CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-075

ADMINISTRATIVE DOCUMENTS

FORM FDA 356h
ESTABLISHMENT INFORMATION

Nutropin Depot™ is manufactured for Genentech, Inc. under contract by [redacted] at the following address:

[redacted]

[redacted] is responsible for the manufacturing and testing of Nutropin Depot Final Product, including the rhGH-Zinc Acetate Powder and [redacted] rhGH Bulk Microspheres.

All testing of [redacted] rhGH Bulk Microspheres and Nutropin Depot Final Product, with the exception of the determination of zinc content of [redacted] rhGH Bulk Microspheres, is performed by [redacted] Quality Control. The testing to determine zinc content of [redacted] rhGH Bulk Microspheres is performed for [redacted] by [redacted]

[redacted] Facility Contact: [redacted]

Telephone No.:

Central File No.: Not yet assigned.

Genentech, Inc. is responsible for the manufacture of bulk drug substance, and for the labeling, packaging, final release and distribution of Nutropin Depot Final Product and the Nutropin Depot Kit at the following address:

Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990

Genentech Facility Contact: Robert L. Garnick, Ph.D., Vice President, Regulatory Affairs

Telephone No.: (650) 225 1201

Facility License No.: 1048

Central File No.: 2917293/SAN

All sites are currently ready for inspection.

DSI

NOT NEEDED

APPEARS THIS WAY
ON ORIGINAL

NEW DRUG APPLICATION: **ITEM 13**

NUTROPIN DEPOT™ [somatropin (rDNA origin) for injectable suspension]

13. PATENT INFORMATION ON ANY PATENT WHICH CLAIMS THE DRUG

Nutropin Depot™ falls within the scope of the claims of the patents described in the table below. Copies of these patents are appended to this section.

Patent No.	Expiry Date	Patent Type	Patent Owner	Patent Agent
US 5,656,297	July 25, 2014	Pharmaceutical Composition, Method of Use	Alkermes Controlled Therapeutics, Inc.	Hamilton, Brook, Smith, and Reynolds
US 5,654,101	August 5, 2014	Composition and Method of Use	Alkermes, Inc.	Hamilton, Brook, Smith, and Reynolds

Patent Nos. US 5,656,297 and US 5,654,101 cover the formulation, composition, and or method of use of Nutropin Depot. This product is the subject of this application for which approval is being sought.

APPEARS THIS WAY
ON ORIGINAL

US005654010A

United States Patent [19]

Johnson et al.

[11] Patent Number: 5,654,010

[45] Date of Patent: Aug. 5, 1997

[54] COMPOSITION FOR SUSTAINED RELEASE OF HUMAN GROWTH HORMONE

[75] Inventors: **OluFunmi Lily Johnson**, Cambridge, Mass.; **Medha M. Ganmukhi**, Wexford, Pa.; **Howard Bernstein**, Cambridge; **Henry Auer**, Belmont, both of Mass.; **M. Amin Khan**, Dowington, Pa.

[73] Assignee: **Alkermes, Inc.**, Cambridge, Mass.

[21] Appl. No.: **473,544**

[22] Filed: **Jun. 7, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 984,323, Dec. 2, 1992, abandoned.

[51] Int. Cl.^{6} **A61F 2/02**; A61K 9/14; A61K 9/50; A61K 38/24

[52] U.S. Cl. 424/502; 424/423; 424/426; 424/489; 424/499; 424/500; 424/501; 514/21; 530/399; 530/839

[58] **Field of Search** 424/423, 426, 424/489, 499, 500, 501, 502; 514/21; 530/399, 839

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Attorney, Agent, or Firm—Hamilton, Brook, Smith & Reynolds, P.C.

[57] **ABSTRACT**

A composition, and methods of forming and using said composition, for the sustained release of biologically active, stabilized human growth hormone (hGH). The sustained release composition of this invention comprises a polymeric matrix of a biocompatible polymer and particles of biologically active, stabilized hGH, wherein said particles are dispersed within the biocompatible polymer. The method of the invention for producing a composition for the sustained release of biologically active hGH, includes dissolving a biocompatible polymer in a polymer solvent to form a polymer solution, dispersing particles of biologically active, stabilized hGH in the polymer solution, and then solidifying the polymer to form a polymeric matrix containing a dispersion of said hGH particles. The method for using a composition of the invention is a method for providing a therapeutically effective blood level of biologically active, non-aggregated hGH in a subject for a sustained period. In this method, a subject is administered an effective dose of the sustained release composition of the present invention. The method of using the sustained release composition of the present invention comprises providing a therapeutically effective blood level of biologically active, non-aggregated human growth hormone in a subject for a sustained period by administering to the subject a dose of said sustained release composition.

11 Claims, No Drawings

COMPOSITION FOR SUSTAINED RELEASE OF HUMAN GROWTH HORMONE

RELATED APPLICATIONS

This application is a Continuation-in-Part of U.S. patent application Ser. No. 07/984,323, filed Dec. 2, 1992, now abandoned, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Human growth hormone (hGH) is a protein secreted by the pituitary gland and which can be produced by recombinant genetic engineering. hGH will cause growth in all bodily tissues which are capable of growth.

hGH is typically used to treat patients suffering from hypopituitary dwarfism. Currently, aqueous hGH is administered as a subcutaneous bolus three times a week or once daily to patients to maintain suitable serum levels of hGH. For patients chronically receiving hGH, the frequent injections result in patient compliance problems.

To resolve the problems associated with repetitive injections of aqueous hGH, attempts have been made to formulate controlled release devices containing higher doses of hGH than a bolus injection, encapsulated within a polymeric matrix wherein the hGH would be released in vivo over a period of about a week or more.

However, these controlled release devices often exhibited high initial bursts of hGH release and minimal hGH release thereafter. Further, due to the high concentration of hGH within these controlled release devices, the hGH molecules have tended to aggregate after several days to form aggregated hGH which is immunogenic in vivo and likely has reduced biological activity.

Therefore, a need exists for a means for sustaining the release of biologically active hGH in vivo without causing an immune system response over the release period of the hGH.

SUMMARY OF THE INVENTION

This invention relates to a composition, and methods of forming and using said composition, for the sustained release of biologically active, stabilized human growth hormone (hGH). The sustained release composition of this invention comprises a polymeric matrix of a biocompatible polymer and particles of biologically active, stabilized hGH, wherein said particles are dispersed within the biocompatible polymer.

The method of the invention for forming a composition for the sustained release of hGH, includes dissolving a biocompatible polymer in a polymer solvent to form a polymer solution, dispersing particles of biologically active, stabilized hGH in the polymer solution, and then solidifying the polymer to form a polymeric matrix containing a dispersion of said hGH particles.

The method of using the sustained release composition of the present invention comprises providing a therapeutically effective blood level of biologically active, non-aggregated human growth hormone in a subject for a sustained period by administering to the subject a dose of said sustained release composition.

The advantages of this sustained release formulation for hGH include longer, more consistent in vivo blood levels of hGH, lower initial bursts of hGH, and increased therapeutic benefits by eliminating fluctuations in serum hGH levels.

The advantages also include increased patient compliance and acceptance by reducing the required number of injections. The advantages further include the ability to use smaller amounts of hGH compared to bolus injection regimen because serum hGH levels are maintained closer to therapeutical thresholds.

DETAILED DESCRIPTION OF THE INVENTION

The human growth hormone (hGH) used in this invention is biologically active hGH in its molecular (monomeric or non-aggregated) form. Molecular hGH is typically non-immunogenic.

Aggregated hGH may induce an immune response resulting in antibodies formed against hGH. This may compromise the efficacy of long-term hGH therapy. Additionally, aggregated hGH may stimulate an auto-immune response to endogenous hGH.

A sustained release of biologically active, non-aggregated human growth hormone is a release which results in measurable serum levels of biologically active, monomeric hGH over a period longer than that obtained following direct administration of aqueous hGH. It is preferred that a sustained release be a release of hGH for a period of about a week or more, and more preferably for a period of about two weeks or more.

A sustained release of biologically active, non-aggregated hGH from a polymeric matrix can be continuous or non-continuous release with relatively constant or varying rates of release. The continuity of hGH released and level of hGH released can be established by using, inter-alia, one or more types of polymer compositions, hGH loadings, and/or selection of excipients to produce the desired effect.

Stabilized (hGH) comprises biologically active, non-aggregated hGH which is complexed with at least one type of multivalent metal cation, having a valency of +2 or more, from a metal cation component. Stabilized hGH in the sustained release composition of the present invention is in particulate form.

Suitable multivalent metal cations include metal cations contained in biocompatible metal cation components. A metal cation component is biocompatible if the cation component is non-toxic to the recipient, in the quantities used, and also presents no significant deleterious or untoward effects on the recipient's body, such as an immunological reaction at the injection site.

Typically, the molar ratio of metal cation component to hGH, for the metal cation stabilizing the hGH, is between about 4:1 to about 10:1.

A preferred metal cation used to stabilize hGH is Zn^{+2}. In a more preferred embodiment, the molar ratio of metal cation component, containing Zn^{+2} cations, to hGH is about 6:1.

The suitability of a metal cation for stabilizing hGH can be determined by one of ordinary skill in the art by performing a variety of stability indicating techniques such as polyacrylamide gel electrophoresis, isoelectric focusing, reverse phase chromatography, HPLC and potency tests on hGH lyophilized particles containing metal cations to determine the potency of the hGH after lyophilization and for the duration of release from microparticles. In stabilized hGH, the tendency of hGH to aggregate within a microparticle during hydration in vivo and/or to lose biological activity or potency due to hydration or due to the process of forming a sustained release composition, or due to the chemical characteristics of a controlled release composition. is reduced by complexing at least one type of metal cation with hGH prior contacting the hGH with a polymer solution.

Stabilized hGH is typically stabilized against significant aggregation in vivo over the sustained release period.

Stabilized hGH is typically stabilized against significant aggregation in vivo over the sustained release period. Significant aggregation is defined as an amount of aggregation resulting in aggregation of about 15% or more of the initial amount of encapsulated hGH monomer. Preferably. aggregation is maintained below about 5% of the initial dose of hGH monomer. More preferably. aggregation is maintained below about 2% of the initial dose.

The hGH in a hGH sustained release composition can also be mixed with other excipients. such as bulking agents or additional stabilizing agents. such as buffers to stabilize the hGH during lyophilization.

Bulking agents typically comprise inert materials. Suitable bulking agents are known to those skilled in the art.

A polymer. or polymeric matrix. suitable for the sustained release composition of the present invention must be biocompatible. A polymer is biocompatible if the polymer. and any degradation products of the polymer. are non-toxic to the recipient and also present no significant deleterious or untoward effects on the recipient's body. such as an immunological reaction at the injection site.

The polymer of the hGH sustained release composition must also be bidegradable. Biodegradable as defined herein. means the composition will degrade or erode in vivo to form smaller 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. poly (lactic acid-co-glycolic acid)s. polycaprolactone. polycarbonates. polyesteramides. polyanhydrides. poly (amino acids). polyorthoesters. polycyanoacrylates. poly(p-dioxanone). poly(alkylene oxalate)s. biodegradable polyurethanes. blends and copolymers thereof.

Further. the terminal functionalities of the polymer can be modified. For example. polyesters can be blocked. unblocked or a blend of blocked and unblocked polymers. A blocked polymer is as classically defined in the art. specifically having blocked carboxyl end groups. Generally. the blocking group is derived from the initiator of the polymerization and is typically an alkyl group. An unblocked polymer is as classically defined in the art. specifically having free carboxyl end groups.

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. and rate of dissolution of polymer in solvent. Typically. an acceptable range of molecular weights is of about 2.000 Daltons to about 2.000.000 Daltons. In a preferred embodiment. the polymer is a biodegradable polymer or copolymer. In a more preferred embodiment. the polymer is a poly(lactide-co-glycolide) (hereinafter "PLGA") with a lactide:glycolide ratio of about 1:1 and a molecular weight of about 5.000 Daltons to about 70.000 Daltons. In an even more preferred embodiment. the molecular weight of the PLGA used in the present invention has a molecular weight of about 6.000 to about 31.000 Daltons.

The amount of hGH. which is contained in a dose of sustained release microparticles. or in an alternate sustained release device. containing biologically active. stabilized hGH particles is a therapeutically or prophylactically effective amount. which can be determined by a person of ordinary skill in the art taking into consideration factors such as body weight. condition to be treated. type of polymer used. and release rate from the polymer.

In one embodiment. an hGH sustained release composition contains from about 0.01% (w/w) to about 50% (w/w) of biologically active. stabilized hGH particles. The amount of such hGH particles used will vary depending upon the desired effect of the hGH. the planned release levels. the times at which hGH should be released. and the time span over which the hGH will be released. A preferred range of hGH particle loading is between about 0.1% (w/w) to about 30% (w/w) hGH particles. A more preferred range of hGH particle loading is between about 0.1% (w/w) to about 20% (w/w) hGH particles. The most preferred loading of the biologically active. stabilized hGH particles is about 15% (w/w).

In another embodiment. a hGH sustained release composition also contains a second metal cation component. which is not contained in the stabilized hGH particles. and which is dispersed within the polymer. The second metal cation component preferably contains the same species of metal cation. as is contained in the stabilized hGH. Alternately. the second metal cation component can contain one or more different species of metal cation.

The second metal cation component acts to modulate the release of the hGH from the polymeric matrix of the sustained release composition. such as by acting as a reservoir of metal cations to further lengthen the period of time over which the hGH is stabilized by a matal cation to enhance the stability of hGH in the composition.

