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(73) Patenthaver: **Mapi Pharma Limited, 16 Einstein Street , Weizmann Science Park , P.O. Box 4113, 74140 Ness Ziona, Israel**

(72) Opfinder: **MAROM, Ehud, 7 Aluf Kalman Magen Street, 6107079 Tel Aviv, Israel**
RUBNOV, Shai, 37 Gruzenberg Street, 6581141 Tel Aviv, Israel

(74) Fuldmægtig i Danmark: **Novagraaf Brevets, Bâtiment O2, 2 rue Sarah Bernhardt CS90017, F-92665 Asnières-sur-Seine cedex, Frankrig**

(54) Benævnelse: **DEPOTSYSTEM DER OMFATTER GLATIRAMERACETAT**

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Fortsættes ...

DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to methods for producing a long acting parenteral pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable biodegradable carrier selected from the group consisting of poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA), long acting parenteral pharmaceutical compositions obtainable by such methods and their uses in the treatment of multiple sclerosis.

BACKGROUND OF THE INVENTION

Glatiramer Acetate

[0002] Copolymer-1, also known as glatiramer acetate and marketed under the tradename Copaxone®, comprises the acetate salts of polypeptides containing L-glutamic acid, L-alanine, L-tyrosine and L-lysine. The average molar fractions of the amino acids are 0.141, 0.427, 0.095 and 0.338, respectively, and the average molecular weight of copolymer-1 is between 4,700 and 11,000 daltons. Chemically, glatiramer acetate is designated L-glutamic acid polymer with L-alanine, L-lysine and L-tyrosine, acetate (salt). Its structural formula is:

(Glu, Ala, Lys, Tyr)$x$$CH_3COOH$

$(C_5H_9NO_4\_C_3H_7NO_2\_C_6H_{14}N_2O_2\_C_9H_{11}NO_3)xC_2H_4O_2$ [CAS - 147245-92-9], approx. ratio $Glu_{14}Ala_{43}Tyr_{10}Lyz_{34}x(CH_3COOH)_{20}$. Copaxone® is a clear, colorless to slightly yellow, sterile, nonpyrogenic solution for subcutaneous injection. Each milliliter contains 20mg of glatiramer acetate and 40mg of mannitol. The pH range of the solution is approximately 5.5 to 7.0.

Mechanism of Action

[0003] Glatiramer acetate is a random polymer (average molecular mass 6.4 kD) composed of four amino acids that are found in myelin basic protein. The mechanism of action for glatiramer acetate is unknown, although some important immunological properties of this copolymer have emerged. Administration of copolymer-1 shifts the population of T cells from pro-inflammatory Th1 cells to regulatory Th2 cells that suppress the inflammatory response (FDA Copaxone® label). Given its resemblance to myelin basic protein, copolymer-1 may also act as a decoy, diverting an autoimmune response against myelin. The integrity of the blood-brain barrier,

however, is not appreciably affected by copolymer-1, at least not in the early stages of treatment.

[0004] Copolymer-1 is a non-autoantigen which has been demonstrated to suppress experimental allergic encephalomyelitis (EAE) induced by various encephalitogens including mouse spinal cord homogenate (MSCH) which includes all myelin antigens, such as myelin basic protein (MBP) (Sela M et al., Bull Inst Pasteur (1990) 88 303-314), proteolipid protein (PLP) (Teitelbaum D et al., J Neuroimmunol (1996) 64 209-217) and myelin oligodendrocyte glycoprotein (MOG) (Ben-Nun A et al., J Neurol (1996) 243 (Suppl 1) S14-S22) in a variety of species. EAE is an accepted model for multiple sclerosis.

[0005] Copolymer-1 has been demonstrated to be active when injected subcutaneously, intraperitoneally, intravenously or intramuscularly (Teitelbaum D et al., Eur J Immunol (1971) 1 242-248; Teitelbaum D et al., Eur J Immunol (1973) 3 273-279). In phase III clinical trials, daily subcutaneous injections of copolymer-1 were found to slow the progression of disability and reduce the relapse rate in exacerbating-remitting multiple sclerosis (Johnson KP, Neurology (1995) 1 65-70; www.copaxone.com). Copolymer-1 therapy is presently limited to daily subcutaneous administration. Treatment with copolymer-1 by ingestion or inhalation is disclosed in US 6,214,791, but these routes of administration have not been shown to attain clinical efficacy in human patients.

Efficacy

[0006] Evidence supporting the effectiveness of glatiramer acetate in decreasing the frequency of relapses in patients with Relapsing-Remitting Multiple Sclerosis (RR MS) derives from two placebo-controlled trials, both of which used a glatiramer acetate dose of 20 mg/day. No other dose or dosing regimen has been studied in placebo-controlled trials of RR MS (www.copaxone.com). A comparative trial of the approved 20 mg dose and the 40 mg dose showed no significant difference in efficacy between these doses (The 9006 trial; Cohen JA et al., Neurology (2007) 68 939-944). Various clinical trials in glatiramer acetate are on-going. These include studies with a higher dose of glatiramer acetate (40 mg - the FORTE study); studies in Clinically Isolated Syndrome patients (the PreCISe study) as well as numerous combination and induction protocols, in which glatiramer acetate is given together with or following another active product.

Side effects

[0007] Currently, all specifically approved treatments of multiple sclerosis involve self injection of the active substance. Frequently observed injection-site problems include irritation, hypersensitivity, inflammation, pain and even necrosis (in the case of interferon 1β treatment) and a low level of patient compliance.

[0008] Side effects generally include a lump at the injection site (injection site reaction), aches, fever, and chills. These side effects are generally mild in nature. Occasionally a reaction occurs minutes after injection in which there is flushing, shortness in breath, anxiety and rapid heartbeat. These side effects subside within thirty minutes. Over time, a visible dent at the injection site due to the local destruction of fat tissue, known as lipoatrophy, may develop. Therefore, an alternative method of administration is desirable.

[0009] More serious side effects have been reported for glatiramer acetate, according to the FDA's prescribing label, these include serious side effects to the body's cardiovascular system, digestive system (including liver), hemic and lymphatic system, musculoskeletal system, nervous system, respiratory system, special senses (in particular the eyes), urogenital system; also reported have been metabolic and nutritional disorders; however a link between glatiramer acetate and these adverse effects has not been definitively established (FDA Copaxone® label).

Depot Systems

[0010] The parenteral route by intravenous (IV), intramuscular (IM), or subcutaneous (SC) injection is the most common and effective form of delivery for small as well as large molecular weight drugs. However, pain, discomfort and inconvenience due to needle sticks makes this mode of drug delivery the least preferred by patients. Therefore, any drug delivery technology that can at a minimum reduce the total number of injections is preferred. Such reductions in frequency of drug dosing in practice may be achieved through the use of injectable depot formulations that are capable of releasing drugs in a slow but predictable manner and consequently improve compliance. For most drugs, depending on the dose, it may be possible to reduce the injection frequency from daily to once or twice monthly or even longer (6 months). In addition to improving patient comfort, less frequent injections of drugs in the form of depot formulations smoothes out the plasma concentration-time profile by eliminating the hills and valleys. Such smoothing out of plasma profiles has the potential to not only boost the therapeutic benefit in most cases, but also to reduce any unwanted events, such as immunogenicity etc. often associated with large molecular weight drugs.

[0011] Microparticles, implants and gels are the most common forms of biodegradable polymeric devices used in practice for prolonging the release of drugs in the body. Microparticles are suspended in an aqueous media right before injection and one can load as much as 40% solids in suspensions. Implant/rod formulations are delivered to SC/IM tissue with the aid of special needles in the dry state without the need for an aqueous media. This feature of rods/implants allows for higher masses of formulation, as well as drug content to be delivered. Further, in the rods/implants, the initial burst problems are minimized due to much smaller area in implants compared to the microparticles. Besides biodegradable systems, there are non-biodegradable implants and infusion pumps that can be worn outside the body. Non-biodegradable implants require a doctor's visit not only for implanting the device into the SC/IM

tissue but also to remove them after the drug release period.

[0012] Injectable compositions containing microparticle preparations are particularly susceptible to problems. Microparticle suspensions may contain as much as 40% solids as compared with 0.5-5% solids in other types of injectable suspensions. Further, microparticles used in injectable depot products, range in size up to about 250µm (average, 60-100µm), as compared with a particle size of less than 5µm recommended for IM or SC administration. The higher concentrations of solids, as well as the larger solid particle size require larger size of needle (around 18-21 gauge) for injection. Overall, despite the infrequent uses of larger and uncomfortable needles, patients still prefer less frequently administered dosage forms over everyday drug injections with a smaller needle.

[0013] Biodegradable polyesters of poly(lactic acid) (PLA) and copolymers of lactide and glycolide referred to as poly(lactide-co-glycolide) (PLGA) are the most common polymers used in biodegradable dosage forms. PLA is hydrophobic molecule and PLGA degrades faster than PLA because of the presence of more hydrophilic glycolide groups. These biocompatible polymers undergo random, non-enzymatic, hydrolytic cleavage of the ester linkages to form lactic acid and glycolic acid, which are normal metabolic compounds in the body. Resorbable sutures, clips and implants are the earliest applications of these polymers. Southern Research Institute developed the first synthetic, resorbable suture (Dexon®) in 1970. The first patent describing the use of PLGA polymers in a sustained release dosage form appeared in 1973 (US 3,773,919).

[0014] US 7,195,778 B2 relates to a drug delivery device for oral administration, the device comprising a core comprising the drug (*e.g., inter alia,* copaxone) and a cylindrical plug embedded in the core. The core containing the embedded cylindrical plug is at least partially coated with a coating which is impermeable to the drug and the impermeable coating may be coated with a drug coating. The drug in the drug coating may be the same or different from the drug in the core and the drug coating may be one that erodes to provide a sustained release of the drug. As an example of an eroding drug coating, one that comprises various grades of polyvinylpyrrolidone, hydroxypropyl cellulose or hydroxypropylmethylcellulose is described.

[0015] Today, PLGA polymers are commercially available from multiple suppliers; Alkermes (Medisorb polymers), Absorbable Polymers International [formerly Birmingham Polymers (a Division of Durect)], Purac and Boehringer Ingelheim. Besides PLGA and PLA, natural cellulosic polymers such as starch, starch derivatives, dextran and non-PLGA synthetic polymers are also being explored as biodegradable polymers in such systems.

[0016] At present no long acting dosage forms of glatiramer acetate are available. This is a huge unmet medical need, as these formulations would be extremely beneficial to many patients, particularly to those with neurological symptoms or physical disabilities.

SUMMARY OF THE INVENTION

[0017] The invention relates to the embodiments as described in the claims.

