* February 2002, vol. 19 (2), 175-181 **[0075]**