A metal cation component used in modulating release typically contains at least one type of multivalent metal cation. Examples of second metal cation components suitable to modulate hGH release. include. or contain. for instance. $Mg(OH)_2$. $MgCO_3$ (such as $4MgCO_3.Mg(OH)_2.5H_2O$). $ZnCO_3$ (such as $3Zn(OH)_2.2ZnCO_3$). $CaCO_3$. $Zn_3(C_6H_5O_7)_2$. $Mg(OAc)_2$. $MgSO_4$. $Zn(OAc)_2$. $ZnSO_4$. $ZnCl_2$. $MgCl_2$ and $Mg_3(C_6H_5O_7)_2$. A suitable ratio of second metal cation component-to-polymer is between about 1:99 to about 1:2 by weight. The optimum ratio depends upon the polymer and the second metal cation component utilized.

A polymeric matrix containing a dispersed metal cation component to modulate the release of a biologically active agent from the polymeric matrix is further described in co-pending U.S. patent application Ser. No. 08/237.057. filed May 3. 1994. and co-pending PCT Patent Application PCT/US95/05511. the teachings of which are incorporated herein by reference in their entirety.

The hGH 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 polymeric component having a diameter of less than about one millimeter and having stabilized hGH particles dispersed therein. A microparticle can have a spherical. non-spherical or irregular shape. It is preferred that a microparticle be a microsphere. Typically. the microparticle will be of a size suitable for injection. A preferred size range for microparticles is from about 1 to about 180 microns in diameter.

In the method of this invention for forming a composition for the sustained release of biologically active. non-aggregated hGH. a suitable amount of particles of biologically active. stabilized hGH are dispersed in a polymer solution.

A suitable polymer solution contains between about 1% (w/w) and about 30% (w/w) of a suitable biocompatible polymer, wherein the biocompatible polymer is typically dissolved in a suitable polymer solvent. Preferably, a polymer solution contains about 2% (w/v) to about 20% (w/v) polymer. A polymer solution containing 5% to about 10% (w/w) polymer is most preferred.

A suitable polymer solvent, as defined herein, is solvent in which the polymer is soluble but in which the stabilized hGH particles are substantially insoluble and non-reactive. Examples of suitable polymer solvents include polar organic liquids, such as methylene chloride, chloroform, ethyl acetate and acetone.

To prepare biologically active, stabilized hGH particles, hGH is mixed in a suitable aqueous solvent with at least one suitable metal cation component under pH conditions suitable for forming a complex of metal cation and hGH.

Suitable pH conditions to form a complex of hGH typically include pH values between about 7.0 and about 7.4. Suitable pH conditions are typically achieved through use of an aqueous buffer, such as sodium bicarbonate, as the solvent.

Suitable solvents are those in which the hGH and the metal cation component are each at least slightly soluble, such as in an aqueous sodium bicarbonate buffer. For aqueous solvents, it is preferred that water used be either deionized water or water-for-injection (WFI).

It is understood that the hGH can be in a solid or a dissolved state, prior to being contacted with the metal cation component. It is also understood that the metal cation component can be in a solid or a dissolved state, prior to being contacted with the hGH. In a preferred embodiment, a buffered aqueous solution of hGH is mixed with an aqueous solution of the metal cation component.

Typically, the complexed hGH will be in the form of a cloudy precipitate, which is suspended in the solvent. However, the complexed hGH can also be in solution. In an even more preferred embodiment, hGH is complexed with Zn^{+2}.

The complexed hGH is then dried, such as by lyophilization, to form a particulate of stabilized hGH. The complexed hGH, which is suspended or in solution, can be bulk lyophilized or can be divided into smaller volumes which are then lyophilized. In a preferred embodiment, the complexed hGH suspension is micronized, such as by use of an ultrasonic nozzle, and then lyophilized to form stabilized hGH particles. Acceptable means to lyophilize the complexed hGH mixture include those known in the art.

Preferably, particles of stabilized hGH are between about 1 to about 6 micrometers in diameter. The hGH particles can be fragmented separately, as described in co-pending U.S. patent application Ser. No. 08/006,682, filed Jan. 21, 1993, which describes a process for producing small particles of biologically active agents, which is incorporated herein in its entirety by reference. Alternately, the hGH particles can be fragmented after being added to a polymer solution, such as by means of an ultrasonic probe or ultrasonic nozzle. In another embodiment, a second metal cation component, which is not contained in the stabilized hGH particles, is also dispersed within the polymer solution.

It is understood that a second metal cation component and stabilized hGH can be dispersed into a polymer solution sequentially, in reverse order, intermittently, separately or through concurrent additions. Alternately, a polymer, a second metal cation component and stabilized hGH and can be mixed into a polymer solvent sequentially, in reverse order, intermittently, separately or through concurrent additions.

The method for forming a composition for modulating the release of a biologically active agent from a biodegradable polymer is further described in co-pending U.S. patent application Ser. No. 08/237,057.

In this method, the polymer solvent is then solidified to form a polymeric matrix containing a dispersion of stabilized hGH particles.

One suitable method for forming an hGH sustained release composition from a polymer solution is the solvent evaporation method described in U.S. Pat. No. 3,737,337, issued to Schnoring et al., U.S. Pat. No. 3,529,906, issued to Vranchen et al., U.S. Pat. No. 3,691,090, issued to Kitajima et al., or U.S. Pat. No. 4,389,330, issued to Tice et al. Solvent evaporation is typically used as a method to form hGH sustained release microparticles.

In the solvent evaporation method, a polymer solution containing a stabilized hGH particle dispersion, is mixed in or agitated with a continuous phase, in which the polymer solvent is partially miscible, to form an emulsion. The continuous phase is usually an aqueous solvent. Emulsifiers are often included in the continuous phase to stabilize the emulsion. The polymer solvent is then evaporated over a period of several hours or more, thereby solidifying the polymer to form a polymeric matrix having a dispersion of stabilized hGH particles contained therein.

A preferred method for forming hGH sustained release microparticles from a polymer solution is described in U.S. Pat. No. 5,019,400, issued to Gombotz et al., and co-pending U.S. patent application Ser. No. 08/443,726, filed May 18, 1995, the teachings of which are incorporated herein by reference in their entirety. This method of microsphere formation, as compared to other methods, such as phase separation, additionally reduces the amount of hGH required to produce a controlled release composition with a specific hGH content.

In this method, the polymer solution, containing the stabilized hGH particle dispersion, is processed to create droplets, wherein at least a significant portion of the droplets contain polymer solution and the stabilized hGH particles. These droplets are then frozen by means suitable to form microparticles. Examples of means for processing the polymer solution dispersion to form droplets include directing the dispersion through an ultrasonic nozzle, pressure nozzle, Rayleigh jet, or by other known means for creating droplets from a solution.

Means suitable for freezing droplets to form microparticles include directing the droplets into or near a liquified gas, such as liquid argon and liquid nitrogen to form frozen microdroplets which are then separated from the liquid gas. The frozen microdroplets are then exposed to a liquid non-solvent, such as ethanol, or ethanol mixed with hexane or pentane.

The solvent in the frozen microdroplets is extracted as a solid and/or liquid into the non-solvent to form stabilized hGH containing microparticles. Mixing ethanol with other non-solvents, such as hexane or pentane, can increase the rate of solvent extraction, above that achieved by ethanol alone, from certain polymers, such as poly(lactide-co-glycolide) polymers.

A wide range of sizes of hGH sustained release microparticles can be made by varying the droplet size, for example, by changing the ultrasonic nozzle diameter. If very large microparticles are desired, the microparticles can be extruded through a syringe directly into the cold liquid. Increasing the viscosity of the polymer solution can also increase microparticle size. The size of the microparticles can be produced by this process; for example microparticles ranging from greater than about 1000 to about 1 micrometers in diameter.

Yet another method of forming an hGH sustained release composition, from a polymer solution, includes film casting, such as in a mold, to form a film or a shape. For instance, after putting the polymer solution containing a dispersion of stabilized hGH particles into a mold, the polymer solvent is then removed by means known in the art, or the temperature of the polymer solution is reduced, until a film or shape, with a consistent dry weight, is obtained. Film casting of a polymer solution, containing a biologically active agent, is further described in co-pending U.S. patent application Ser. No. 08/237,057, the teachings of which are incorporated herein by reference in their entirety.

It is believed that the release of the hGH can occur by two different mechanisms. The hGH can be released by diffusion through aqueous filled channels generated in the polymeric matrix, such as by the dissolution of the hGH or by voids created by the removal of the polymer's solvent during the synthesis of the sustained release composition. A second mechanism is the release of hGH due to degradation of the polymer.

The rate of degradation can be controlled by changing polymer properties that influence the rate of hydration of the polymer. These properties include, for instance, the ratio of different monomers, such as lactide and glycolide, comprising a polymer; the use of the L-isomer of a monomer instead of a racemic mixture; and the molecular weight of the polymer. These properties can affect hydrophilicity and crystallinity, which control the rate of hydration of the polymer. Hydrophilic excipients such as salts, carbohydrates and surfactants can also be incorporated to increase hydration and which can alter the rate of erosion of the polymer.

By altering the properties of the polymer, the contributions of diffusion and/or polymer degradation to hGH release can be controlled. For example, increasing the glycolide content of a poly(lactide-co-glycolide) polymer and decreasing the molecular weight of the polymer can enhance the hydrolysis of the polymer and thus, provides an increased hGH release from polymer erosion.

In addition, the rate of polymer hydrolysis is increased in non-neutral pH's. Therefore, an acidic or a basic excipient can be added to the polymer solution, used to form the microsphere, to alter the polymer erosion rate.

The composition of this invention can be administered to a human, or other animal, by injection, implantation (e.g. subcutaneously, intramuscularly, intraperitoneally, intracranially, intravaginally and intradermally), administration to mucosal membranes (e.g., intranasally or by means of a suppository); or in situ delivery (e.g. by enema or aerosol spray) to provide the desired dosage of hGH based on the known parameters for treatment with hGH of the various medical conditions.

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

EXAMPLE 1

Formation of Zn^{+2}-Stabilized hGH

Human growth hormone (hGH), whose DNA sequence is described in U.S. Pat. No. 4,898,830, issued to Goeddel et al. was used in this Example. Human growth hormone was stabilized by forming an insoluble complexes with zinc.

The hGH was dissolved in Samples of a 4 mM sodium bicarbonate buffer (pH 7.2) to form hGH solutions with concentrations between 0.1 and 0.5 mM hGH. A 0.9 mM Zn^{+2} solution was prepared from deionized water and zinc acetate dihydrate and then was added to the hGH solutions to form a Zn^{+2}-hGH complex. The pH of the Zn^{+2}-hGH solution was then adjusted to between 7.0 and 7.4 by adding 1% acetic acid. A cloudy suspended precipitate, comprising Zn^{+2}-stabilized hGH formed.

The suspension of Zn^{+2}-stabilized hGH was then micronized using an ultrasonic nozzle (Type V1A; Sonics and Materials, Danbury, Conn.) and sprayed into a polypropylene tub (17 cm diameter and 8 cm deep) containing liquid nitrogen to form frozen particles. The polypropylene tub was then placed into a −80° C. freezer until the liquid nitrogen evaporated. The frozen particles, which contained Zn^{+2}-stabilized hGH, were then lyophilized to form Zn^{+2}-stabilized hGH particles.

EXAMPLE 2

Preparation of PLGA Microspheres Containing Biologically Active, Zn^{+2}-Stabilized hGH

Microspheres containing Zn^{+2}-stabilized human growth hormone (hGH), were prepared from hydrophilic poly (lactice-co-glycolide) polymer RG502H having free carboxyl end groups (hereinafter "unblocked-PLGA") (50:50 PLGA, 9,300 Daltons; Boehringer Ingelheim Chemicals, Inc.) or a more hydrophobic PLGA polymer having blocked carboxyl end groups (hereinafter "blocked-PLGA") (50:50 PLGA, 10,000 Daltons; Lot #115-56-1, Birmingham Polymers, Inc., Birmingham, Ala.).

The polymer was dissolved in methylene chloride at room temperature. The lyophilized hGH particles were added to the polymer solution and zinc carbonate was also added. The mixture was then sonicated to give a homogeneous suspension. The suspension was atomized through a sonicating nozzle on to a bed of frozen ethanol, overlaid with liquid nitrogen. The vessel containing the microspheres was stored at −80° C. to extract the methylene chloride and then freeze-dried to give a free-flowing powder.

EXAMPLE 3

Analysis of Encapsulated hGH Protein

The integrity of encapsulated hGH was determined by dissolving unhydrated microspheres into methylene chloride and acetone, collecting the protein, freeze-drying and re-constituting in HEPES buffer containing 10 mM EDTA. Appropriate controls were run to ensure that the extraction process did not affect the integrity of the protein.

The integrity of the encapsulated hGH was analyzed by measuring the percent of hGH monomer contained in the hGH sample after encapsulation by size exclusion chromatography (SEC).

The results of SEC analyses of the hGH integrity of hGH sustained release microspheres are provided below.

Formulation (polymer; % Zinc Carbonate)	% Monomer (SEC)
31K unblocked; 6% ZnCO3	98.6
31K unblocked; 6% ZnCO3	99.2
31K unblocked; 3% ZnCO3	97.7
31K unblocked; 3% ZnCO3	97.8
31K unblocked; 1% ZnCO3	97.6
31K unblocked; 0% ZnCO3	97.8
31K unblocked; 0% ZnCO3	97.1

-continued

Formulation (polymer; % Zinc Carbonate)	% Monomer (SEC)
10K blocked; 1% ZnCO3	98.2
10K blocked; 1% ZnCO3	98.4
8K unblocked; 0% ZnCO3	98.5
10K blocked; 1% ZnCO3	98.4

The results showed that the encapsulation process did not cause aggregation of the protein. The yield percent protein recovered by the extraction procedure (relative to the amount measured by nitrogen content of the microspheres) ranged from about 40 to 98%.