[0018] In particular, the invention relates to the following:

1. 1. A method for producing a long acting parenteral pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable biodegradable carrier selected from the group consisting of poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA); the method comprising the steps of:
    1. (i) dispersing a concentrated solution of glatiramer acetate in a solution of the pharmaceutically acceptable biodegradable carrier in water-immiscible volatile organic solvent, thereby obtaining a water-in-oil emulsion,
    2. (ii) dispersing said water-in-oil emulsion in a continuous external water phase containing a surfactant to form water-in oil-in water double emulsion droplets,
    3. (iii) evaporating said organic solvent, thereby obtaining solid microparticles,
    4. (iv) collecting said microparticles by filtration or centrifugation.
2. 2. The method of item 1, further comprising the step of
   (v) washing said collected microparticles with purified water.
3. 3. The method of item 1 or 2, further comprising the step of
   (vi) lyophilizing said collected microparticles.
4. 4. The method of item 3, further comprising the step of
   (vii) reconstituting said lyophilized microparticles.
5. 5. The method of items 1 to 4, wherein the pharmaceutically acceptable biodegradable carrier is PLGA.
6. 6. The method of items 1 to 5, wherein the surfactant is selected from the group consisting of polyvinyl alcohol (PVA), polysorbate, polyethylene oxide-polypropylene oxide block copolymers and cellulose esters.
7. 7. The method of items 1 to 6, wherein lyophilizing said microparticles is in the presence of a cryoprotectant, preferably mannitol.
8. 8. A long acting parenteral pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable biodegradable carrier selected from the group consisting of poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA), obtainable by the method according to any one of items 1 to 7.
9. 9. The pharmaceutical composition of item 8, wherein the pharmaceutically acceptable biodegradable carrier is PLGA.
10. 10. The pharmaceutical composition of items 8 or 9, wherein the glatiramer acetate comprises L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in molar ratios of 0.14 glutamic acid, 0.43 alanine, 0.10 tyrosine and 0.33 lysine.
11. 11. The pharmaceutical composition according to items 8 to 10, which is in depot form suitable for implantation at a medically acceptable location in a subject in need thereof and/or which is suitable for a dosing schedule from once every 2 weeks to once monthly.
12. 12. The pharmaceutical composition of items 8 to 11 for use in the treatment of multiple sclerosis.
13. 13. The pharmaceutical composition for use in the treatment of multiple sclerosis

according to item 12, wherein the composition provides equal or superior therapeutic efficacy to the commercially available daily injectable dosage forms of glatiramer acetate, with reduced incidence and/or severity of side effects at the local and/or systemic level; and

wherein the composition further provides prolonged release or prolonged action of glatiramer in a subject as compared to a substantially similar dose of an immediate release formulation of glatiramer acetate.

14. 14. The pharmaceutical composition for use in the treatment of multiple sclerosis according to item 12 or 13, wherein the glatiramer acetate is administered in combination with at least one other active agent.

BRIEF DESCRIPTION OF THE FIGURES

[0019]

Figure 1. Release of glatiramer acetate from PLGA microparticulate formulations MPG-02 - 07 in PBS at 37°C. Data represented are normalized to standard peptide solution stored in same conditions.

Figure 2. Release of glatiramer acetate from PLGA microparticulate formulations MPG-05R, 08-11 and tocopheryl succinate salt of glatiramer (1:1) in PBS at 37°C. Data represented are normalized to standard peptide solution stored in same conditions.

Figure 3. Release of glatiramer acetate from PLGA microparticulate formulations MPG-12 - 15 in PBS at 37°C. The data presented are normalized to standard peptide solution stored in same conditions.

Figure 4. Release of glatiramer acetate from PLGA microparticulate formulations MPG-14SU-1 and MPG-15SU-1 in vitro in PBS at 37°C, pH 7.4.

Figure 5. Release of glatiramer acetate from PLGA microparticulate formulations MPG-14SU-2 and MPG-15SU-2 in vitro in PBS at 37°C, pH 7.4.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The present invention provides methods for producing a long acting parenteral pharmaceutical composition comprising glatiramer acetate and a pharmaceutically acceptable biodegradable carrier selected from the group consisting of poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA); the method comprising the steps of:

1. (i) dispersing a concentrated solution of glatiramer acetate in a solution of the pharmaceutically acceptable biodegradable carrier in water-immiscible volatile organic solvent, thereby obtaining a water-in-oil emulsion,
2. (ii) dispersing said water-in-oil emulsion in a continuous external water phase containing a surfactant to form water-in oil-in water double emulsion droplets,
3. (iii) evaporating said organic solvent, thereby obtaining solid microparticles,
4. (iv) collecting said microparticles by filtration or centrifugation.

[0021] The long acting parenteral pharmaceutical compositions obtainable by said methods afford equal or superior therapeutic efficacy to the daily injections and thus result in improved patient compliance. In addition to providing the same therapeutic efficacy, the long acting injections or implants reduce the glatiramer side effects (local and/or systemic), resulting from frequent injections.

[0022] The long acting parenteral pharmaceutical composition obtainable by the methods of the invention may comprise a therapeutically effective amount of glatiramer acetate or any other pharmaceutically acceptable salt of glatiramer. The term "parenteral" as used herein refers to routs selected from subcutaneous (SC), intravenous (IV), intramuscular (IM), intradermal (ID), intraperitoneal (IP) and the like. The term "therapeutically effective amount" as used herein is intended to qualify the amount of copolymer that will achieve the goal of alleviation of the symptoms of multiple sclerosis.
However, it is understood that the amount of the copolymer administered will be determined by a physician, according to various parameters including the chosen route of administration, the age, weight, and the severity of the patient's symptoms. The therapeutically effective amount of the at least one copolymer may range from 1 mg to 500 mg/day. Alternatively, such therapeutically effective amounts of the at least one copolymer may range from 20 mg to 100 mg/day.

[0023] The long acting pharmaceutical composition obtainable by the methods of the invention may comprise a therapeutically effective amount of glatiramer acetate or any other pharmaceutically acceptable salt of glatiramer in a depot form suitable for administration at a medically acceptable location in a subject in need thereof. The term "long acting" as used herein refers to a composition which provides prolonged, sustained or extended release of the glatiramer salt to the general systemic circulation of a subject or to local sites of action in a subject. This term may further refer to a composition which provides prolonged, sustained or extended duration of action (pharmacokinetics) of the glatiramer salt in a subject. In particular, the long acting pharmaceutical compositions obtainable by the methods of the invention may provide a dosing regimen which ranges from once weekly to once every 6 months. The dosing regimen may range from once a week, twice monthly (approximately once in every 2 weeks) to once monthly.

[0024] The depot formulations obtainable by the methods of the invention may include

suspensions of glatiramer or a pharmaceutically acceptable salt thereof in water, oil or wax phase; poorly soluble polyelectrolyte complexes of glatiramer or a pharmaceutically acceptable salt thereof; "in-situ" gel-forming matrices based on the combination of water-miscible solvent with glatiramer or a pharmaceutically acceptable salt thereof; and biodegradable polymeric microparticles with incorporated glatiramer or a pharmaceutically acceptable salt thereof. In particular, the compositions obtainable by the methods of the invention are in the form of injectable microparticles wherein the glatiramer or pharmaceutically acceptable salt thereof is entrapped in a biodegradable or non-biodegradable carrier. The microparticulate compositions obtainable by the methods of the invention may comprise a water-in oil-in water double emulsion. The microparticulate composition obtainable by the methods of the invention may comprise an internal aqueous phase comprising glatiramer or any pharmaceutically acceptable salt thereof, an oil phase or water-immiscible phase comprising a biodegradable or non-biodegradable polymer and an external aqueous phase. The external aqueous phase may further comprise a surfactant, preferably polyvinyl alcohol (PVA), polysorbate, polyethylene oxide-polypropylene oxide block copolymers or cellulose esters. The terms "oil phase" and "water-immiscible phase" may be used interchangeably herein.

[0025] The present invention further provides a long acting pharmaceutical composition obtainable by the methods of the invention for use in treating multiple sclerosis. Also disclosed herein for illustration purposes only (i.e. aspect not according to the invention) is a method of treating multiple sclerosis, by administration into an individual in need thereof, glatiramer acetate or any other pharmaceutically acceptable salt of glatiramer in a depot form. The term "treating" as used herein refers to suppression or alleviation of symptoms after the onset of multiple sclerosis. Common symptoms after the onset of multiple sclerosis include, but are not limited to, reduced or loss of vision, stumbling and uneven gait, slurred speech, as well as urinary frequency and incontinence. In addition, multiple sclerosis can cause mood changes and depression, muscle spasms and severe paralysis. The "subject" to which the drug is administered is a mammal, preferably, but not limited to, a human. The term "multiple sclerosis" as used herein refers to an auto-immune disease of the central nervous system which is accompanied by one or more of the symptoms described hereinabove.

[0026] The term "glatiramer acetate" as used herein refers to a compound formerly known as Copolymer 1 that is sold under the trade name Copaxone® and consists of the acetate salts of synthetic polypeptides, containing four naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine, and L-lysine with an average molar fraction of 0.141, 0.427, 0.095, and 0.338, respectively. The average molecular weight of glatiramer acetate in Copaxone® is 4,700-11,000 daltons (FDA Copaxone® label) and the number of amino acid ranges between 15 to 100 amino acids. The term also refers to chemical derivatives and analogues of the compound. Typically the compound is prepared and characterized as specified in any of US Patent Nos. 5,981,589; 6,054,430; 6,342,476; 6,362,161; 6,620,847; and 6,939,539.

[0027] The composition obtainable by the methods of the invention may comprise any other pharmaceutically acceptable salt of glatiramer including, but not limited to, sulfate, pyrosulfate,

bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, hydrochloride, hydrobromide, hydroiodide, acetate, nitrate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caprate, heptanoate, propiolate, oxalate, malonate, succinate, tocopheryl succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, terephthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, β-hydroxybutyrate, glycollate, tartrate, methanesulfonate, propanesulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, mandelate and the like salts.

[0028] The copolymers can be made by any procedure available to one of skill in the art. For example, the copolymers can be made under condensation conditions using the desired molar ratio of amino acids in solution, or by solid phase synthetic procedures. Condensation conditions include the proper temperature, pH, and solvent conditions for condensing the carboxyl group of one amino acid with the amino group of another amino acid to form a peptide bond. Condensing agents, for example, dicyclohexylcarbodiimide, can be used to facilitate the formation of the peptide bond.

[0029] Blocking groups can be used to protect functional groups, such as the side chain moieties and some of the amino or carboxyl groups against undesired side reactions. The process disclosed in U.S. Patent No. 3,849,550 can be used for preparing the copolymers. For example, the N-carboxyanhydrides of tyrosine, alanine, γ- benzyl glutamate and N, ε-trifluoroacetyl-lysine may be polymerized at ambient temperatures in anhydrous dioxane with diethylamine as an initiator. The γ-carboxyl group of the glutamic acid can be deblocked by hydrogen bromide in glacial acetic acid. The trifluoroacetyl groups are removed from lysine by one molar piperidine. One of skill in the art readily understands that the process can be adjusted to make peptides and polypeptides containing the desired amino acids, that is, three of the four amino acids in Copolymer 1, by selectively eliminating the reactions that relate to any one of glutamic acid, alanine, tyrosine, or lysine. U.S. Patent Nos. 6,620,847; 6,362,161; 6,342,476; 6,054,430; 6,048,898 and 5,981,589, disclose improved methods for preparing glatiramer acetate (Cop-1). As used herein, the terms "ambient temperature" and "room temperature" typically means a temperature ranging from 20°C to 26°C.