EXAMPLE 4

Determination of the Effect of Zinc Carbonate on In vitro Release Kinetics

The microspheres were formed as described in Example 2 and contained 15% w/w hGH (6:1 Zn:hGH protein complex); 0%, 1%, 6%, 10% or 20% w/w zinc carbonate; and poly(lactide-co-glycolide) polymer.

In vitro release kinetics of the hGH sustained release microsphere formulations containing various concentrations of zinc carbonate were determined by suspending an aliquot (10 mg) of each type of microsphere in different 1.5 ml samples of HEPES buffer (50 mM Hepes, 10 mM KCl 0.1% NaN3) pH 7.2 and then incubating at 37° C. The amount of protein released was quantitated by sampling the buffer at 1, 3, 7, 10, 14, 21, 28 days after incubation and replenishing with fresh buffer after each sampling.

A curve of-cumulative percent released (relative to initial hGH content in the starting mass of microspheres) versus time was plotted. Released protein samples from each time point were assayed for hGH monomer content by size exclusion chromatography.

Zinc carbonate is thought to act as a reservoir of zinc ions so that the formation of the Zn-hGH complex is favored and dissociation into soluble hGH disfavored. Because the aqueous solubility of zinc carbonate is low, the release of zinc ions from the reservoir is slow thus modulating the solubility of the protein.

In the absence of zinc carbonate, the rate of release of the encapsulated hGH was very rapid and all the protein was released in a very short period.

EXAMPLE 5

Assay for hGH After in Vivo Degradation of Blocked-PLGA Zn^{+2}-Stabilized hGh Microspheres

Microspheres of blocked-PLGA, containing 16% w/v Zn^{+2}-stabilized hGH and 0%, 6%, 10% or 20% $ZnCO_3$ were formed by the method of Example 2. Groups of test rats were injected subcutaneously with 50 mg samples of the different hGH microspheres. The rats were sacrificed after 60 days and the skin samples were excised from the injection sites. The excised skin samples were placed in 10% Neutral Buffered Formalin for at least 24 hours. They were then trimmed with a razor blade to remove excess skin and placed in PBS. Tissue samples were processed by Pathology Associates, Inc. (Frederick, Md.). The skin samples were embedded in glycomethacrylate, sectioned and assayed for the presence of hGH using a HistoScan/LymphoScan Staining Kit (Product #24-408M; Accurate Chemical & Scientific Corp., Westbury, N.Y.) according to the manufacturer's instructions. Tissue samples were scored for the presence or absence of staining which was indicative of the presence or absence of hGH in the sample. All skin samples, associated with hGH microsphere injections, tested positive for the presence of hGH thus indicating that the blocked-PLGA microspheres still contained hGH after 60 days in vivo.

The method described in Example 2 was used to form microspheres by encapsulating 0% or 15% w/w hGH, in the form of Zn:hGH complex, and also 0%, 1% or 6% w/w $ZnCO_3$ salt, within blocked-PLGA and within unblocked-PLGA.

In vivo degradation of unblocked-PLGA microspheres versus blocked-PLGA microspheres were compared by injecting samples of microspheres into rats and then analyzing the microspheres remaining at the injection site at various times post-injection. Three rats were assayed at each time point for each microsphere sample. On the day of administration of the microspheres, 750 µl of vehicle (3% carboxymethyl cellulose (low viscosity) and 1% Tween-20 in saline) was added to vials containing 50±1 mg of microspheres. Immediately, the vials were shaken vigorously to form a suspension which was then aspirated into a 1.0 cc syringe without a needle.

Rats (Sprague-Dawley males) were anesthetized with a halothane and oxygen mixture. The injection sites (intrascapular region) were shaven and marked with a permanent tatoo to provide for the precise excision of skin at the sampling time points. Each rat was injected with an entire vial of microspheres using 18 to 21 gauge needles.

On designated days (days 15, 30, 59 and 90 post-injection for animal receiving blocked-PLGA microspheres, or days 7, 14, 21, 28 and 45 post-injection for animals receiving unblocked-PLGA microspheres the rats were sacrificed by asphyxiation with CO_2 gas and the skin at the injection sites (including microspheres) was excised. Since the microspheres tended to clump at the injection sites, the presence or absence of microspheres was determined visually.

The visual inspections found that the unblocked-PLGA microspheres degraded substantially faster than the blocked-PLGA microspheres, and that the addition of $ZnCO_3$ to the blocked-PLGA substantially slowed polymeric degradation. For example, in the rats injected with unblocked-PLGA microspheres containing 0% hGH and 0% or 1% $ZnCO_3$, no microspheres were visible on day 21. In addition, for rats injected with blocked-PLGA microspheres containing 0% hGH and 0% $ZnCO_3$, a few microspheres were visible on day 60 and none were visible on day 90. Furthermore, for rats injected with blocked-PLGA microspheres containing 0% or 15% hGH and 6% $ZnCO_3$, microspheres were visible on day 90.

EXAMPLE 6

In Vivo Pharmacokinetic Studies of hGH Sustained Release Microspheres in Rats

Studies were conducted in rats to screen various hGH microsphere formulations, determine pharmacokinetic parameters following intravenous (IV), subcutaneous (SC) and SC osmotic pump (Alzet) administration of hGH, and to evaluate serum profiles and in vivo release rate various hGH microsphere formulations.

Sprague-Dawley rats were divided into groups of three each, randomized by body weight, and one hGH microsphere formulation was administered to each group. Rats were injected subcutaneously with approximately 7.5 mg hGH in 50 mg of microspheres, suspended in 0.75 ml of an aqueous injection vehicle. The vehicle composition was 3%

CMC (low viscosity). 1 Polysorbate 20. in 0.9% NaCl. The microsphere dose delivered was determined indirectly by weighing the residual dose in the injection vial and correcting for residual injection vehicle. The hGH dose was then computed from the protein loading of the microspheres determined by nitrogen analysis.

Blood samples were collected at pre-determined intervals for up to 10 days after injection. Blood samples of 250 μl were collected during the first 24 hours and at least 400 μl at time points after 24 hours. Blood samples were clotted and hGH concentrations in serum were determined using a radio-immuno assay (RIA) kit from ICN.

For the determination of pharmacokinetic parameters. hGH in saline was administered to rats by subcutaneous bolus injection. intravenously or delivered via an osmotic pump (Alzet Model 2ML4) which was implanted subcutaneously.

Three groups of rats received single subcutaneous injections of hGH in 0.9% NaCl at 0.5 or 7.5 mg/kg at a dose volume of 1.0 ml/kg and two groups received single intravenous bolus injections of hGH in 0.9% NaCl solution at about 1.0 mg and 5.0 mg of hGH per kg rat with a dose volume of 1.0 ml/kg. For the Alzet pump study. rats were divided into four groups of three rats each. randomized by body weight and dosed with about 20 mg/ml and 40 mg/ml hGH in 0.9% saline solution loaded into pumps (Alzet Model 2002. 200 μL. 14 days release). and with about 4 mg/ml and 12 mg/ml hGH in 0.9% saline solution loaded into pumps (Alzet Model 2ML4. 2 ml. 28 days release). Expected release rates from the pumps correspond to about 2% and 4 to 6% of the dose (about 15 mg/kg) per day. respectively. The Alzet pumps were implanted subcutaneously in the inter-scapular region after soaking for 1–2 minutes in sterile saline.

The formulations of hGH sustained release microspheres. synthesized as described in Example 2 contained 15% w/w hGH complexed with Zn in a ratio of 6:1 Zn:hGH; 0%. 1%. 3% or 6% w/w zinc carbonate; and 8K unblocked PLGA. 10K blocked PLGA or 31K unblocked PLGA. To evaluate the various hGH sustained release formulations. Cmax. Cd5 and Cmax/Cd5 were the in vivo indices used. where Cmax is the maximum serum concentration observed. and Cd5 is the serum concentration at day 5 which should approximate the steady state concentration. The results were as follows:

Formulation	'Burst' in vitro (%)	% Monomer Day 7	Cmax (ng/ml)	C day 5 (ng/ml)	Cmax/Css
8K unblocked PLGA 0% ZnCO3	22.0 ± 0.9	99.3*	323.3 ± 98.6	20.4 ± 14.2	19.5 ± 10.6
8K unblocked PLGA 1% ZnCO3	16.4 ± 1.6	97.3*	309.0 ± 67.1	20.4 ± 14.2	39.5 ± 17.7
8K unblocked PLGA 3% ZnCO3	15.9 ± 6.9	98.7	670.5 ± 244.4	9.0 ± 4.2	44.8 ± 22.6
8K unblocked PLGA 6% ZnCO3	17.6 ± 2.7	99.3	358.0 ± 58.9	18.8 ± 14.7	42.4 ± 6.8
31K unblocked PLGA 0% ZnCO3	12.3 ± 1.1	98.2	592 ± 318.2	4.5 ± 1.5	132.5 ± 47.9
31K unblocked PLGA 1% ZnCO3	11.4 ± 1.3	98.8	432.7 ± 91.6	5.1 ± 0.3	84.1 ± 14.9
31K unblocked PLGA 3% ZnCO3	7.9 ± 1.9	99.4	643.6 ± 203.9	8.0 ± 2.6	93.3 ± 62.0
31K unblocked PLGA 6% ZnCO3	15.8 ± 0.5	99.8	1691.8 340.0±	6.6 ± 0.8	262.2 ± 83.5
10K unblocked PLGA 1% ZnCO3	12.7 ± 0.1	99.3	615.9 ± 384.3	4.5 ± 1.0	155.0 ± 126.8
10K blocked PLGA 3% ZnCO3	18.1 ± 3.2	99.6	1053.2 ± 293.3	3.6 ± 0.8	291.7 ± 71.1
10K blocked PLGA 6% ZnCO3	9.9 ± 1.4	99.0	1743.5 ± 428.4	4.9 ± 2.7	516.1 ± 361.6

*Value obtained from duplicate batch of the same formulation.

The results of the screening showed that the two unblocked polymers 8K and 31K had different in vivo release kinetics compared to the original formulation. Which used blocked 10K PLGA and 6% zinc carbonate. Cmax values were generally lower with the unblocked polymer

formulations than with the original formulation which suggested that the in vivo 'burst' may be lower with the unblocked polymer formulations. The 'burst' was defined as the percent of hGH released in the first 24 hours after injection. The in vitro 'burst' values were between 8–22%. The zinc carbonate content of the formulations did not appear to have an effect on the 'burst' or the in vitro release profile. The serum concentrations between days 4 and 6 were maintained at a fairly constant level above baseline (or the pre-bleed levels) with the unblocked polymer formulations. while serum concentrations with the blocked formulations. at the same time points were close to the baseline levels. The in vitro release data for up to 7 days showed that the released hGH protein was monomeric. Useful data could not be obtained beyond day 6 because of anti-hGH antibody formulation in the rats.

EXAMPLE 7

Rhesus Monkey Pharmacokinetics Study

The objective of this primate study was to evaluate the pharmacokinetic profiles of different hGH sustained release formulations as compared to more traditional methods of administering hGH (e.g., bolus sc injections. daily sc injections and sc injection combined with the use of an osmotic pump) and to determine which hGH sustained release formulatin gave the optimal hGH blood concentration profile.

The formulations for the hGH sustained release microspheres tested were 1) 15% hGH (complexed with Zn at a 6:1 Zn:hGH ratio). 6% w/w zinc carbonate and 10K blocked PLGA; 2) 15% hGH (complexed with Zn at a 6:1 Zn:hGH ratio). 1% w/w zinc carbonate and 8K unblocked PLGA ("RG502H" PLGA polymer); and 3) 15% hGH (complexed with Zn at a 6:1 Zn:hGH ratio). 1% w/w zinc carbonate and 31K unblocked PLGA ("RG503H" PLGA polymer).

There were four monkeys per group and each animal received a single subcutaneous injection into the dorsal cervical region on Day 1. A dose of 160 mg of hGH sustained release microspheres (24 mg of hGH) was administered to each monkey in 1.2 ml of injection vehicle through a 20 gauge needle. The injection vehicle was an aqueous vehicle containing 3% w/v Carboxymethyl Cellulose (sodium salt). 1% v/v Tween 20 (Polysorbate 20) and 0.9% sodium chloride.

The hGH dose was intended to provide measurable hGH serum concentrations for pharmacokinetic analysis. To obtain pharmacokinetic parameters additional study groups of four monkeys each were included. specifically 1) a single subcutaneous injection (24 mg hGH). 2) daily subcutaneous injections (24 mg/28 days=0.86 mg hGH/day). 3) a subcutaneous injection (3.6 mg hGH) combined with an Alzet osmotic pump (20.4 mg hGH)(total dose of 24 mg hGH). and 4) a subcutaneous injection of the injection vehicle as a control (only used 3 monkeys for the vehicle control group).

Blood samples were collected at the following times for hGH. IGF1 and anti-hGH antibody analyses: −7. −5. −3 days pre-dose and 0.5. 1. 2. 3. 5. 8. 10. 12. 24. 28. 32 and 48 hours. 5. 4. 6. 8. 11. 14. 17. 20. 23. 26. 29. 32. 25. 28. 41. 44. 47. 50. 53. 56 days post-dose.

The concentrations of IGF-1 and hGH in the serum were then measured. An IRMA kit from RADIM (distributed by: Wein Laboratories. P.O. Box 227. Succasunna. N.J.) was used to quantify hGH in monkey serum. The IRMA assay had a limit of quantification in PBS buffer of 0.1 ng/mL and in pooled juvenile rhesus monkey serum of 1.5 ng/mL with a basal GH level of about 4 ng/mL.

The results showed that the hGH sustained release microspheres were releasing significant. sustained levels of hGH over a one month period while the subcutaneous injections were not able to maintain the same serum levels.