[0030] The molecular weight of the copolymers can be adjusted during polypeptide synthesis or after the polymers have been made. To adjust the molecular weight during polypeptide synthesis, the synthetic conditions or the amounts of amino acids are adjusted so that synthesis stops when the polypeptide reaches the approximate desired length. After synthesis, polypeptides with the desired molecular weight can be obtained by any available size selection procedure, such as chromatography of the polypeptides on a molecular weight sizing column or gel, and collection of the molecular weight ranges desired. The present polypeptides can also be partially hydrolyzed to remove high molecular weight species, for example, by acid or enzymatic hydrolysis, and then purified to remove the acid or enzymes.

[0031] Copolymers with a desired molecular weight may be prepared by a process which

includes reacting a protected polypeptide with hydrobromic acid to form a trifluoroacetyl-polypeptide having the desired molecular weight profile. The reaction is performed for a time and at a temperature which is predetermined by one or more test reactions. During the test reaction, the time and temperature are varied and the molecular weight range of a given batch of test polypeptides is determined. The test conditions which provide the optimal molecular weight range for that batch of polypeptides are used for the batch. Thus, a trifluoroacetyl-polypeptide having the desired molecular weight profile can be produced by a process which includes reacting the protected polypeptide with hydrobromic acid for a time and at a temperature predetermined by the test reaction.
The trifluoroacetyl-polypeptide with the desired molecular weight profile is then further treated with an aqueous piperidine solution to form a deprotected polypeptide having the desired molecular weight.

[0032] A test sample of protected polypeptide from a given batch may be reacted with hydrobromic acid for 10-50 hours at a temperature of 20-28°C. The best conditions for that batch are determined by running several test reactions. For example, the protected polypeptide may be reacted with hydrobromic acid for 17 hours at a temperature of 26°C.

[0033] The dosage forms obtainable by the methods of the invention may include biodegradable injectable depot systems such as, PLGA based injectable depot systems; non-PLGA based injectable depot systems, and injectable biodegradable gels or dispersions. The term "biodegradable" as used herein refers to a component which erodes or degrades at its surfaces over time due, at least in part, to contact with substances found in the surrounding tissue fluids, or by cellular action. In particular, the biodegradable component may be a polymer such as lactic acid-based polymers such as polylactides e.g. poly (D,L-lactide) i.e. PLA; glycolic acid-based polymers such as polyglycolides (PGA) e.g. Lactel® from Durect; poly (D,L-lactide-co-glycolide) i.e. PLGA, (Resomer® RG-504, Resomer® RG-502, Resomer® RG-504H, Resomer® RG- 502H, Resomer® RG-504S, Resomer® RG-502S, from Boehringer, Lactel® from Durect); polycaprolactones such as Poly(e-caprolactone) i.e. PCL (Lactel® from Durect); polyanhydrides; poly(sebacic acid) SA; poly(ricenolic acid) RA; poly(fumaric acid), FA; poly(fatty acid dimmer), FAD; poly(terephthalic acid), TA; poly(isophthalic acid), IPA; poly(p-{carboxyphenoxy}methane), CPM; poly(p- {carboxyphenoxy} propane), CPP; poly(p-{carboxyphenoxy}hexane)s CPH; polyamines, polyurethanes, polyesteramides, polyorthoesters {CHDM: cis/trans- cyclohexyl dimethanol, HD:1,6-hexanediol. DETOU: (3,9-diethylidene-2,4,8,10- tetraoxaspiro undecane)}; polydioxanones; polyhydroxybutyrates; polyalkylene oxalates; polyamides; polyesteramides; polyurethanes; polyacetals; polyketals; polycarbonates; polyorthocarbonates; polysiloxanes; polyphosphazenes; succinates; hyaluronic acid; poly(malic acid); poly(amino acids); polyhydroxyvalerates; polyalkylene succinates; polyvinylpyrrolidone; polystyrene; synthetic cellulose esters; polyacrylic acids; polybutyric acid; triblock copolymers (PLGA-PEG-PLGA), triblock copolymers (PEG-PLGA-PEG), poly (N-isopropylacrylamide) (PNIPAAm), poly (ethylene oxide)- poly (propylene oxide)-poly (ethylene oxide) tri-block copolymers (PEO-PPO-PEO), poly valeric acid; polyethylene glycol; polyhydroxyalkylcellulose; chitin; chitosan; polyorthoesters and copolymers,

terpolymers; lipids such as cholesterol, lecithin; poly(glutamic acid-co-ethyl glutamate) and the like, or mixtures thereof.

[0034] The compositions obtainable by the methods of the invention may comprise a biodegradable polymer selected from, but not limited to, PLGA, PLA, PGA, polycaprolactone, polyhydroxybutyrate, polyorthoesters, polyalkaneanhydrides, gelatin, collagen, oxidized cellulose, polyphosphazene and the like.

[0035] The biodegradable polymer may be a lactic acid-based polymer, such as polylactide, or poly (D, L-lactide-co-glycolide) i.e. PLGA. The biodegradable polymer may be present in an amount between 10% to 98% w/w of the composition. The lactic acid-based polymer may have a monomer ratio of lactic acid to glycolic acid in the range of 100:0 to 0:100, such as 100:0 to 10:90 and may have an average molecular weight of from 1,000 to 200,000 daltons. However, it is understood that the amount of biodegradable polymer is determined by parameters such as the duration of use and the like.

[0036] The compositions obtainable by the methods of the invention may further comprise one or more pharmaceutically acceptable excipient(s) selected from co-surfactants, solvents/co-solvents, water immiscible solvents, water, water miscible solvents, oily components, hydrophilic solvents, emulsifiers, preservatives, antioxidants, anti-foaming agents, stabilizers, buffering agents, pH adjusting agents, osmotic agents, channel forming agents, osmotic adjustment agents, or any other excipient known in the art. Suitable co-surfactants include polyethylene glycols, polyoxyethylene- polyoxypropylene block copolymers known as "poloxamer", polyglycerin fatty acid esters such as decaglyceryl monolaurate and decaglyceryl monomyristate, sorbitan fatty acid ester such as sorbitan monostearate, polyoxyethylene sorbitan fatty acid ester such as polyoxyethylene sorbitan monooleate (Tween), polyethylene glycol fatty acid ester such as polyoxyethylene monostearate, polyoxyethylene alkyl ether such as polyoxyethylene lauryl ether, polyoxyethylene castor oil and hardened castor oil such as polyoxyethylene hardened castor oil, and the like or mixtures thereof. Suitable solvents/co-solvents include alcohols, triacetin, dimethyl isosorbide, glycofurol, propylene carbonate, water, dimethyl acetamide, and the like or mixtures thereof. Suitable anti-foaming agents include silicon emulsions or sorbitan sesquioleate. Suitable stabilizers to prevent or reduce the deterioration of the components in the compositions obtainable by the methods of the invention include antioxidants such as glycine, α-tocopherol or ascorbate, BHA, BHT, and the like or mixtures thereof. Suitable tonicity modifiers include mannitol, sodium chloride, and glucose. Suitable buffering agents include acetates, phosphates, and citrates with suitable cations.

[0037] The compositions obtainable by the methods of the invention can be prepared by any manner known in the art. An example is the incorporation of the glatiramer or salt thereof copolymer into a colloidal delivery system, e.g., biodegradable microparticles, thus allowing release retardation by diffusion through polymeric walls of the particle and by polymer degradation in water media or biological fluids in the body. According to one aspect of the present invention, the long acting parenteral pharmaceutical compositions comprising glatiramer acetate and a pharmaceutically acceptable biodegradable carrier selected from the

group consisting of poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) are prepared in the form of injectable microparticles by a process known as the "double emulsification". Briefly, the concentrated solution of the water-soluble copolymer is dispersed in a solution of the biodegradable or non-biodegradable polymer in water-immiscible volatile organic solvent (e.g. methylene chloride, chloroform and the like). The thus obtained "water-in-oil" (w/o) emulsion is then dispersed in a continuous external water phase containing surfactant (e.g. polyvinyl alcohol - PVA, polysorbates, polyethylene oxide-polypropylene oxide block copolymers, cellulose esters and the like) to form "water-in oil-in water (w/o/w) double emulsion" droplets. After evaporation of the organic solvent, the microparticles solidify and are collected by filtration or centrifugation. The collected microparticles (MPs) are washed with purified water to eliminate most of the surfactant and non-bonded peptide and centrifugated again. The washed MPs are collected and lyophilized without additives or with the addition of cryoprotectant (mannitol) to facilitate their subsequent reconstitution.

[0038] The particle size of the "water-in oil-in water (w/o/w) double emulsion" can be determined by various parameters including the amount of applied force at this step, the speed of mixing, surfactant type and concentration, etc. Suitable particle sizes range from 1 to 100 µm.

[0039] Suitable forms of the compositions obtainable by the methods of the invention include biodegradable or non-biodegradable microspheres, implantable rods, implantable capsules, and implantable rings. Other suitable forms are prolonged release gel depot and erodible matrices. Suitable implantable systems are described for example in US 2008/0063687. Implantable rods can be prepared as is known in the art using suitable micro-extruders such as those described for example in http://www.randcastle.com/prodinfo.html.

[0040] According to the principles of the present invention, the long acting pharmaceutical compositions obtainable by the methods of the invention provide equal or superior therapeutic efficacy to the commercially available daily injectable dosage forms, with reduced incidence of side effects and with reduced severity of side effects at the local and/or systemic level. In some embodiments, the compositions of the present disclosure provide prolonged release or prolonged action of glatiramer in a subject as compared to a substantially similar dose of an immediate release formulation of glatiramer acetate.

[0041] Encompassed by the present invention is the long acting pharmaceutical compositions obtainable by the methods of the invention for use in the treatment of multiple sclerosis wherein the glatiramer acetate is administered in combination with at least one other active agent. Active agents include interferons, e.g. pegylated or non-pegylated α-interferons, or β-interferons, e.g. interferon β-1a or interferon β-1b, or τ-interferons; immunosuppressants with optionally antiproliferative/antineoplastic activity, e.g. mitoxantrone, methotrexate, azathioprine, cyclophosphamide, or steroids, e.g. methylprednisolone, prednisone or dexamethasone, or steroid-secreting agents, e.g. ACTH; adenosine deaminase inhibitors, e.g. cladribine; IV immunoglobulin G (e.g. as disclosed in Neurology, 1998, May 50(5):1273-81) monoclonal antibodies to various T-cell surface markers, e.g. natalizumab (ANTEGREN®) or alemtuzumab;

TH2 promoting cytokines, e.g. IL-4, IL-10, or compounds which inhibit expression of TH1 promoting cytokines, e.g. phosphodiesterase inhibitors, e.g. pentoxifylline; antispasticity agents including baclofen, diazepam, piracetam, dantrolene, lamotrigine, rifluzole, tizanidine, clonidine, beta blockers, cyproheptadine, orphenadrine or cannabinoids; AMPA glutamate receptor antagonists, e.g. 2,3-dihydroxy-6-nitro-7- sulfamoylbenzo(f)quinoxaline, [1 ,2,3,4,-tetrahydro-7-morpholin-yl-2,3-dioxo-6- (trifluoromethyl)quinoxalin-i -yl]methylphosphonate, 1 -(4-aminophenyl)-4-methyl-7,8- methylene-dioxy-5H-2,3-benzodiazepine, or (-)1 -(4-aminophenyl)-4-methyl-7,8- methylene-dioxy-4,5-dihydro-3-methylcarbamoyl-2,3-benzodiazepine; inhibitors of VCAM-1 expression or antagonists of its ligand, e.g. antagonists of the α4β1 integrin VLA-4 and/or α-4-β-7 integrins, e.g. natalizumab (ANTEGREN®); anti-macrophage migration inhibitory factor (Anti-MIF); xii) Cathepsin S inhibitors; xiii) mTor inhibitors. In particular, the other active agent may be FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl] propane-1 ,3-diol; fingolimod) belonging to the class of immunosuppressants.

[0042] The following examples are presented in order to more fully illustrate certain embodiments of the invention.

Examples

Example 1: General preparation methods

PLGA based injectable depot particles

[0043] Microparticles were prepared by solvent extraction/evaporation method (single emulsion). A solution of 50:50, dichloromethane/ethanol containing 250mg PLGA and 200 mg glatiramer acetate was slowly poured into an aqueous solution (200ml) containing 2% PVA and emulsified using a mechanical stirrer (300 rpm) at 25°C. The organic solvent was evaporated under stirring (100 rpm) for 2h. The thus formed microparticles were collected by centrifugation and washed with distilled water to remove excessive emulsifier. The final suspension was then freeze-dried to obtain a fine powder.

Polycaprolactone based injectable depot particles

[0044] Microparticles were prepared by solvent extraction/evaporation method (single emulsion). A solution of 70:30, dichloromethane/acetone containing 500mg polycaprolactone and 200 mg glatiramer acetate was slowly poured into an aqueous solution (200ml) containing 2% PVA, 1% Tween 80 and emulsified using mechanical stirrer (500 rpm) at 25°C. The organic

solvent was evaporated under stirring (300 rpm) for 4h. The formed microparticles were collected by centrifugation and washed with distilled water to remove excessive emulsifiers. The final suspension was then freeze dried to obtain a fine powder.

PLGA based implant-rods

[0045] PLGA based biodegradable rod shaped implants, 20mm in length and 2mm in diameter, were prepared by solvent extraction/evaporation method. A solution of 50:50, dichloromethane/ethanol containing 250mg PLGA and 200 mg glatiramer acetate was slowly poured into special rod shaped mold. The organic solvent was evaporated in vacuum oven during 12hrs at room temperature. Alternatively the rod shaped implant was prepared by extrusion of the mixture of 250 mg PLGA and 200 mg of glatiramer at 85-90°C, using a screw type extruder (Microtruder Rancastle RCP-0250 or similar), with die diameter 0.8 or 1.0 mm.

Example 2: Analytical method - assay of glatiramer acetate

Equipment

[0046]

Spectrophotometer

Analytical balance, capable of accurately weighing to 0.01 mg

Materials and Reagents

[0047]

Glatiramer acetate 83% as a reference standard

2, 4, 6-trinitrobenzenesulfonic acid (TNBS, picrylsulfonic acid, 170.5 mM) 5% in MeOH

0.1 M borate buffer pH 9.3 (sodium tetraborate decahydrate MW 381.37)

water, purified

volumetric pipettes for 0.5, 1.0, 2.0 and 7.0 mL

miscellaneous glassware.

Preparations

Preparation of glatiramer stock solution 400 µg/mL

[0048] 4.8 mg of glatiramer acetate (potency 83% as base for reference standard) were weighed into a 10ml volumetric flask. Approximately 7ml of 0.1M borate buffer were added to afford dissolution of the glatiramer acetate in ultrasonic bath. The solution was further diluted with 0.1M borate buffer to obtain glatiramer stock solution 400 µg/ml (as base).

Preparation of 0.25% TNBS working solution

[0049] Prior to the use, 5% stock solution of TNBS was diluted with water (20 times; e.g. 50 µl and 950 µl of water) to obtain 0.25% TNBS working solution.

Calibration curve standards preparation

[0050] Eight glatiramer calibration standard solutions (cSTD; 4ml each) were prepared according to Table 1.

Table 1. Standard solutions of glatiramer acetate

cSTD #	Concentration of glatiramer µg/ml (as base)	Volume of glatiramer stock solution (ml)	Volume of glatiramer Std 3 (ml)	Volume of 0.1M Borate buffer (ml)
Std 0	0		-	4
Std 1	2		0.4	3.6
Std 2	10		2	2
Std 3	20	0.2		3.8
Std 4	50	0.5		3.5
Std 5	100	1		3
Std 6	200	2		2
Std 7	400	4		-

Optical density measurement

[0051] 1.0 ml of each glatiramer calibration standard solutions, samples (in duplicate) and reagent blank (0.1M borate buffer) were transferred into 1.5 ml polypropylene centrifuge tube, to which 50 µl of 0.25% TNBS working solution was added. The solution was thoroughly mixed and kept at room temperature for 30 minutes. The optical densities of each of the obtained solutions were read at 420nm and 700nm and the difference of these densities were calculated to avoid error due to light dispersion in colloidal systems. A calibration curve for the selected range of concentrations was calculated.

Acceptance criteria

[0052] The difference between results for duplicate sample preparations was NMT 5%, calculated by following equation:

$$D = \frac{(Rspl1 - Rspl2) \times 2}{Rspl1 + Rspl2} \times 100,$$

in which Rspl1 is the result obtained for sample 1 and Rspl2 is the result obtained for sample 2.

Example 3: Preparation of PLGA microparticles loaded with glatiramer acetate

[0053] External (continuous) water phase: 30 ml of 0.75% NaCl solution in purified water, further containing 0.5% partially hydrolyzed (87-89%) polyvinyl alcohol (PVA) as a surfactant, 0.2% polysorbate-80 (Tween-80) for MPG-10 and 2% PVA for blank MP preparation.

[0054] Internal water phase (for peptide solution): 150-200 µl of purified water per 25-30 mg of glatiramer acetate. The glatiramer acetate was dissolved in water using an ultrasonic bath.

[0055] Organic polymeric solution (oil phase): 165-300 mg of PLGA in 2-5 mL of methylene chloride. Optionally, a counter-ion was further dissolved or dispersed in the organic phase.

Preparation proceedings

[0056] Water in oil (w/o) emulsion preparation: Internal water phase, containing dissolved glatiramer acetate, was mixed directly in the test tube with the oil phase containing PLGA solution in CH_2Cl_2. The mixture was thoroughly shaken and treated with ultrasonic indenter (titanium tip, max. power 120 watt, working power 10-15%, 3-5 cycles of 5 seconds). Cooling was optionally applied using ice or ice water to avoid boiling of methylene chloride.

[0057] Double emulsion (w/o/w) preparation: The thus obtained w/o emulsion of the glatiramer acetate solution in polymeric PLGA organic solution, was further treated with high shear mixer (small mixer, VDI-12, shaft diameter 10 mm, and bigger mixer, OMNI-1100, shaft diameter 18 mm) at various speeds for 30-120 seconds.

[0058] Solvent elimination: an open beaker with the thus formed double emulsion was placed on the magnetic plate stirrer and stirred for 3-4 hours at room temperature in a fume hood until all methylene chloride evaporated and the microparticles had solidified.

[0059] Centrifugation of microparticles: The suspension of solidified microparticles was centrifugated at 2000 - 5000g for 10 minutes, the supernatant was transferred into a separate vessel and analyzed for glatiramer acetate content to estimate the peptide incorporation and binding.

[0060] Washing of microparticles: the sedimented microparticles from the above described procedure were suspended in 10 ml of purified water using vortex and an ultrasonic bath and shaken or sonicated for 2-3 minutes. The suspension of the microparticles was centrifuged again at 2000 - 5000g for 10 minutes, the supernatant was transferred to a separate vessel and analyzed for glatiramer acetate content.

[0061] Lyophilization: The washed precipitate of microparticles was re-suspended in 3-5 ml of purified water or 5% mannitol, transferred to 10 ml pre-weighed glass vials, frozen using lyophilizer plate set at -37-43°C and lyophilized (main drying for 16-48 hours at -20°C and vacuum 0.05 bar, final drying for 12-16 hours at +20°C and 0.025 bar). Vials after lyophilizing were weighed, closed with bromobutyl rubber stoppers and stored at refrigerator storage conditions until use.

[0062] Particle size estimation: particle size of the microparticles was evaluated using light field and phase contrast microscopy (Leutz Orthoplan™, Germany) with objectives 40x and 10x and stage micrometer with range of 1-1000 µm.

[0063] All microparticle formulations were prepared using water phase containing 0.75% sodium chloride to increase the external osmotic pressure and to improve the incorporation of the water-soluble charged drug. Blank (empty) microparticles (first experiment) were obtained with 2% PVA as a surfactant, whereas for the preparation of all peptide loaded formulations 0.5% PVA was used.