The IGF-1 serum profile showed that serum IGF-1 concentrations were elevated above the baseline values between days 2 and 29 after administering the microparticles. This shows that enough hGH was being released from the hGH sustained release microspheres to cause a pharmacodynamic effect. This also indicates that the hGH released was biologically active which suggest that the encapsulation process had not adversely affected the biopotency of hGH.

EQUIVALENTS

Those skilled in the art will recognize. or be able to ascertain using no more than routine experimentation. many equivalents to specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

The invention claimed is:

1. A composition for the sustained release of human growth hormone from a polymeric matrix. comprising:

a) a biocompatible polymer; and

b) particles of metal cation-complexed human growth hormone. wherein said particles are dispersed within the biocompatible polymer.

2. A 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. poly (lactic acid-co-glycolic acid)s. polycaprolactone. polycarbonates. polyesteramides. polyanhydrides. poly

(amino acids). polyorthoesters. polycyanoacrylates. poly(p-dioxanone). poly(alkylene oxalate)s. biodegradable polyurethanes. blends and copolymers thereof.

3. A sustained release composition of claim 2 wherein said polymer comprises poly (lactide-co-glycolide).

4. A sustained release composition of claim 1 wherein the metal cation-complexed human growth hormone contains a multivalent metal cation component.

5. A sustained release composition of claim 4 wherein the metal cation component contains Zn^{+2}.

6. A sustained release composition of claim 1 further comprising a second metal cation component. wherein the second metal cation component is not complexed to said human growth hormone. and wherein the second metal cation component is dispersed within the biocompatible polymer to modulate the release of human growth hormone from the polymeric matrix.

7. A composition for the sustained release of human growth hormone from a polymeric matrix. comprising:

a) poly(lactide-co-glycolide);

b) particles of zinc-complexed human growth hormone. with a zinc-to-human growth hormone molar ratio between 4:1 and 100:1. wherein said particles are dispersed within the poly(lactide-co-glycolide. and wherein the proportion of human growth hormone in the sustained release composition is between 10 and 30 weight percent; and

c) a second metal cation component. wherein the second metal cation component is not complexed to said human growth hormone. and wherein the second metal cation component is dispersed within the polymeric matrix at a concentration of at least about 1% by weight of polymer.

8. A sustained release composition of claim 7 wherein the particles of zinc-complexed human growth hormone also contain sodium bicarbonate.

9. A method for providing a therapeutically effective blood level of biologically active. non-aggregated human growth hormone in a subject for a sustained period. comprising administering to the subject a dose of the sustained release composition of claim 1.

10. A sustained release composition of claim 7 wherein said second metal cation component is zinc carbonate.

11. A sustained release composition of claim 10 wherein said zinc carbonate is dispersed within the polymeric matrix at a concentration of at least about 3% by weight of polymer.

* * * * *

US005656297A

United States Patent [19]

Bernstein et al.

[11] **Patent Number: 5,656,297**

[45] **Date of Patent: *Aug. 12, 1997**

[54] **MODULATED RELEASE FROM BIOCOMPATIBLE POLYMERS**

[75] Inventors: **Howard Bernstein**; **Yan Zhang**, both of Cambridge, Mass.; **M. Amin Khan**, Downingtown, Pa.; **Mark A. Tracy**, Arlington, Mass.

[73] Assignee: **Alkermes Controlled Therapeutics, Incorporated**, Cambridge, Mass.

[*] Notice: The portion of the term of this patent subsequent to Jul. 25, 2014, has been disclaimed.

[21] Appl. No.: **237,057**

[22] Filed: **May 3, 1994**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 849,754, Mar. 12, 1992, abandoned.

[51] **Int. Cl.**^{6} **A61K 9/14**; A61K 9/16

[52] **U.S. Cl.** 424/484; 424/486; 424/487; 424/488; 424/489; 514/772.3; 514/772.6; 514/781; 514/805; 514/965

[58] **Field of Search** 424/489, 426, 424/425, 428, 486, 484, 487, 488; 514/772.3, 772.6, 781, 965

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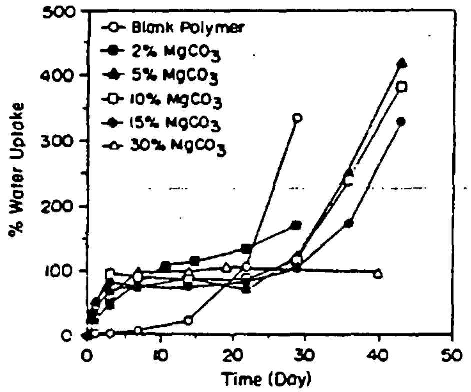
Primary Examiner—Thurman K. Page
Assistant Examiner—James M. Spear
Attorney, Agent, or Firm—Hamilton, Brook, Smith & Reynolds, P.C.

[57] **ABSTRACT**

The present invention relates to a composition for the modulated release of a biologically active agent. The composition comprises a biocompatible polymeric matrix, a biologically active agent which is dispersed within the polymeric matrix, and a metal cation component which is separately dispersed within the polymeric matrix, whereby the metal cation component modulates the release of the biologically active agent from the polymeric matrix. The present invention also relates to a method for modulating the release of a biologically active agent from a biocompatible polymeric matrix, comprising the steps of dissolving a biocompatible polymer in a solvent to form a polymer solution and also separately dispersing a metal cation component and a biologically active agent within the polymer solution. The polymer solution is then solidified to form a polymeric matrix, wherein at least a significant portion of the metal cation component is dispersed in the polymeric matrix separately from the biologically active protein, and whereby the metal cation component modulates the release of the biologically active agent from the polymeric matrix.

23 Claims, 9 Drawing Sheets



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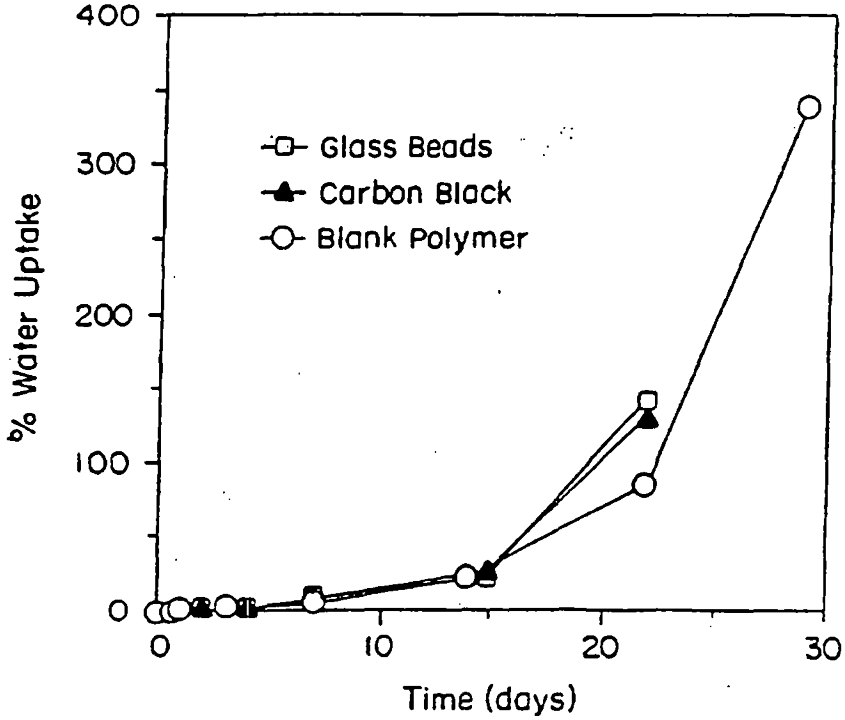

400
300
200
100
0
% Water Uptake
-□- Glass Beads
-▲- Carbon Black
-○- Blank Polymer
0
10
20
30
Time (days)

FIG. 1

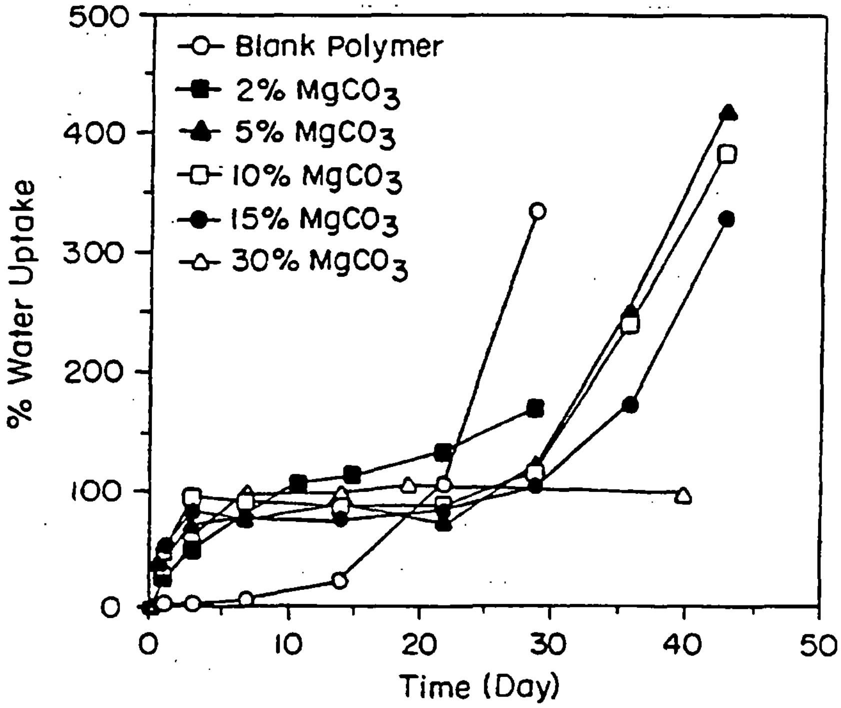
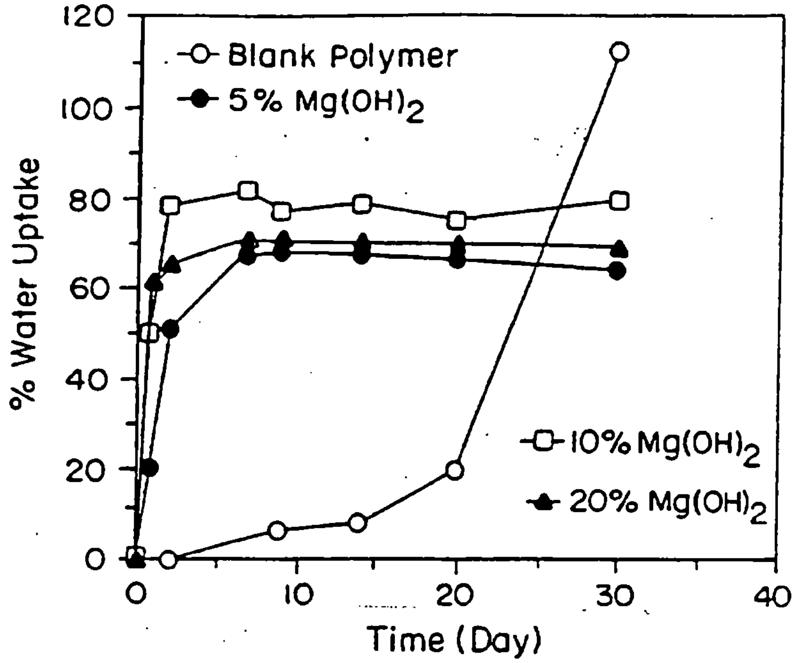
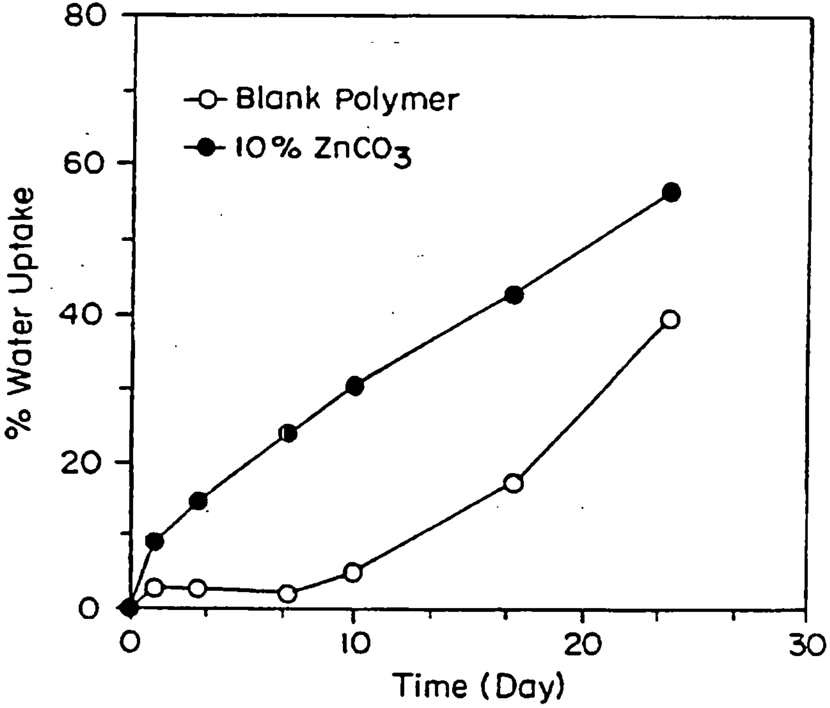

500
400
300
200
100
0
% Water Uptake
-○- Blank Polymer
-■- 2% MgCO3
-▲- 5% MgCO3
-□- 10% MgCO3
-●- 15% MgCO3
-△- 30% MgCO3
0
10
20
30
40
50
Time (Day)

FIG. 2



120
100
80
60
40
20
0
% Water Uptake
0
10
20
30
40
Time (Day)
-○- Blank Polymer
-●- 5% Mg(OH)2
-□- 10% Mg(OH)2
-▲- 20% Mg(OH)2

FIG. 3



80
60
40
20
0
% Water Uptake
0
10
20
30
Time (Day)
-○- Blank Polymer
-●- 10% ZnCO3

FIG. 4

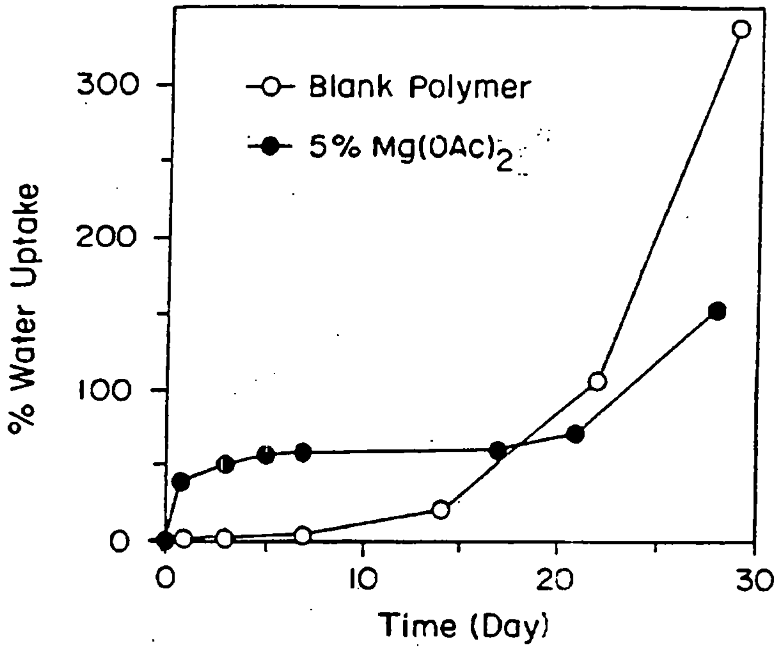

-○- Blank Polymer
-●- 5% $Mg(OAc)_2$
% Water Uptake
0
100
200
300
0
10
20
30
Time (Day)

FIG. 5

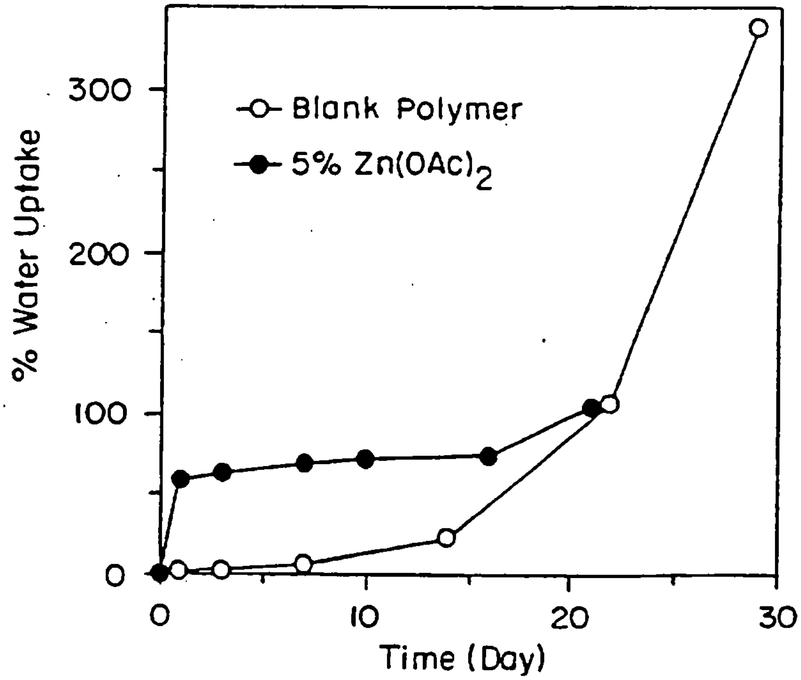
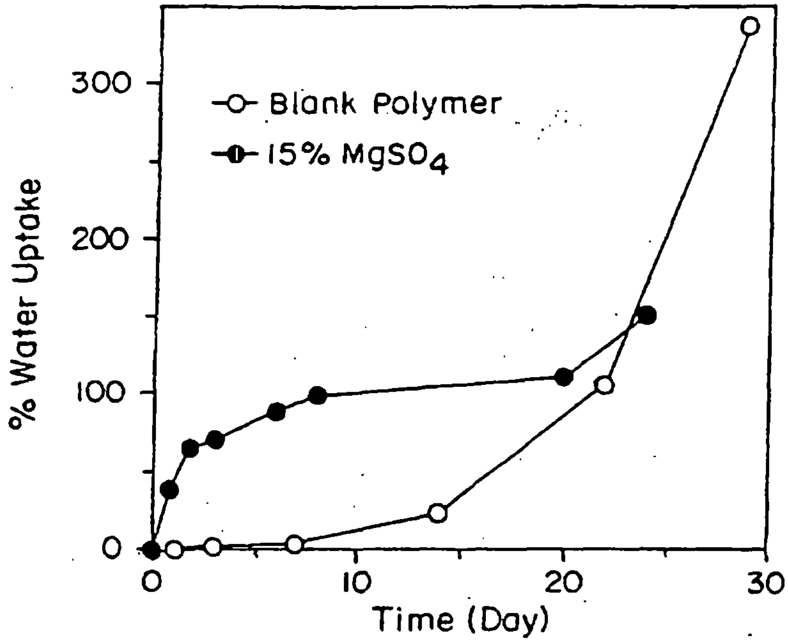
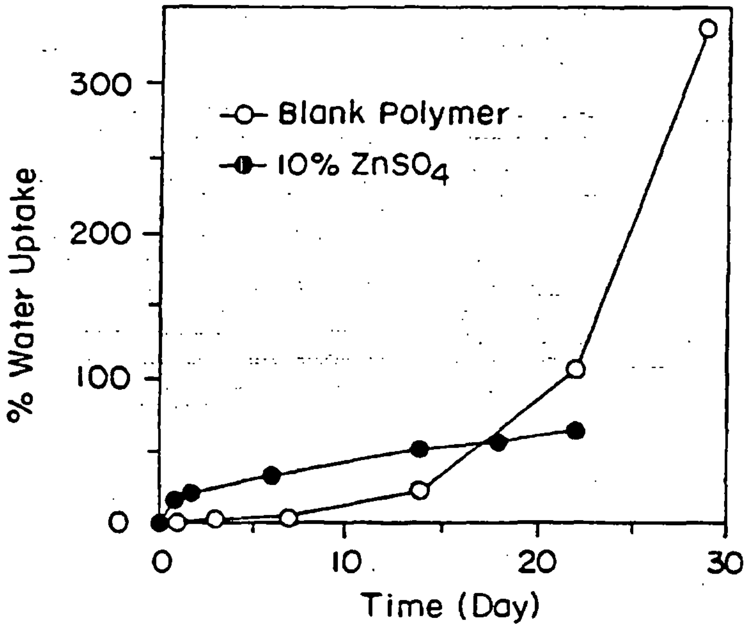

-○- Blank Polymer
-●- 5% $Zn(OAc)_2$
% Water Uptake
0
100
200
300
0
10
20
30
Time (Day)

FIG. 6



300
200
100
0
% Water Uptake
0
10
20
30
Time (Day)
-○- Blank Polymer
-●- 15% $MgSO_4$

FIG. 7



300
200
100
0
% Water Uptake
0
10
20
30
Time (Day)
-○- Blank Polymer
-●- 10% $ZnSO_4$

FIG. 8

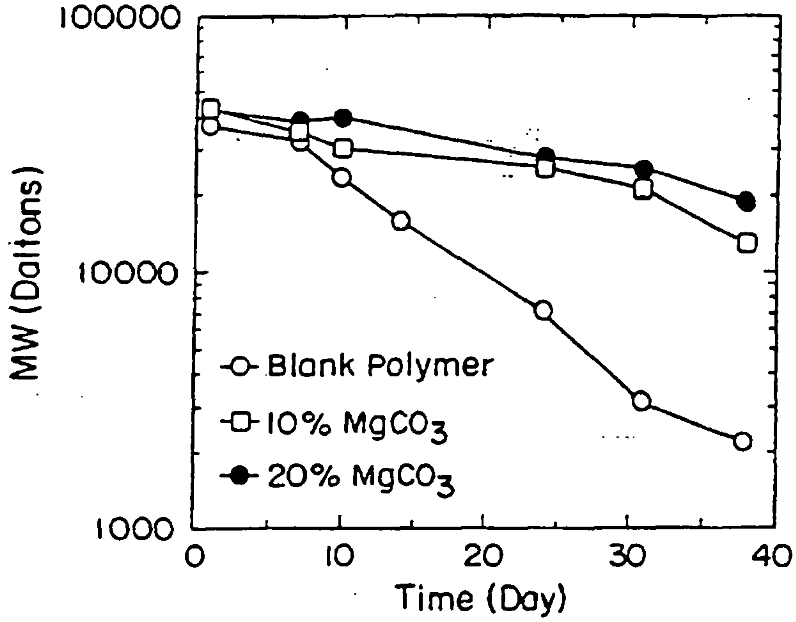

100000
10000
1000
MW (Daltons)
0
10
20
30
40
Time (Day)
-○- Blank Polymer
-□- 10% $MgCO_3$
-●- 20% $MgCO_3$

FIG. 9

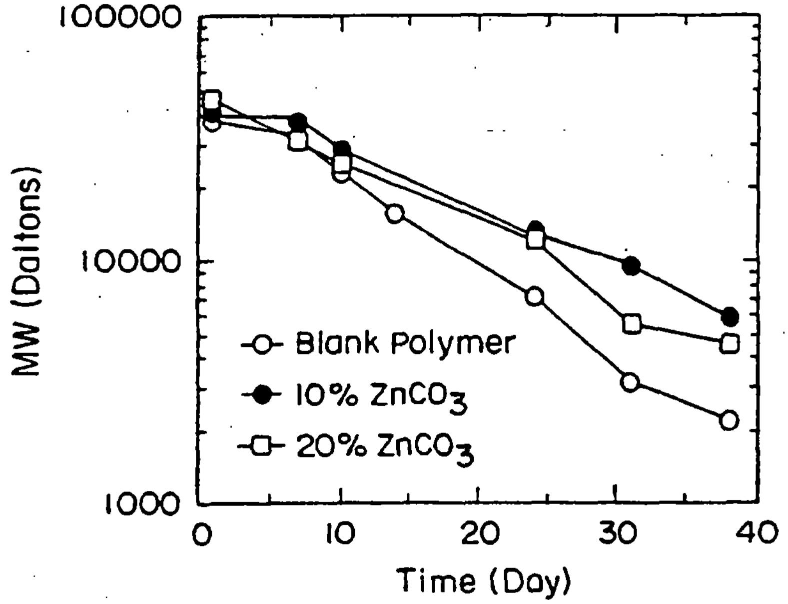

100000
10000
1000
MW (Daltons)
0
10
20
30
40
Time (Day)
-○- Blank Polymer
-●- 10% $ZnCO_3$
-□- 20% $ZnCO_3$

FIG. 10

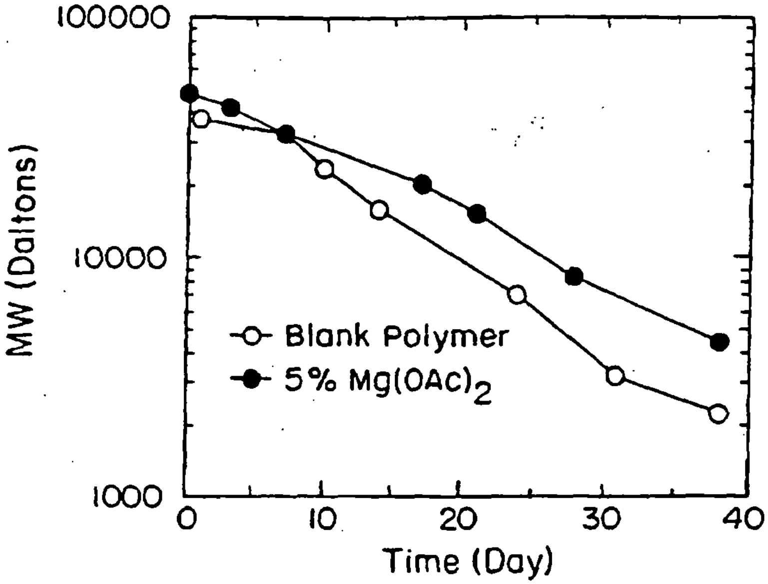

100000
10000
1000
MW (Daltons)
0
10
20
30
40
Time (Day)
Blank Polymer
5% Mg(OAc)2