[0064] Compositions and parameters of the preparation process are presented in Tables 2-5.
Table 2. PLGA microparticles for sustained release of glatiramer acetate (GA) (formulations 1-4)

	MP Blank	MPG-**01**	MPG-**02**	MPG-**03**	MPG-**04**
Internal water phase					
GA, mg		17	60.25	30	18.7
GA dry base, mg	0	14.11	50.0	24.9	15.5
Water for GA, µl		100	400	200	100+50 µl of 2%

	MP Blank	MPG-**01**	MPG-**02**	MPG-**03**	MPG-**04**
					PVA
Polymer in the oil phase					
PLGA RG 502H, mg	215	270			165
PLGA RG 502, mg			500	220	
Oil phase					
Tocopheryl succinate		100	120	50	65
Methylene chloride	2ml (2.3g)	4.5 g	9 g	3.2 g	3 g
External water phase					
PVA (2% or 0.5%)	23 ml 2%	65 ml 0.5%	60 ml 0.5%	30 ml 0.5%	30 ml 0.5%
NaCl	0	0.5 g	0.5 g	0.25 g	0.25 g
Preparation process description (processor, speed set, evaporation duration)	IKA VDI-12 #5 30 sec, evap. overnight RT magnetic stirrer	IKA VDI-12 #5 2 min, evap. overnight RT magnetic stirrer	IKA VDI-12 #5 2 min, evap. overnight RT magnetic stirrer	IKA VDI-12 #5 2 min, evap. 4 hr RT magnetic stirrer	IKA VDI-12 #5 2 min, evap. 4hr RT magnetic stirrer
Microparticles description	spherical MP 10-50 µm smooth surface	spherical MP 5-20 µm porous surface	aggregate MP 10-30 µm porous surface	spherical MP 10-15 µm slightly porous surface	spherical MP 10-15 µm slightly porous surface
Binding (association with MPs)		86%	34%	61%	70%

[0065] VWR VDI-12 high shear mixer from IKA Germany with small diameter of the stator (shaft 12mm) and speed range 8-30,000 rpm was set in position #5 (approx. 24,000 rpm). Short treatment (30 sec) of approx. 10% PLGA solution in methylene chloride in 2% PVA phase was used to prepare blank MP sample, which resulted in smooth spherical microparticles with relatively wide size distribution (10-50 µm). Due to foaming, further process was carried out at lower concentration of surfactant. Homogenization time was also extended (1 or 2 minutes

treatment) to obtain a more narrow size distribution.

[0066] Due to the presence of internal water phase in the double emulsion, all the microparticles prepared with the glatiramer peptide had visible inclusions and porosity signs either on the MP surface or inside the particle, when observed under optical microscope.

Table 3. PLGA microparticles for sustained release of glatiramer acetate (GA) (formulations 5-7)

	MPG-**05**	MPG-**06**	MPG-**07**
Internal water phase			
GA, mg	30.8	20	20
GA drv base, mg	25.6	16.6	16.6
Water for GA, µl	100+50 PVA 2%	166	175
Polymer in the oil phase			
PLGA RG 502H, mg	165		
PLGA RG 502, mg		200	250
Oil phase			
Dicetylphosphate		75	
Dimyristoylphosphatidyl glycerol sodium (DMPG Na)			60
Methylene chloride	2.7 g	2.5 g	3.25 g
External water phase			
PVA (0.5%)	30 ml	30 ml	30 ml
NaCl	0.25 g	0.25 g	0.25 g
Observations and comments		Flakes formed from DCP and GA	DMPG Na is poorly soluble in CH_2Cl_2
Preparation process description (processor, speed set, evaporation duration)	IKA VDI-12 #5 2 min, evaporation 4hr RT magetic stirrer	IKA VDI-12 #5 2 min, evaporation 4hr RT magnetic stirrer	IKA VDI-12 #5 2 min, evaporation 4hr RT magnetic stirrer
Microparticles description	spherical MP 10-15 µm slightly porous surface	irregular particles	spherical MP 5-15 µm
Binding (association with MPs)	81%	76%	84%

[0067] The formed glatiramer acetate loaded microparticles were centrifugated; the pellet was

re-suspended in purified water, washed and repeatedly centrifuged. Supernatant and in some cases washing water were analyzed for glatiramer acetate content. The centrifugated precipitate was re-suspended in purified water or 5% mannitol solution and lyophilized.

Table 4: PLGA microparticles for sustained release of glatiramer acetate (GA) (formulations 05R, 08-011 and tocopheryl succinate salt of glatiramer)

	MPG-**08**	MPG-**09**	MPG-**10**	MPG-**11**	MPG-**05R**	Tocopheryl succinate salt 1:1
Internal water phase						
GA, mg	30.1	30.1	30.1	30.1	30.9	30.1
GA dry base, mg	25.0	25.0	25.0	25.0	25.6	25.0
Water, µl	150	200	200	200	200	200
Polymer						
PLGA RG 502H, mg	165	165			165	
PLGA RG-503, mg			165	165		0
Oil phase						
Tocopheryl succinate, mg	20	50	20	50	0	10
Methylene chloride	2.7 g	2.7 g	2.7 g	3.2 g	3.7 g	2 g
External water phase						
Surfactant	30 ml 0.5% PVA	30 ml 0.5% PVA	30 ml 0.2% Tw80	30 ml 0.5% PVA	30 ml 0.5% PVA	20 ml H_2O
NaCl	0.25 g	0.25 g	0.25 g	0.25 g	0.25g	-
Preparation process description (processor, speed set, evaporation duration)	OMNI GLH #4 1 min, evap. 4hr RT mag. stirrer	OMNI GLH #4 1 min, evap. 4hr RT mag. stirrer	OMNI GLH #4 1 min, evap.4hr RT mag. stirrer	OMNI GLH #4 1 min, evap.4hr RT mag. stirrer	IKA VDI-12 #5 2 min, evap.4hr RT mag. stirrer	22 kHz Titanium indenter sonication 13W 60 sec
Microparticles description	Spherical MP 2-5 µm smooth	Spherical MP 1-3 µm smooth	Spherical MP 3-5&20 µm smooth	Spherical MP 2-4 µm smooth	Spherical MP 1-10 µm with inclusions	Spherical agglomerate 30-100 µm

	MPG-**08**	MPG-**09**	MPG-**10**	MPG-**11**	MPG-**05R**	Tocopheryl succinate salt 1:1
Binding (association with MPs)	82%	87%	46%	85%	93%	89%

[0068] Formulation of an equimolar complex (salt) of tocopheryl succinate (MW 530, one COOH eq. 265 Dalton) and glatiramer acetate (MW 4,700-11,000, one NH_2 eq. ~693 Dalton) was prepared by suspending an aqueous solution of glatiramer in methylene chloride with previously dissolved equimolar amount of tocopheryl succinate with the help of an ultrasonic indenter for 60 seconds (6×10sec) with ice cooling. After evaporation of the organic solvent and water, the thus formed water insoluble product was collected, washed with purified water and with dry ethanol and used for further investigations without additional purification.

Table 5. PLGA microparticles for sustained release of glatiramer acetate (GA) (formulations MPG-12 - 15)

	MPG-**12**	MPG-**13**	MPG-**14**	MPG-**15**
Internal water phase				
GA, mg	31.8	31.8	31.5	31.7
GA drv base, mg	26.4	26.4	26.1	26.3
Water, µl	200	200	200	200
Polymer in the oil phase				
PLGA RG 502H, mg	200	250	300	165
Oil phase				
Tocopheryl succinate, mg				9
Methylene chloride	2.6 g	2.7 g	2.7 g	2.6 g
External water phase				
0.5% PVA solution	30 ml	30 ml	30 ml	30 ml
NaCl	0.25 g	0.25 g	0.25 g	0.25 g
Preparation process description (processor, speed set, evaporation duration)	IKA VDI-12 #5 (1 min), evaporation 4hr RT magnetic stirrer	IKA VDI-12 #5 (1 min), evaporation 4hr RT magnetic stirrer	IKA VDI-12 #5 (1 min), evaporation 4hr RT magnetic stirrer	IKA VDI-12 #6 20sec, #5 40sec, evaporation 4hr RT magnetic stirrer

	MPG-**12**	MPG-**13**	MPG-**14**	MPG-**15**
Microparticles description	spherical particles 10-15 µm with inclusions	spherical particles 10-18 µm with inclusions	spherical particles 10-15 µm with inclusions	spherical particles 6-10 µm with inclusions; aggregates
Binding (association with MPs)	85.4%	94.9%	96.4%	70.9%
Burst (release at 1 hour)	18.9%	8.5%	9.5%	13.6%
Amount of GA, released between day 4 and day 11	25.6%	21.0%	32.7%	19.6%

Lyophilization

[0069] Microparticulate formulations after centrifugation and washing were lyophilized either "as is", following sediment re-suspension in purified water, or in some cases, with the addition of cryoprotectant (sediment was re-suspended in a 5% mannitol solution). Samples were frozen for 1 hour at -37--43°C using the lyophilizer plate, and freeze-dried using lyophilizer "Alpha 2-4 LSC" (Christ, Germany) for 24-48 hours at pressure 0.050 mbar and -20°C, final drying at 0.025 mbar and +20°C for 10-16 hours. In both re-suspension procedures the lyophilized product could be easily reconstituted. The use of mannitol lead to a readily reconstituted product as compared to formulations without the cryoprotectant, but such compositions contained significant amount of ballast material and required more complex calculations to determine the real concentration of the active material.

Example 4: In vitro release of glatiramer acetate from PLGA microparticles

Equipment

[0070]

20 ml vials

multi-point magnetic stirrer

Incubator

Pipettors

UV-Vis spectrophotometer Shimadzu 1601

Reagents and plastic/glassware

Test-articles

[0071] Formulations MPG-02, 03, 04, 05, 05R, 06, 07, 12, 13, 14, and 15 - 50 mg of dry lyophilized microparticles.

[0072] Formulations MPG-08, 09, 10, and 11 - amount corresponding to 50 mg of dry microparticles, lyophilized with 5% mannitol.

Control glatiramer acetate solution 20-50 µg/mL (as base) in PBS with 0.05% sodium azide)

Temperature: 37°C

In order to evaluate the release of incorporated glatiramer acetate from biodegradable PLGA microparticles loaded with glatiramer acetate (various formulations), the following process had been employed.

[0073] Process description: 20 ml of PBS (0.01M phosphate, 0.05% NaN_3) pH 7.4 were added to each vial. The vials were placed at 37°C and stirred with a small magnet. 600 µl samples were centrifugated at 10,000g for 5 minutes. 500 µl of supernatant were transferred to a 1.5 ml microtube followed by the addition of 500µl of 0.1M borate buffer (2-fold dilution) and 50 µl TNBS. The resulting composition was torturously mixed and was kept on the bench for 30 minutes. Analysis was performed using TNBS method.

[0074] The remaining precipitated particles, re-suspended with 500 µl of fresh PBS (with NaN_3), were returned to the vial. Correct calculation for released amount of glatiramer acetate was performed in further release process for 2.5% for each time-point.

[0075] The release of the incorporated glatiramer acetate was carried out in tightly closed 20ml glass vials, using incubator at 37°C, equipped with a multi-point magnetic stirrer. Phosphate buffered saline (PBS) with pH 7.4 was used as a release media.

[0076] The release of the glatiramer acetate was tested over a period of 10-32 days.

[0077] The equation for the calibration curve in the range 1-200µg/ml was calculated

(Shimadzu UV-1601) as:
$OD=0.035+0.0132*C$ ($r^2 = 0.9985$)

Where OD - optical density (difference at 420 and 700 nm)

C - concentration of glatiramer acetate base, µg/ml

[0078] Results of peptide release of formulations MPG01-MPG07 are shown in figure 1. The fastest release of the incorporated glatiramer acetate (40% for days 1-10) was obtained in formulation MPG-05, based on low molecular weight PLGA polymer with acidic end groups (Resomer RG 502H) and without hydrophobic counter-ion. Neutral polymer RG 502 with relatively small amount of tocopheryl succinate as a counter-ion (MPG-03) also demonstrated significant release (~30% for days 2-12), but with lower absolute release values. Formulations containing higher amounts of counter-ions showed suppression of the drug release. Without being bound by any theory or mechanism of action, this might be attributed to the high hydrophobicity of the formed complex. Additionally, the preparation of the microparticles with DCP or DMPG was associated with the formation of aggregates and a wide particle size distribution.