FIG. 11

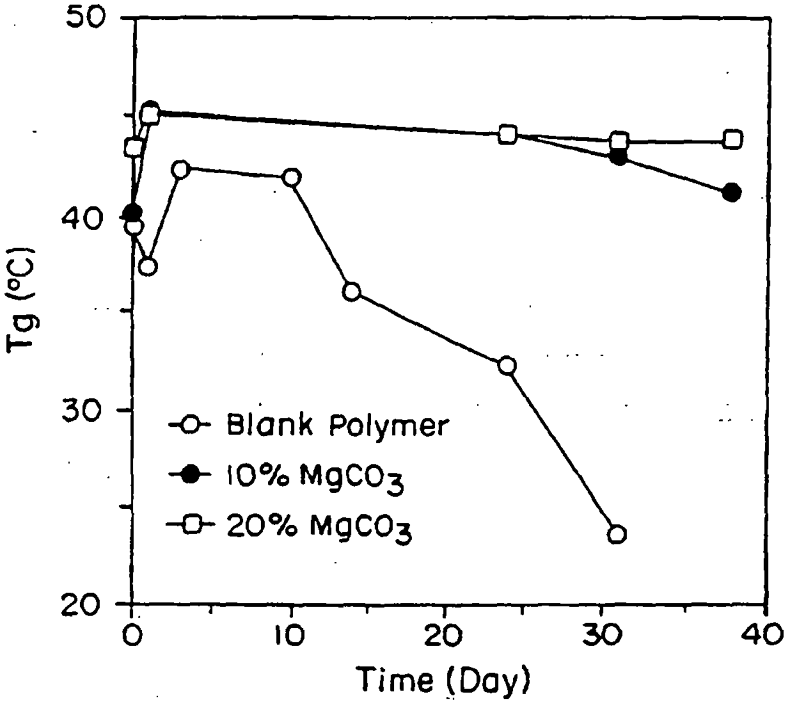

50
40
30
20
Tg (°C)
0
10
20
30
40
Time (Day)
Blank Polymer
10% MgCO3
20% MgCO3

FIG. 12

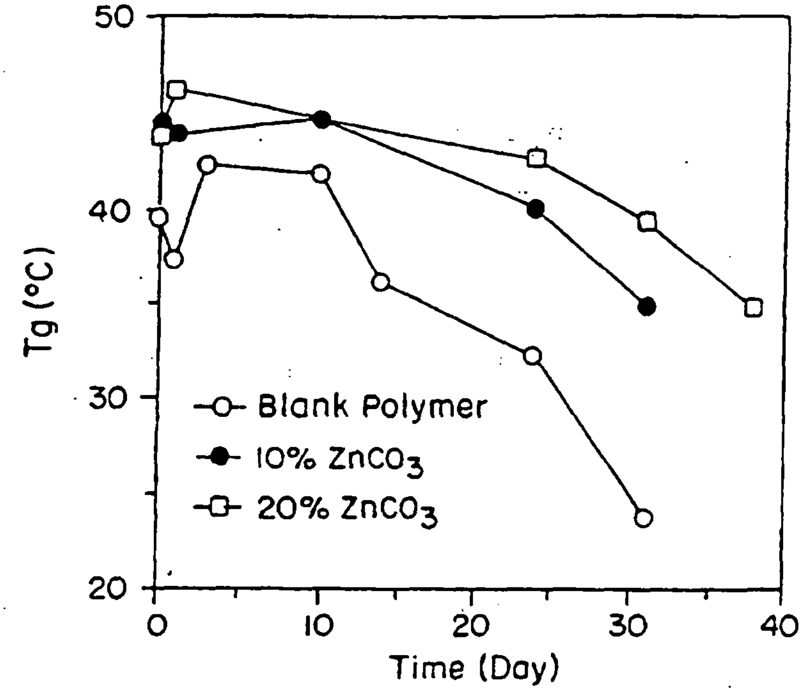

50
40
30
20
Tg (°C)
0
10
20
30
40
Time (Day)
-○- Blank Polymer
-●- 10% $ZnCO_3$
-□- 20% $ZnCO_3$

FIG. 13

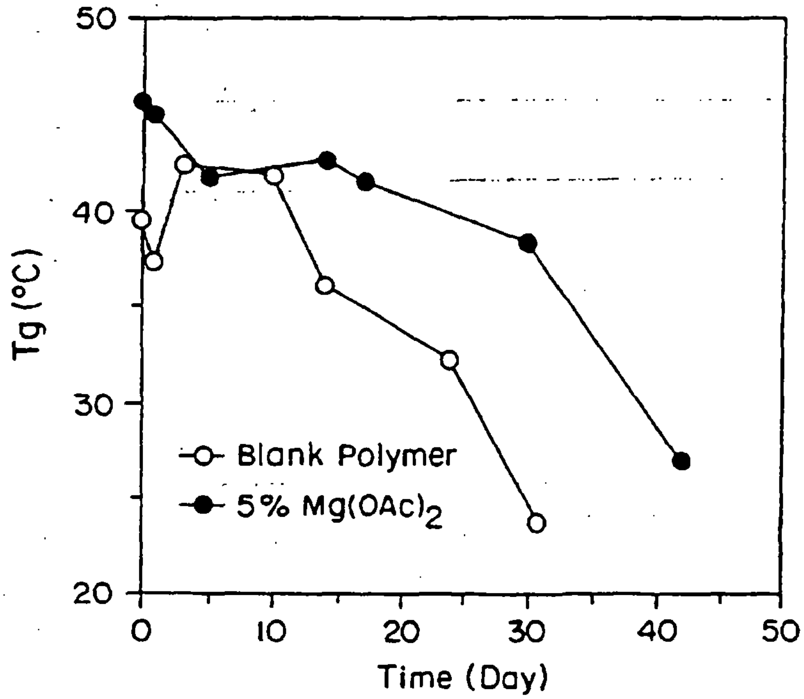
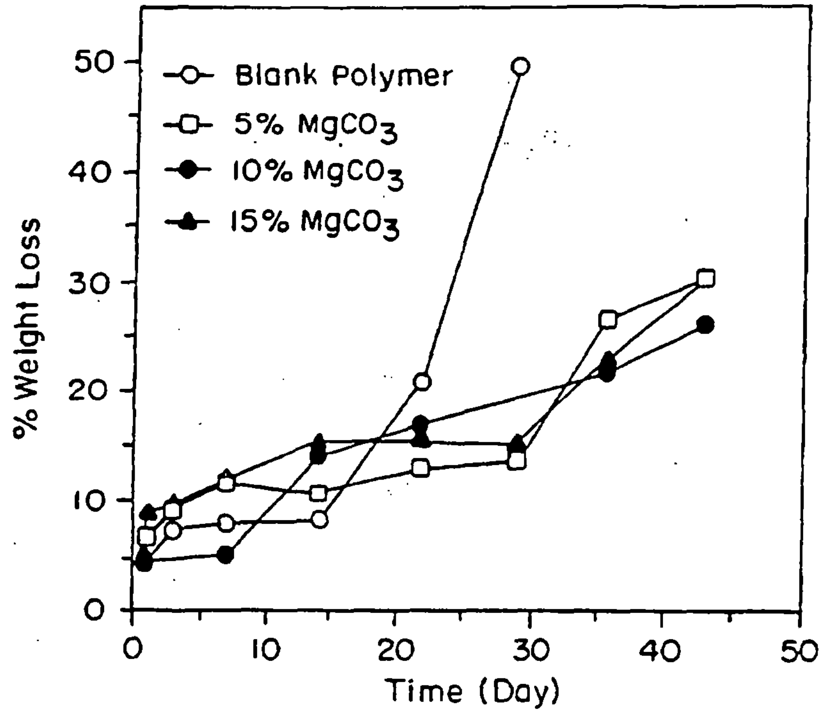
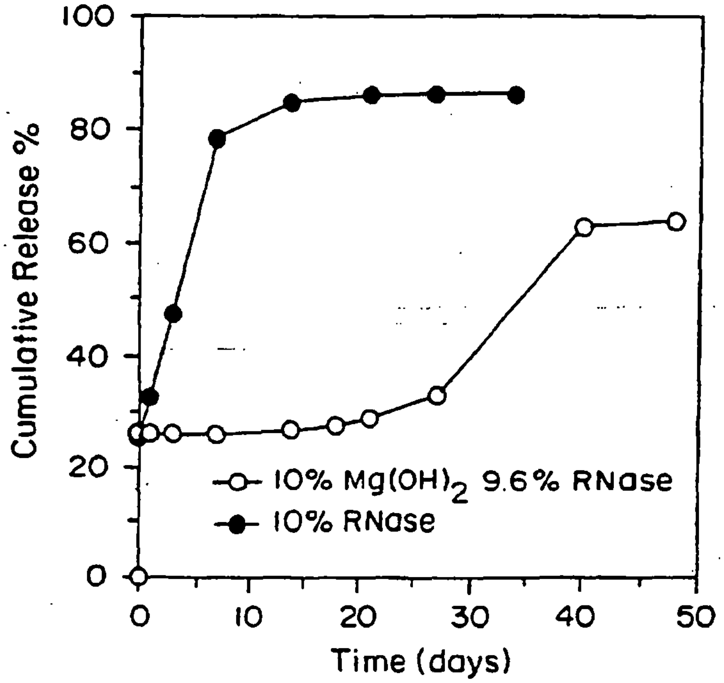

50
40
30
20
Tg (°C)
0
10
20
30
40
50
Time (Day)
-○- Blank Polymer
-●- 5% $Mg(OAc)_2$

FIG. 14



Blank Polymer
5% $MgCO_3$
10% $MgCO_3$
15% $MgCO_3$
% Weight Loss
0
10
20
30
40
50
Time (Day)
0
10
20
30
40
50

FIG. 15



100
80
60
40
20
0
Cumulative Release %
10% $Mg(OH)_2$ 9.6% RNase
10% RNase
0
10
20
30
40
50
Time (days)

FIG. 16

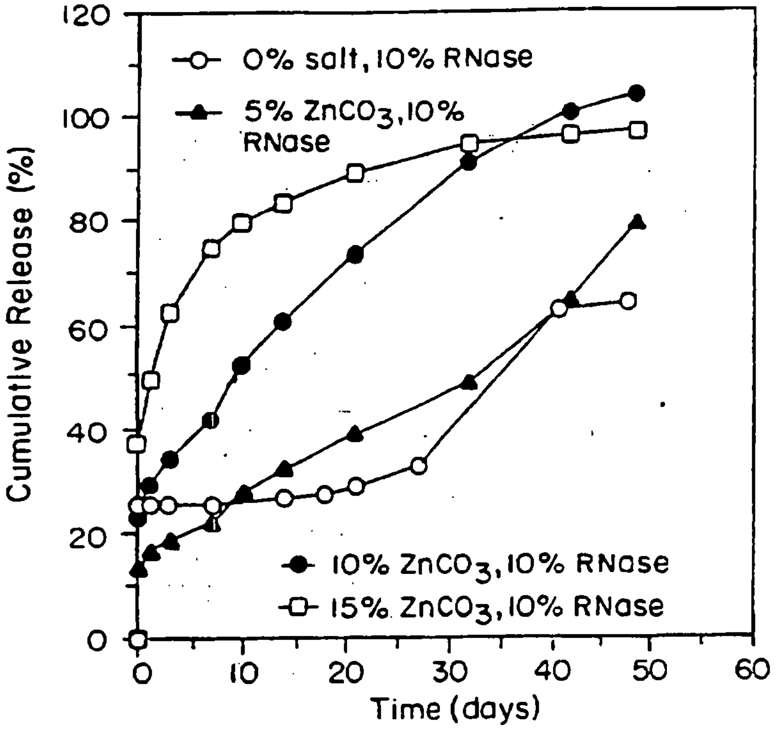

120
100
80
60
40
20
0
Cumulative Release (%)
0
10
20
30
40
50
60
Time (days)
0% salt, 10% RNase
5% $ZnCO_3$, 10% RNase
10% $ZnCO_3$, 10% RNase
15% $ZnCO_3$, 10% RNase

FIG. 17

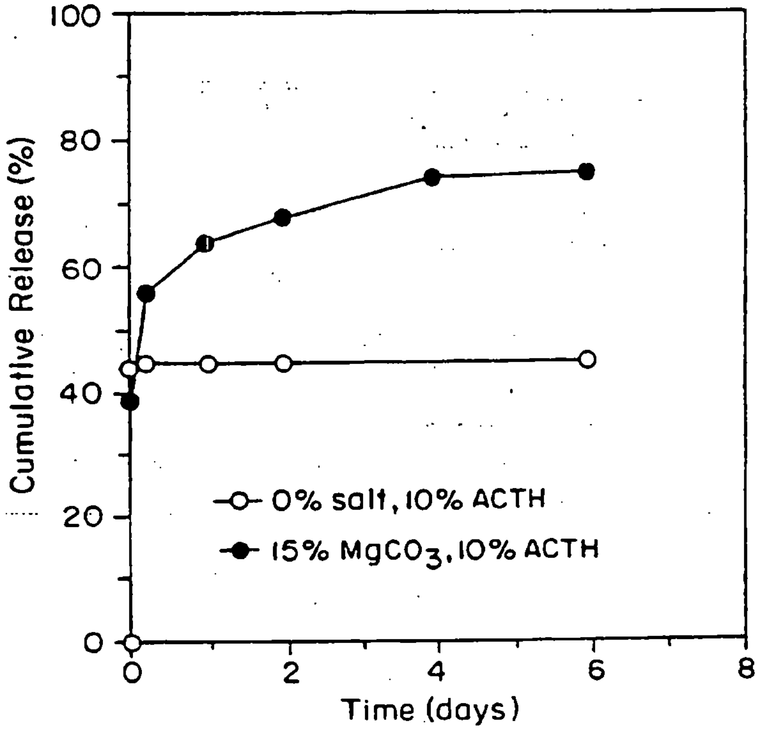

100
80
60
40
20
0
Cumulative Release (%)
0
2
4
6
8
Time (days)
0% salt, 10% ACTH
15% $MgCO_3$, 10% ACTH

FIG. 18

MODULATED RELEASE FROM BIOCOMPATIBLE POLYMERS

RELATED APPLICATIONS

This is a continuation-in-part of U.S. patent application Ser. No. 07/849.754. filed Mar. 12. 1992. now abandoned the teachings of which are hereby incorporated by reference.

BACKGROUND

Many illnesses or conditions require a constant level of medicaments or agents in vivo to provide the most effective prophylactic. therapeutic or diagnostic results. In the past. medicaments were given in doses at intervals which resulted in fluctuating medication levels.

Attempts to control and steady medication levels have more recently included the use of many biodegradable substances. such as poly(lactide) or poly(lactide-co-glycolide) microspheres containing the medicament. The use of these microspheres provided an improvement in the controlled release of medicaments by utilizing the inherent biodegradability of the polymer to improve the release of the medicament and provide a more even. controlled level of medication. However. in some cases. biodegradable polymers under in vivo conditions can have an initial level of medicament release. which is too high or too low. and after a period of hydration can substantially degrade to thereby limit the effective life of the controlled release microspheres. Therefore. a need exists for a means of modulating the controlled release of medicament from a biodegradable polymer to provide a higher level of initial medicament release and to provide longer periods of fairly consistent medicament release levels in vivo.

SUMMARY OF THE INVENTION

The present invention relates to a composition for the modulated release of a biologically active agent. The composition comprises a biocompatible polymeric matrix. a biologically active agent which is dispersed within the polymeric matrix. and a metal cation component which is separately dispersed within the polymeric matrix. whereby the metal cation component modulates the release of the biologically active agent from the polymeric matrix.

The present invention also relates to a method for modulating the release of a biologically active agent from a polymeric matrix. comprising the steps of dissolving a biocompatible polymer in a solvent to form a polymer solution and also separately dispersing a metal cation component and a biologically active agent within said polymer solution. The polymer solution is then solidified to form a polymeric matrix. wherein at least a significant portion of the metal cation component is dispersed in the polymeric matrix separately from the biologically active protein. and whereby the metal cation component modulates the release of the biologically active agent from the polymeric matrix.

This invention has the advantage of modulating the release of a biologically active agent in vivo from a biodegradable polymer. thereby enhancing the control of the level of prophylactic. therapeutic and diagnostic agents released in vivo and lengthening the period during which controlled release can be maintained for a single dose.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a plot of percent water uptake (%w/w) as a function of time in 10 mM HEPES for the following polymer films: a) blank PLGA. b) PLGA containing glass beads. and c) PLGA containing carbon black. illustrating the effect of glass beads and carbon black on PLGA film water absorption.

FIG. 2 is a plot of percent water uptake (%w/w) as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA. b) PLGA containing 2% $MgCO_3$. c) PLGA containing 5% $MgCO_3$. d) PLGA containing 10% $MgCO_3$. e) PLGA containing 15% $MgCO_3$. and f) PLGA containing 30% $MgCO_3$. illustrating the effect of $MgCO_3$ at different concentrations on PLGA film water absorption.

FIG. 3 is a plot of percent water uptake (%w/w) as a function of hydration time in 50 mM PBS for the following polymer films: a) blank PLGA. b) PLGA containing 5% $Mg(OH)_2$. c) PLGA containing 10% $Mg(OH)_2$. and d) PLGA containing 20% $Mg(OH)_2$. illustrating the effect of at different concentrations on PLGA film water absorption.

FIG. 4 is a plot of percent water uptake (%w/w) versus hydration time in 50 mM PBS for the following polymer films: a) blank PLGA and b) PLGA. containing 10% $ZnCO_3$. illustrating the effect of $ZnCO_3$ on PLGA film water absorption.

FIG. 5 is a plot of percent water uptake (%w/w) versus hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA and b) PLGA. containing 5% $Mg(OAc)_2$. illustrating the effect of $Mg(OAc)_2$ on PLGA film water absorption.

FIG. 6 is a plot of percent water uptake (%w/w) versus hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA and b) PLGA. containing 5% $Zn(OAc)_2$. illustrating the effect of $Zn(OAc)_2$ on PLGA film water absorption.

FIG. 7 is a plot of percent water uptake (%w/w) versus hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA and b) PLGA. containing 15% $MgSO_4$. illustrating the effect of $MgSO_4$ on PLGA film water absorption.

FIG. 8 is a plot of percent water uptake (%w/w) versus hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA and b) PLGA. containing 10% $ZnSO_4$. illustrating the effect of $ZnSO_4$ on PLGA film water absorption.

FIG. 9 is a plot of molecular weight as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA. b) PLGA containing 10% $MgCO_3$. and c) PLGA containing 20% $MgCO_3$. illustrating the effects of $MgCO_3$ at different concentrations on the changes in molecular weight of PLGA films due to hydration.

FIG. 10 is a plot of molecular weight as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA. b) PLGA containing 10% $ZnCO_3$. and c) PLGA containing 20% $ZnCO_3$. illustrating the effects of $ZnCO_3$ at different concentrations on the changes in molecular weight of PLGA films due to hydration.

FIG. 11 is a plot of molecular weight (Mw) as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA and b) PLGA containing 10% $Mg(OAc)_2$. illustrating the effects of $Mg(OAc)_2$ on the molecular weight of PLGA.

FIG. 12 is a plot of glass transition temperature (Tg) as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA. b) PLGA containing 10% $MgCO_3$. and c) PLGA containing 20% $MgCO_3$. illustrating the effects of $MgCO_3$ at different concentrations on the changes in the glass transition temperature of PLGA due to hydration.

FIG. 13 is a plot of glass transition temperature (Tg) as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA. b) PLGA containing 10% $ZnCO_3$. and c) PLGA containing 20% $ZnCO_3$. illustrating the effects of $ZnCO_3$ at different concentrations on the changes in the glass transition temperature of PLGA due to hydration.

FIG. 14 is a plot of glass transition temperature (Tg) as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA and b) PLGA containing 5% $Mg(OAc)_2$. illustrating the effects of $Mg(OAc)_2$ on the changes in glass transition temperature of PLGA due to hydration.

FIG. 15 is a plot of percent weight loss as a function of hydration time in 10 mM HEPES for the following polymer films: a) blank PLGA. b) PLGA containing 5% $MgCO_3$. c) PLGA containing 10% $MgCO_3$. and d) PLGA containing 15% $MgCO_3$. illustrating the effects of $MgCO_3$ at different concentrations on the degradation of PLGA due to hydration.

FIG. 16 is a plot of the cumulative percent release of RNase-A in 10 mM HEPES from PLGA microspheres containing 10% RNase-A and either 0% $Mg(OH)_2$ or 10% $Mg(OH)_2$. illustrating the effects $Mg(OH)_2$ on RNase-A release kinetics from PLGA microspheres due to hydration.

FIG. 17 is a plot of the cumulative percent release of RNase-A in 10 mM HEPES from PLGA microspheres containing 10% RNase-A and either 0% $ZnCO_3$. 5% $ZnCO_3$. 10% $ZnCO_3$. or 15% $ZnCO_3$. illustrating the effects $ZnCO_3$ on RNase-A release kinetics from PLGA microspheres due to hydration.

FIG. 18 is a plot of the cumulative percent release of adrenocorticotropin hormone (ACTH) in 50 mM PBS from PLGA microspheres containing 10% ACTH and either 0% $MgCO_2$ or 15% $MgCO_3$. illustrating the effects $MgCO_3$ on ACTH release kinetics from PLGA microspheres due to hydration.

DETAILED DESCRIPTION OF THE INVENTION

A modulated release of a biologically active agent. as defined herein. is a release of a biologically active agent from a biocompatible polymeric matrix containing a dispersed metal cation component which is separate from the biologically active agent. In a modulated release. at least one release characteristic. such as initial release level of said agent. subsequent agent release levels. the amount of agent released and/or the extent of the release period. are changed from the release characteristic(s) demonstrated for said biologically active agent from a polymeric matrix not containing a dispersed metal cation component.

A polymer of the polymeric matrix of this composition is a biocompatible polymer which can be either a biodegradable or non-biodegradable polymer. or blends or copolymers thereof.

Biodegradable. as defined herein. means the composition will degrade or erode in vivo to form smaller chemical species. Degradation can result. for example. by enzymatic. chemical and physical processes. Suitable biocompatible. biodegradable polymers include. for example. poly(lactide)s. poly(glycolide)s. poly(lactide-co-glycolide)s. polyanhydrides. polyorthoesters. polyetheresters. polycaprolactone. polyesteramides. blends and copolymers thereof.

Biocompatible. non-biodegradable polymers suitable for the modulated release composition of this invention 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. polyvinyl chloride. polyvinyl fluoride. poly(vinyl imidazole). chlorosulphonate polyolefins. polyethylene oxide. blends and copolymers thereof.

A polymer. or polymeric matrix. is biocompatible if the polymer. and any degradation products of the polymer. are non-toxic to the recipient and also present no significant deleterious or untoward effects on the recipient's body.

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. and rate of dissolution of polymer in solvent. Typically. an acceptable range of molecular weights is of about 2.000 Daltons to about 2.000.000 Daltons. In a preferred embodiment. the polymer is a biodegradable polymer or copolymer. In a more preferred embodiment. the polymer is a poly(lactide-co-glycolide) (hereinafter "PLGA") with a lactide:glycolide ratio of about 1:1 and a molecular weight of about 5.000 Daltons to about 70.000 Daltons. In an even more preferred embodiment. the molecular weight of the PLGA used in the present invention has a molecular weight of about 5.000 Daltons to about 42.000 Daltons

A biologically active agent. as defined herein. is an agent which possesses therapeutic. prophylactic or diagnostic properties in vivo. Examples of suitable therapeutic and/or prophylactic biologically active agents include proteins. such as hormones. antigens. growth factors. etc.; nucleic acids. such as antisense molecules; and small molecules. such as antibiotics. steroids. decongestants. neuroactive agents. anesthetics and sedatives. Examples of suitable diagnostic and/or therapeutic biologically active agents include radioactive isotopes and radiopaque agents.

In the modulated release composition of the present invention. an effective amount of particles of a biologically active agent is dispersed within a polymeric matrix. An effective amount of a biologically active agent is a therapeutically. prophylactically or diagnostically effective amount. which can be determined by a person of ordinary skill in the art taking into consideration factors such as body weight; age; physical condition; therapeutic. prophylactic or diagnostic goal desired. type of agent used. type of polymer used. initial burst and subsequent release levels desired. and release rate desired. Typically. a polymeric matrix for modulating the release of a biologically active agent will contain from about 0.01% (w/w) biologically active agent to about 50% (w/w) biologically active agent. by weight.

In one embodiment. a biologically active agent is a protein. Preferred proteins for inclusion in a modulated release composition include. for example. nucleases. erythropoietin. human growth hormone. interferons. interleukins. tumor necrosis factor. adrenocorticotropic hormone. growth factors. and colony-stimulating factors.

A modulated controlled release composition may also contain more than one biologically active agent. for instance. two different proteins. such as erythropoietin and granulocyte-macrophage colony-stimulating factor.

A metal cation component. as defined herein. is a component containing at least one kind of multivalent metal cation in a non-dissociated state. a dissociated state. or a combination of non-dissociated and dissociated states. Suitable metal cation components include. for instance. metal salts. metal hydroxides. and basic (pH of about 7 or higher) salts of weak acids wherein the salt contains a metal cation. It is preferred that the metal cation be divalent.

In the modulated release composition of the present invention. a suitable concentration of a metal cation component is dispersed within a polymer matrix. A suitable concentration of a metal cation component is any concentration of a metal cation component which will modulate the release of a biologically active agent from a polymeric matrix. In one embodiment. suitable proportions of a metal cation component to be dispersed in a polymer is between about 2% (w/w) to about 30% (w/w). The optimum ratio depends upon the polymer. the metal cation component and the biologically active agent utilized. In a preferred embodiment. suitable amounts of a metal cation component to be dispersed in a polymer is between about 5% (w/w) to about 20% (w/w).

In one embodiment. the metal cation component is substantially insoluble in aqueous fluids. Substantial insolubility in aqueous fluids. as defined herein means that the metal cation component is generally not soluble. or is of low solubility. in water or fluids. such as PBS. HEPES or alimentary track fluids. Examples of suitable insoluble metal cation components include. or contain. for instance. $Mg(OH)_2$. $MgCO_3$ (such as $4MgCO_3{\cdot}Mg(OH)_2{\cdot}5H_2O$). $ZnCO_3$ (such as $3Zn(OH)_2{\cdot}2ZnCO_3$). $CaCO_3$ and $Zn_3(C_6H_5O_7)_2$ (hereinafter zinc citrate).

In an alternate embodiment. the metal cation component is substantially soluble in aqueous fluids. Substantial solubility in aqueous fluids. as defined herein means that the metal cation component is generally soluble in water or fluids. such as PBS. HEPES or alimentary track fluids. Suitable soluble metal cation components include. or can contain. for example. $Mg(OAc)_2$. $MgSO_4$. $Zn(OAc)_2$ $ZnSO_4$. $ZnCl_2$. $MgCl_2$ and $Mg_3(C_6H_5O_7)_2$ (hereinafter magnesium citrate).

In one embodiment of the method for modulating the release of a biologically active agent from a polymeric matrix. a suitable polymer is dissolved in a solvent to form a polymer solution. Examples of suitable solvents include. for instance. polar organic solvents such as methylene chloride. chloroform. tetrahydrofuran. dimethyl sulfoxide and hexafluoro-isopropanol.

Particles of at least one metal cation component are then dispersed within the polymer solution. Suitable means of dispersing a metal cation component within a polymer solution include sonication. agitation. mixing and homogenization. It is understood that a metal cation component can be added directly to the polymer solution as a solid. preferentially in particulate form. wherein the metal cation component will either then be suspended as solid particles dispersed within the polymer solution or the metal cation component will then dissociate within the polymer solution to form free metal cations. It is also understood that. before addition to a polymer solution. a metal cation component can be suspended as solid particles or dissolved in a second solvent. wherein the second solvent is then added to the polymer solution. A second solvent is suitable if it is the same solvent as the polymer's solvent. or if the second solvent is miscible with the polymer's solvent and the polymer is soluble in the second solvent. An example of a suitable second solvent is acetone.