[0079] The use of a bigger and more powerful high shear mixer OMNI GLH (shaft diameter 20 mm, 5000-30000 rpm instead of VDI-12 (12 mm shaft) leads to a significant decrease in the size of microparticles (formulations 8-11) and increased surface smoothness. Increasing of amount of the organic solvent (MPG-02) caused decreased peptide incorporation into the microparticles. Without being bound by any theory or mechanism of action, this is possibly attributed to the increase of the intermediate o/w/o double emulsion droplet size. Similarly, the use of polysorbate as non-ionic surfactant also negatively affected the drug loading (MPG-10 with 0.2% Tween-80). The addition of hydrophobic counter-ions (tocopheryl succinate, dimyristoylphosphatidylglycerol DMPG, dicetylphosphate DCP) significantly retarded peptide release from the polymeric microparticles in comparison to formulations without counter-ion (MPG-05, MPG-05R). Without being bound by any theory or mechanism of action, the addition of the hydrophobic counter-ions may provide microparticles with compromised properties (MPG-06).

[0080] The chemical structure of the polymer used showed a greater impact on the release properties than the molecular weight of the PLGA. Resomers RG 502H and RG 502 (MW about 17,000 Dalton) had very similar diffusion coefficients, but the main factor determining the release of the included peptide form the polymeric matrix was a multi-point ionic interaction between positively charged Lys moieties of glatiramer acetate and carboxylic end groups in PLGA polymer. Neutral Resomer® RG 502 showed a low binding capacity even in the presence of a counter-ion (MPG-02, 03) while neutral Resomer® RG 503 with higher molecular weight demonstrated better binding but very slow release (MPG-10, 11).

[0081] Repeated release experiments from separately prepared identical formulations (MPG-

05 and MPG-05R) showed reasonably similar behavior and a good reproducibility for such small-scale batches. Formulations of glatiramer acetate with Resomer® RG 502H demonstrated a similar burst effect (~30%), good initial peptide binding and fast drug release (Figure 2).

[0082] Formulation of an equimolar complex (salt) of tocopheryl succinate had a high binding and extremely low water solubility (~ 5 µg/ml). Without being bound by any theory or mechanism of action, this may be caused by an ionic cross-linking of the diacid (tocopheryl succinate) and the polyamine molecule of the polymer. Release of the polymer from this salt in PBS was extremely slow. For polymeric microparticles, when tocopheryl succinate incorporated into the PLGA matrix, only part of this diacid can interact with the polymer, and for complete release suppression higher amount of tocopheryl succinate is required. So release rate may be regulated by the ratio between the glatiramer and PLGA. The amount of organic solvent used may also be of importance but to a lower extent.

[0083] Formulations 12-15, based on Resomer® RG 502H with different ratios between the drug and the polymer, showed that the ratio plays an important role in controlling the initial burst effect, the binding level and the release rate. The adjustment of the amount of the PLGA and the peptide as well as the addition of a hydrophobic counter-ion, such as tocopheryl succinate, allows the preparation of microparticulate formulations (MPG-12 - 15) with high binding, low initial burst and reasonable release rates (Figure 3).

Example 5: Up scaling

Lyophilized samples of glatiramer acetate microparticulate formulations

[0084] MPG-14 SU-1 - formulation of MPG-014, was produced using a bigger reaction vessel and a bigger homogenizer (OMNI GLH) at low speed.

[0085] Total - 13 vials; each vial contained approximately 235mg of lyophilized formulation with total content of glatiramer acetate of ~18.2 mg per vial, equal to ~ 75 µg/mg of the lyophilized formulation.

[0086] MPG-15 SU-1 - formulation of MPG-015, was produced using a bigger reaction vessel and a bigger homogenizer (OMNI GLH) at low speed.

[0087] Total - 10 vials; each vial contained approximately 145mg of lyophilized formulation with total content of glatiramer acetate of ~14.9 mg per vial, equal to ~ 100 µg/mg of the lyophilized formulation.

[0088] MPG-14 SU-2 - formulation of MPG-014, was produced using the same reaction vessel,

the same homogenizer (VDI 12) and the same parameters, process repeated several times. Composition was washed thoroughly to decrease initial burst.

[0089] Total - 12 vials; each vial contained approximately 88mg of lyophilized formulation with total content of glatiramer acetate of ~6.3 mg per vial, equal to ~ 72 µg/mg of the lyophilized formulation.

[0090] MPG-15 SU-2 - formulation of MPG-015, was produced using the same reaction vessel, the same homogenizer (VDI 12) and the same parameters, process repeated several times. Composition was washed thoroughly to decrease initial burst.

[0091] Total - 12 vials; each vial contained approximately 55mg of lyophilized formulation with total content glatiramer acetate of ~5.6 mg per vial, equal to ~ 100 µg/mg of the lyophilized formulation.

[0092] All lyophilized samples were stored in a refrigerator at +4°C and were reconstituted before use.

[0093] The ratio between the formulation and the diluent (glucose solution) was at least 1:5, preferably 1:10 and higher. Vigorous shaking was performed prior to the administration of the reconstituted sample. Release profiles of these formulations are shown in Figures 4 and 5.

[0094] Thus, the incorporation of the highly water soluble peptide of glatiramer acetate into a biodegradable polymeric microparticles was demonstrated. The microparticles showed good binding of the polymer, reasonable drug loading and reduced initial release burst which can be regulated by employing different compositions and processes of preparation. PLGA microparticles, made of Resomer® 502H and loaded with glatiramer acetate, provide in vitro release of the incorporated peptide with release rate of 3-5% per day for 10-15 days in a stirred aqueous media (phosphate buffered saline, pH 7.4) at 37°C.

Example 6: Experimental autoimmune encephalomyelitis (EAE) model

[0095] Experimental autoimmune encephalomyelitis (EAE) is an inflammatory autoimmune demyelinating disease which can be induced in laboratory animals by injection of myelin basic protein. Such disease has become the standard laboratory model for studying clinical and experimental autoimmune diseases. In fact, numerous articles (e.g., Abramsky et. al., J Neuroimmunol (1982) 2 1 and Bolton et al., J Neurol Sci. (1982) 56 147) note that the similarities of chronic relapsing EAE in animals to multiple sclerosis in humans especially implicates the value of EAE for the study of autoimmune demyelinating diseases such as multiple sclerosis. As such, the EAE test model is employed to establish the activity of the formulations of the present invention against multiple sclerosis. Such testing is conducted according to the following procedure.

[0096] Female Lewis rats are injected in their footpads with 12.5 µg of myelin basic protein (MBP) (prepared form guinea-pig spinal cord) in Complete Freunds adjuvant. The formulation of the present invention is given by injection every week/two weeks/once a month at various dosages to the test animals. A control formulation is given to certain other test animals. The animals are then weighed and scored daily for symptoms of EAE according to a scale of 0 to 3 (0=no change; 1=flaccid tail; 2=hind limb disability and 3=hind quarter paralysis/moribund). Animals are then sacrificed if a score of 3 is reached.

Example 7: In vivo studies using the EAE model

[0097] To determine the effect of the formulations of the present invention on the murine model of MS, experimental autoimmune encephalomyelitis (EAE) is performed. 25-hydroxyvitamin D_3-1α-hydroxylase knockout mice (1α -OH KO) are maintained on a purified diet containing 0.87% calcium and 1 ng 1,25-$(OH)_2D_3$ (Vit D) for two to three weeks prior to EAE immunization. EAE is induced to mice at six to ten weeks of age, by subcutaneous immunization of 200 µg of the immunodominant peptide to myelin oligodendrocyte glycoproprotein (MOG 35-55).

[0098] The peptide is synthesized using standard 9-fluorenyl-methoxy-carbonyl chemistry. The peptide is dissolved in Freund's complete adjuvant (CFA; Sigma) containing 4 mg/ml of heat-inactivated Mycobacterium tuberculosis H837a.

[0099] The mice are examined daily for clinical signs of EAE utilizing the following scoring system: 0, no sign; 1, limp tail; 2, hindlimb weakness; 3, hindlimb paralysis; 4, forelimb paralysis; 5, moribund or death.

[0100] Mice that develop clinical signs of EAE with scores ≥2 are treated with the formulation of the present invention which is administered by injection every week/two weeks/once a month at various dosages. Control groups are treated either with placebo or with Gold standard regimen of glatiramer acetate [e.g. PNAS, 2005, vol. 102, no. 52, 19045-19050]. Mice are then weighed and scored daily for symptoms of EAE. Statistical analysis is performed using the two-tailed Fisher exact probability test on incidence rates and the unpaired Student's t-test on all other measurements. Values of $P<0.05$ are considered statistically significant.

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- PNAS, 2005, vol. 102, 5219045-19050 [0100]

Patentkrav

1. Fremgangsmåde til fremstilling af en langtidsvirkende parenteral farmaceutisk sammensætning, der omfatter glatirameracetat og en farmaceutisk acceptabel, biologisk nedbrydelig bærer valgt fra gruppen bestående af poly(D,L-lactid) (PLA) og poly(D,L-lactid-co-glycolid) (PLGA); hvilken fremgangsmåde omfatter trinnene til:

(i) dispergering af en koncentreret glatirameracetatopløsning i en opløsning af den farmaceutisk acceptable, biologisk nedbrydelige bærer i vandblandbart flygtigt organisk opløsningsmiddel, hvorved der opnås en vand-i-olie-emulsion,

(ii) dispergering af vand-i-olie-emulsionen i en kontinuert, ekstern vandfase, der indeholder et overfladeaktivt middel, så der dannes vand-i-olie-i-vand-dobbeltemulsionsdråber.

(iii) fordampning af det organiske opløsningsmiddel, hvorved der opnås faste mikropartikler,

(iv) indsamling af mikropartiklerne ved filtrering eller centrifugering.

2. Fremgangsmåde ifølge krav 1, der endvidere omfatter trinnene til

(v) vask af de indsamlede mikropartikler med renset vand.

3. Fremgangsmåde ifølge krav 1 eller 2, der endvidere omfatter trinnene til

(vi) lyofilisering af de indsamlede mikropartikler.

4. Fremgangsmåde ifølge krav 3, der endvidere omfatter trinnene til

(vii) rekonstitution af de lyofiliserede mikropartikler.

5. Fremgangsmåde ifølge krav 1 til 4, hvor den farmaceutisk acceptable, biologisk nedbrydelige bærer er PLGA.

6. Fremgangsmåde ifølge krav 1 til 5, hvor det overfladeaktive middel er valgt fra gruppen bestående af polyvinylalkohol (PVA), polysorbat, polyethylenoxid-polypropylenoxid-blokcopolymerer og celluloseestere.

7. Fremgangsmåde ifølge krav 1 til 6, hvor lyofilisering af mikropartiklerne er i nærvær af et beskyttelsesadditiv, fortrinsvis mannitol.

8. Langtidsvirkende parenteral farmaceutisk sammensætning, der omfatter glatirameracetat og en farmaceutisk acceptabel, biologisk nedbrydelig bærer valgt fra gruppen bestående af poly(D,L-lactid) (PLA) og poly(D,L-lactid-co-glycolid) (PLGA), hvilken sammensætning kan opnås ved fremgangsmåden ifølge et hvilket som helst af kravene 1 til 7:

9. Farmaceutisk sammensætning ifølge krav 8, hvor den farmaceutisk acceptable, biologisk nedbrydelige bærer er PLGA.

10. Farmaceutisk sammensætning ifølge krav 8 eller 9, hvor glatirameracetatet omfatter L-alanin, L-glutaminsyre, L-lysin og L-tyrosin i molforhold på 0,14 glutaminsyre, 0,43 alanin, 0,10 tyrosin og 0,33 lysin.

11. Farmaceutisk sammensætning ifølge krav 8 til 10, der er på depotform, som er egnet til implantation på et lægeligt acceptabelt sted hos et individ med behov derfor, og/eller som er egnet til en doseringsplan fra en gang hver anden uge til en gang om måneden.

12. Farmaceutisk sammensætning ifølge krav 8 til 11 til anvendelse ved behandling af multipel sklerose.

13. Farmaceutisk sammensætning til anvendelse ved behandling af multipel sklerose ifølge krav 12, hvor sammensætningen tilvejebringer en lige så stor eller større terapeutisk effektivitet i forhold til de kommercielt tilgængelige doseringsformer af glatirameracetat

til daglige injektioner med nedsat incidens og/eller sværhed af bivirkningerne på lokalt og/eller systemisk niveau; og hvor sammensætningen endvidere tilvejebringer langvarig frigivelse eller langvarig virkning af glatiramer hos et individ sammenlignet med en i det væsentlige tilsvarende dosis af en formulering af glatirameracetat med øjeblikkelig frigivelse.

14. Farmaceutisk sammensætning til anvendelse ved behandling af multipel sklerose ifølge krav 12 eller 13, hvor glatirameracetatet indgives i kombination med mindst et andet aktivt middel.

DRAWINGS

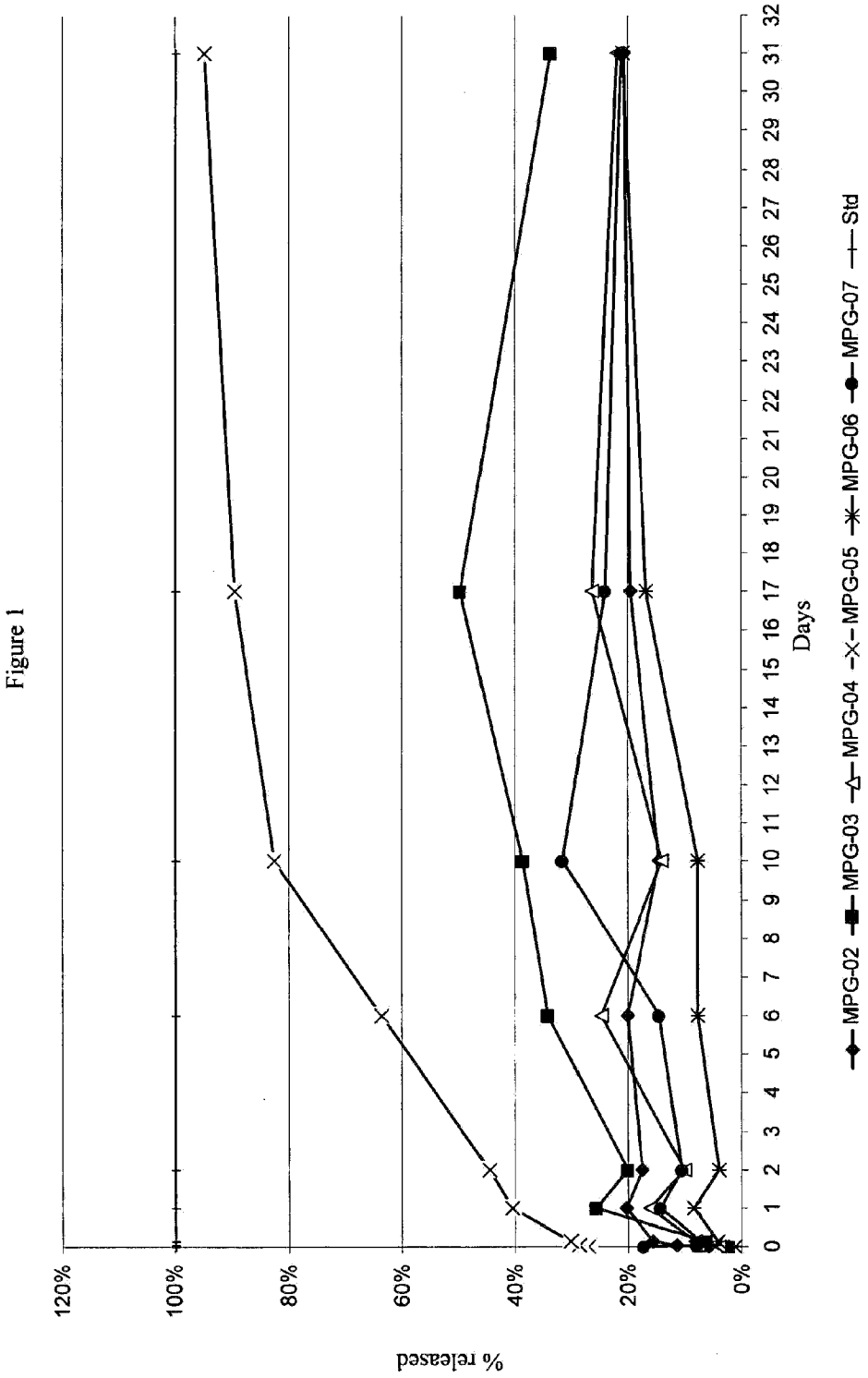
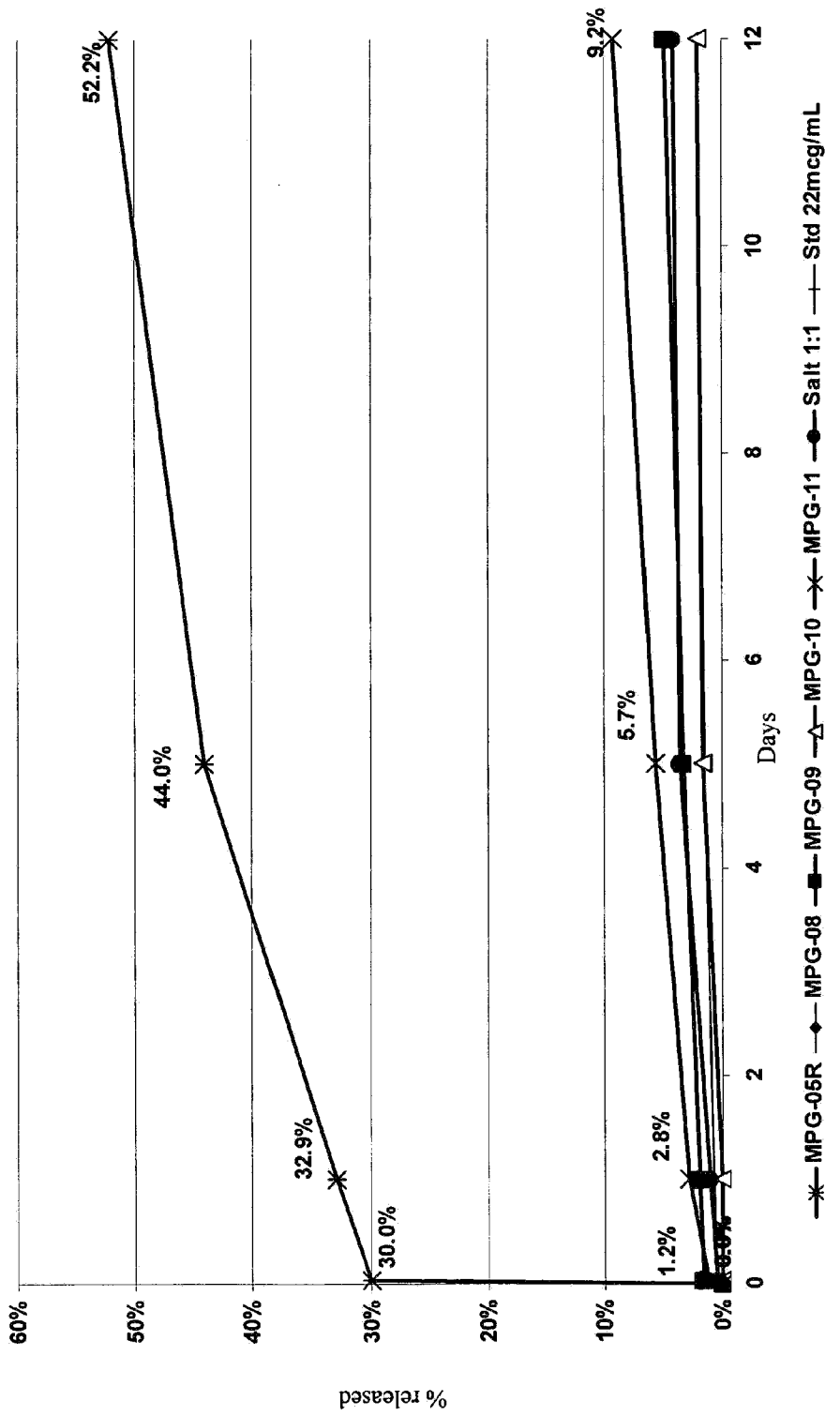


Figure 1

Figure 2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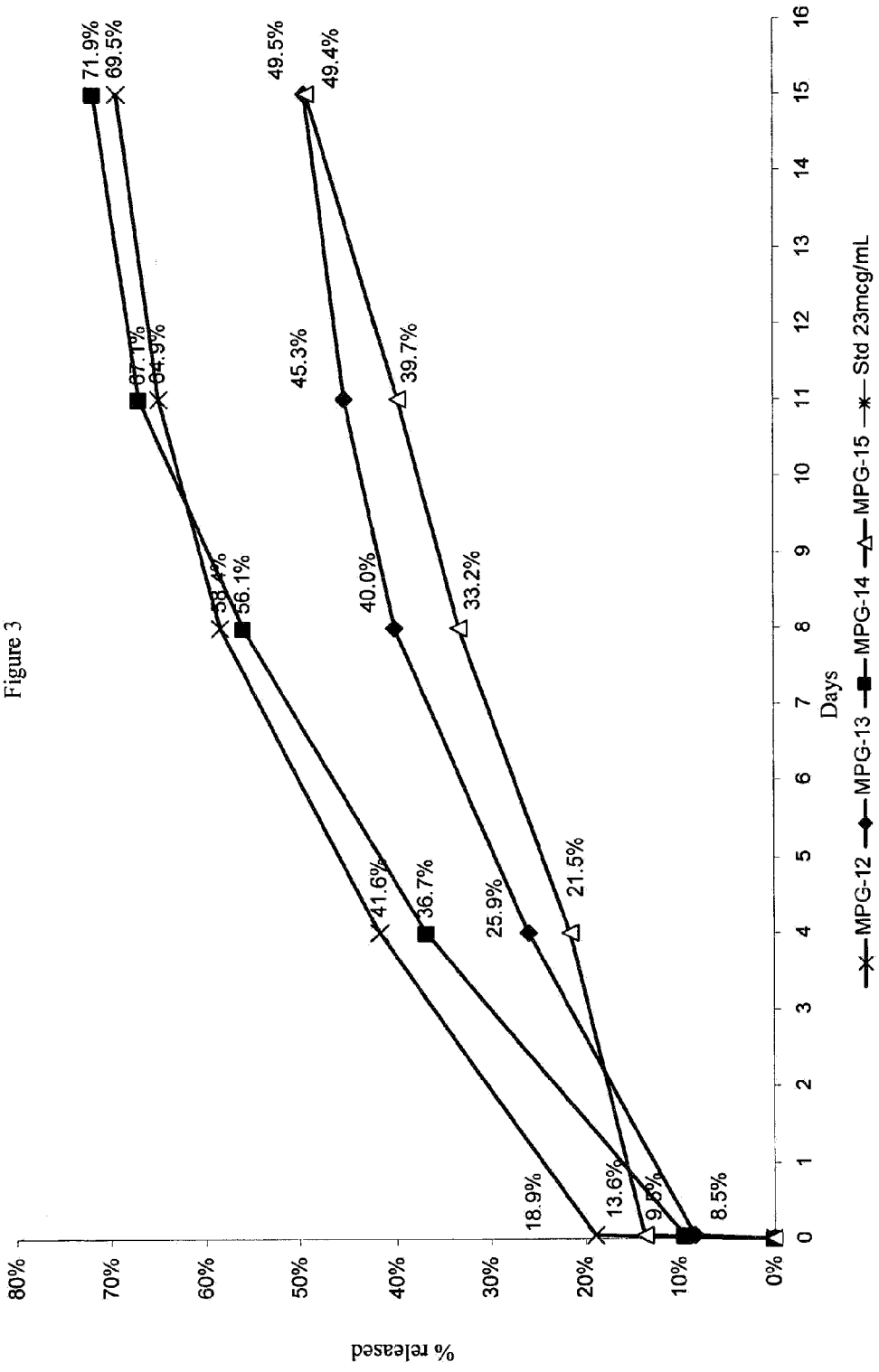
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
12
Days
52.2%
44.0%
32.9%
30.0%
9.2%
5.7%
2.8%
1.2%
MPG-05R
MPG-08
MPG-09
MPG-10
MPG-11
Salt 1:1
Std 22mcg/mL

Figure 3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70%
60%
50%
40%
30%
20%
10%
0%
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4
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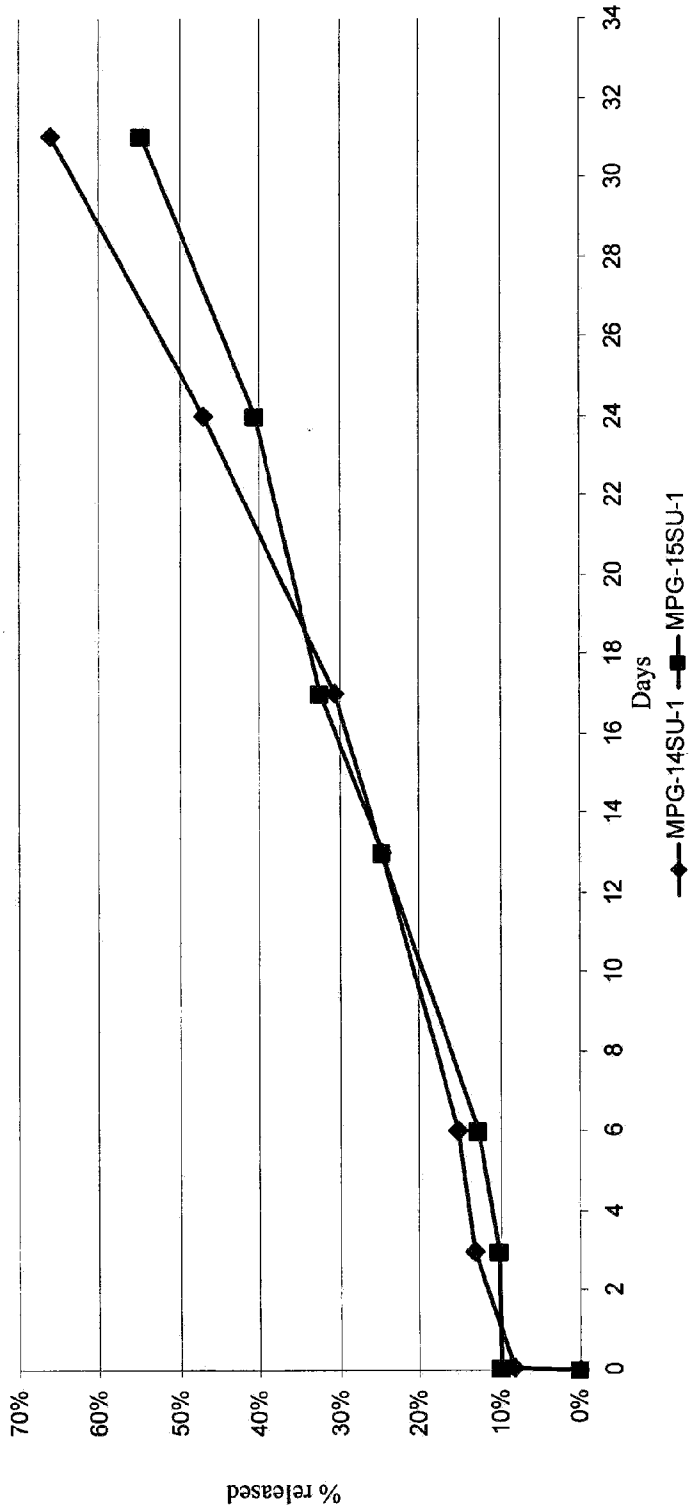
7
8
9
10
11
12
13
14
15
16
Days
71.9%
69.5%
49.5%
49.4%
45.3%
39.7%
40.0%
33.2%
41.6%
36.7%
25.9%
21.5%
18.9%
13.6%
9.6%
8.5%
MPG-12
MPG-13
MPG-14
MPG-15
Std 23mcg/mL

Figure 4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40%
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20%
10%
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12
14
16
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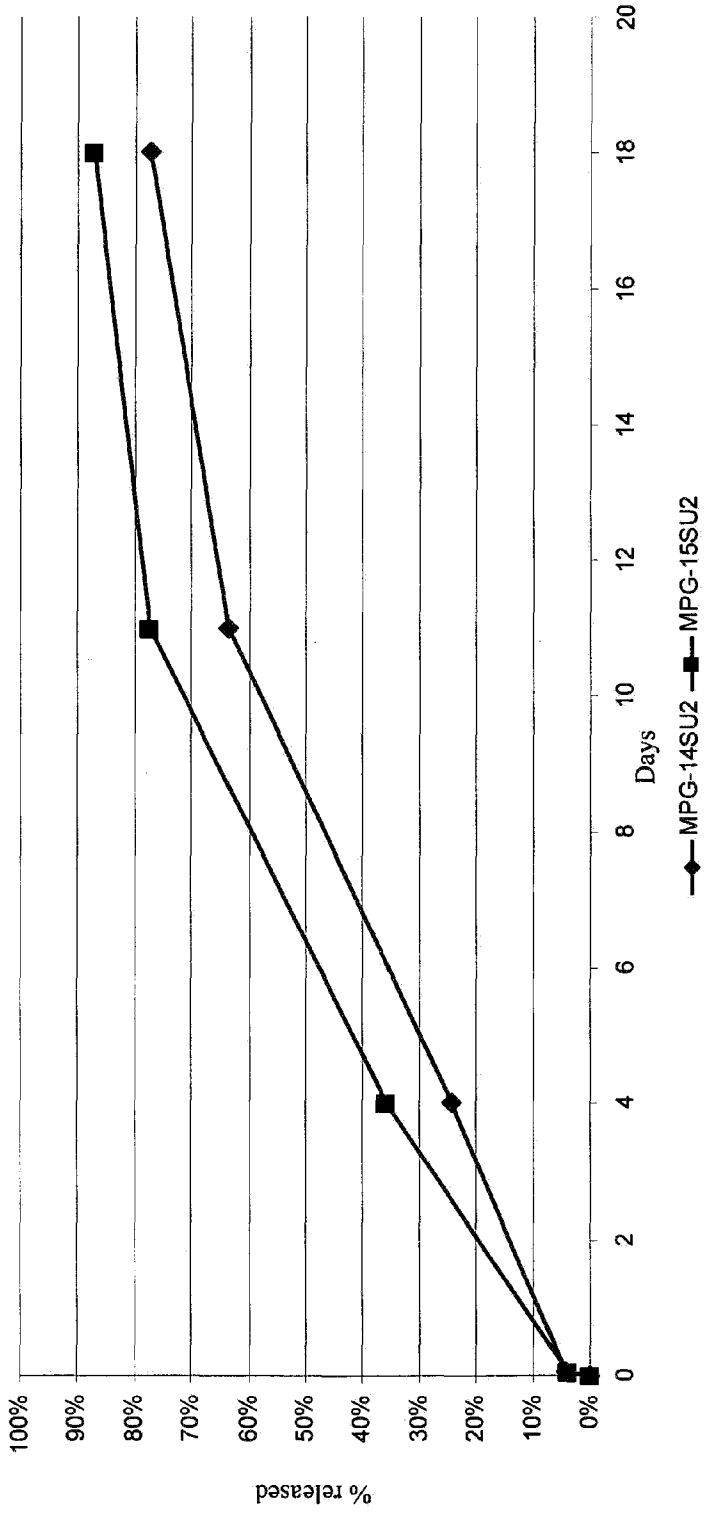
20
22
24
26
28
30
32
34
Days
MPG-14SU-1
MPG-15SU-1

Figure 5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0
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6
8
10
12
14
16
18
20
Days
MPG-14SU2
MPG-15SU2