In another embodiment. a metal cation component can be suspended or dissolved in a solvent. after which. a suitable polymer is then dissolved in said solvent.

At least one biologically active agent is also added to the polymer solution separately from the addition of the metal cation component. metal cation component suspension. or metal cation component solution. In one embodiment. the biologically active agent is dissolved in a solvent. which is also suitable for the polymer. and then mixed into the polymer solution.

It is to be understood that a metal cation component and a biologically active agent can be added to the polymer solution sequentially. in reverse order. intermittently or through separate. concurrent additions. It is also understood that a biologically active agent can be suspended in a solution. or suspension. of a metal cation component in a solvent before dissolving the polymer in said solvent.

The amount of a biologically active agent added to the polymer solution can be determined empirically by comparative in vitro tests of polymeric matrices containing different concentrations of at least one metal cation component and of at least one biologically active agent. The amount used will vary depending upon the particular agent. the desired effect of the agent at the planned release levels. and the time span over which the agent will be released.

In an alternate embodiment. the protein added to the polymer solution can be mixed with an excipient. such as at least one stabilizing agent as is known in the art.

The formation of a polymeric matrix microparticle for modulating the release of RNase-A is further described in Example VII. The effectiveness of the method of modulating the release of RNase-A from a polymeric matrix is also described in Example VII.

The polymeric matrix 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 particle having a diameter of less than about one millimeter containing particles of a biologically active agent dispersed therein. A microparticle can have a spherical. non-spherical or irregular shape. The preferred microparticle shape is a sphere.

In a preferred embodiment. the method includes forming a modulated release polymeric matrix as a microparticle. A suitable metal cation component is dispersed as solid particles or free dissociated cations. and a biologically active agent is separately dispersed as solid particles in a polymer solution containing about 5–30% polymer by weight. In a more preferred embodiment. the polymer solution contains about 5–15% polymer by weight. Biodegradable polymers are preferred. while PLGA is more preferred.

A microparticle is then formed from the polymer solution. A suitable method for forming an acceptable microsphere from a polymer solution is described in U.S. Pat. No. 5.019.400. issued to Gombotz et al. The teachings of U.S. Pat. No. 5.019.400 are incorporated herein by reference.

In another embodiment. a modulated release composition is prepared by the solvent evaporation method described in U.S. Pat. No. 3.737.337. issued to Schnoring et al.. U.S. Pat. No. 3.523.906. issued to Vranchen et al.. U.S. Pat. No. 3.691.090. issued to Kitajima et al.. or U.S. Pat. No. 4.389. 330. issued to Tice et al.. which are incorporated herein by reference.

In the solvent evaporation method a polymer solution. which contains a dispersed metal cation component and a dispersed biologically active agent. is mixed in or agitated with a continuous phase. in which the polymer's solvent is substantially immiscible. to form an emulsion. The continuous phase is usually an aqueous solvent. Emulsifiers are often included in the continuous phase to stabilize the emulsion. The polymer's solvent is then evaporated over a period of several hours or more. thereby solidifying the polymer to form a polymeric matrix having a metal cation component and a biologically active agent separately dispersed therein.

In another embodiment. the method includes forming a modulated release polymeric matrix as a film or any other shape. A polymer solution and metal cation component. in particulate or dissociated form. is mixed. for instance by sonication. until the metal cations are generally dispersed throughout the polymer solution. The polymer solution is subsequently cast in a mold. such as a petri dish. The solvent is then removed by means known in the art until a film or form. with a constant dry weight. is obtained. The formation of polymeric matrix films is further described in Example I.

Several other methods of using the composition of this invention can be used to modulate physical properties of polymers. One embodiment of the method of use consists of a method for modifying the water absorption. or hydration capacity without significant polymer degradation. The method comprises forming a solution of a polymer and then dispersing a metal cation component into the polymer solution. The polymer solution is then solidified to form a polymer matrix wherein the metal cation component is dispersed therein. See Example II for a further description of this method of enhancing initial hydration.

A further embodiment of the method of use consists of a method for significantly stabilizing the glass transition temperature for a polymer during hydration. comprising the steps of forming a solution of a polymer and a solvent and then dispersing a metal cation component within said polymer solution. The polymer solution is then solidified to form a polymer matrix wherein particles of the metal cation component are dispersed therein.

Glass transition temperature (Tg) could be an indirect indicator of polymeric degradation since Tg is a function of the molecular weight of the polymer and usually decreases as molecular weight decreases. Glass transition temperature (Tg) is defined as the temperature at which a polymer converts from a glass phase to a rubbery phase. Tg is affected by the molecular weight of the polymer. See Example IV for further description of this method of stabilizing Tg during polymer hydration. In the embodiment wherein the polymeric matrix is in the form of microparticles. the stabilization of Tg maintains the mechanical properties of the polymer. thereby enhancing the control of agent release.

Yet another embodiment of the method of use consists of a method for increasing the porosity of a polymer without significant polymer degradation. This method includes the steps of forming a solution of a polymer and a solvent and then dispersing a metal cation component into said polymer solution. The polymer solution is then solidified to form a polymer matrix wherein the metal cation compound is dispersed therein and subsequently hydrated to form at least one gap within said polymeric matrix. thereby increasing the porosity of the polymer. Gaps. as defined herein comprise pores and/or voids. See Example V for a further description of this method of use.

An alternate embodiment of the method of use consists of a method for slowing the rate of degradation of a polymer. In this method a solution is formed of a polymer and a metal cation component is then dispersed within said polymer solution. The polymer solution is subsequently solidified to form a polymeric matrix having a metal cation component dispersed therein. Example III provides additional description of the slowing of the polymeric degradation rate.

The composition of this invention can be administered to a human. or other animal. by injection and/or implantation

subcutaneously. intramuscularly. intraperitoneally. intradermally. intravenously. intraarterially or intrathecally; by administration to mucosal membranes. such as intranasally or by means of a suppository. or by in situ delivery to provide the desired dosage of a biologically active agent based on the known parameters for treatment of the various medical conditions with said agent.

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

EXEMPLIFICATION

EXAMPLE I

Preparation of Polymer Films Containing Salts

PLGA (50:50) with a molecular weight of 42.000 Daltons (I.V. 0.7 dL/g Birmingham Polymers. Birmingham Ala.) was used for all film studies. The polymer films were produced by a film casting technique. The polymer was dissolved in methylene chloride (5% w/v) at room temperature for up to 24 hours.

Films were prepared using both water insoluble and soluble salts containing divalent cations. The salts were incorporated either as particulates or by cosolubilizing the salts with the polymer in an appropriate cosolvent. The fabrication procedure is described below.

Three salts with low water solubility. $MgCO_3$. $Mg(OH)_2$ and $ZnCO_3$ (Spectrum Chemical MFG. Corp.. Gardena. Calif.) and two water soluble salts. $MgSO_4$ and $ZnSO_4$ (Spectrum Chemical MFG. Corp.. Gardena. Calif.) were incorporated into films as particulates. MgCOs. $Mg(OH)_2$ and $ZnCO_3$ were sieved prior to film casting using a 38 micron U.S.A. standard testing sieve to control the particle size. The average particle diameter of the sieved salts prior to encapsulation is provided in Table 1.

TABLE 1

Salt	Formula	Diameter (μm)
$MgCO_3$	$4MgCO_3Mg(OH)_2 5H_2O$	2.5
$Mg(OH)_2$	$Mg(OH)_2$	2.5
$ZnCO_3$	$3Zn(OH)_2 2ZnCO_3$	4.0

As non-ionic water insoluble particulates. either carbon black or glass particles (20 micron diameter. Polysciences Inc. Warrington. Pa.) were used. Polymer films were prepared by adding the sieved salt to the polymer solution to a final concentration in the 0–30% (w/w. salt/polymer) range. The salt polymer suspension was sonicated for approximately four minutes to disperse the salt particles. A sample of 100 mL of the suspension was then cast in 9×5×1 inch teflon petri dish (Plastic Structures Co.. Wilmington. Mass.). The films were cast in two layers to avoid settling of the salt particles. The methylene chloride was evaporated at room temperature in a hood for the first 24 hours at atmospheric pressure. The films were transferred to a vacuum oven and were dried at 30° C. for 6 hours. 40° C. for 3 days. and then at 50° C. for 3 days. No further reduction in dry weight was observed at the end of this drying cycle.

Polymer films containing the water soluble salts magnesium acetate and zinc acetate were prepared by cosolubilizing the salts with PLGA in acetone. A 10% solution of polymer was prepared by dissolving 5 g of polymer in 50 mL of acetone at room temperature. A solution of $Mg(OAc)_2$ or $Zn(OAc)_2$ was prepared by dissolving 0.26 g of either salt in 50 mL of room temperature acetone. Equal volumes of the salt solution and the polymer solution were combined and the mixture was sonicated for approximately four minutes. One hundred milliliter samples of the salt-polymer solution were poured into the teflon petri dishes. The methylene chloride was evaporated as described previously.

EXAMPLE II

Water Uptake in Polymeric Films

Water uptake studies were conducted on the polymeric films made in Example I. The buffer solutions used in this study were HEPES (10 mM HEPES. 130 mM NaCl. 0.1% NaN_3. 1% Pluronics F68. pH 7.3) or PBS (50 mM Sodium Phosphate. 78 mM NaCl. 0.1% NAN_3. 1% Pluronics F68. pH 7.2). Film samples (50–80) mg were incubated in buffer (.5 ml/mg film) at 37° C. Duplicate film samples were utilized for each of the time points to enable both dry and wet weight measurements.

Samples were recovered at the specified time intervals. the surface water removed with absorbent paper and the samples were weighed. The samples were then frozen at −80° C. and subsequently lyophilized for 3–4 days until constant dry weight. The weights of the dried films were measured after lyophilization. Buffer solution was replaced in full for the film samples being incubated for the later water uptake determinations.

Water uptake was calculated at each time point using the following equation:

$$\%\ H_2O\ \text{Uptake} = \frac{\text{Wt. hydrated film} - \text{Wt. dried film}}{\text{Wt. dried film}} \times 100$$

Values obtained for duplicate samples were averaged.

The effects of different salts on the water uptake of the PLGA films are shown in FIGS. 1–8. The control films (blank films) without incorporated salts showed a slow. gradual increase in the amount of water absorbed during the first 15 to 20 days (FIG. 1). After this time. a large increase in water uptake was observed. This secondary phase of water uptake was associated with polymer degradation (see Example III). Films containing inert particles (carbon black or glass particles) exhibited water uptake profiles similar to the control polymer films (FIG. 1).

Films containing insoluble salts ($MgCO_3$. $Mg(OH)_2$ and $ZnCO_3$) all exhibited a greater initial water uptake than control films (FIGS. 2–4). Following the initial uptake phase. about 3 days. the amount of water absorbed by the films containing $MgCO_3$ and $Mg(OH)_2$ did not change until after 30 days. The second phase of water uptake occurred approximately 2 weeks later than was observed with control polymer films.

$ZnCO_3$ films exhibited a more continuous water uptake of a magnitude greater than that of control films (FIG. 4). There was no clear distinction between initial and secondary water uptake phases in the $ZnCO_3$ films.

$Mg(OAc)_2$ containing films showed an initial water uptake that was larger than the blank films (FIG. 5). but not as large as those with the insoluble magnesium salts. No additional water uptake was observed until after 21 days. when a second phase of water uptake took place. The onset of secondary water uptake was delayed by a few days relative to the blank film. Water uptake behavior by $Zn(OAc)_2$. $MgSO_4$ and $ZnSO_4$ films was similar to that of the $Mg(OAc)_2$ film samples (FIGS. 6–8).

EXAMPLE III

Effect of Salts on Polymer Degradation

The effects of encapsulated salts on polymer degradation rates were assessed by molecular weight determinations by gel permeation chromatography (GPC). The films prepared in Example I were hydrated as described in Example II. The film samples were redissolved in chloroform (5–10 mg/mL) and were filtered through a 0.22 micron filter. GPC was conducted using a MIXED column (300×10 mm. Polymer Labs) with chloroform as eluent and refractive index for detection. Molecular weights were calculated using polystyrene as standards (580 to 950.000 Daltons) and the universal calibration method.

The molecular weight of the control films decreased from 42000 to 3000 Daltons after 30 days as shown in FIG. 9.

In contrast. the rate of decrease in molecular weight of the films containing $MgCO_3$ were smaller than for the control film (see FIG. 9). The molecular weight decrease in films with $ZnCO_3$ was slower than in control films (FIG. 10). but more rapid than in films containing $MgCO_3$. Similar degradation kinetics were observed with $Mg(OAc)_2$ containing films (FIG. 11).

EXAMPLE IV

Effect of Salts on Glass Transition Temperature

The glass transition temperature (Tg) of the films was determined using a differential scanning calorimeter (DSC) (DSC 7 Serial. Perkin Elmer. Norwalk. Conn.) under nitrogen and using indium as a standard. Each sample was cooled to 0° C. before heating to 60° C. at 10° C./min. Tg measurements were performed on the film samples after lyophilization as described in Example II.

The time course of Tg decrease for control films is plotted in FIG. 12. The drop in Tg observed between 10 and 15 days corresponds to the point at which the polymer MW decreases to less than 20.000 Daltons.

In contrast. the rates of Tg decrease in polymer films that contained Mg and Zn salts (FIGS. 12–14) were either negligible (in the case of $MgCO_3$. FIG. 12). or significantly slower ($ZnCO_3$ and $Mg(OAc)_2$: FIGS. 13 and 14. respectively) than those of control films. In $MgCO_3$ and $ZnCO_3$ containing films. a trend toward a slower Tg decrease with increasing salt content was observed.

EXAMPLE V

Effect of Salts on Film Porosity

SEM was used to observe qualitative changes in film porosity and to monitor morphology changes of the film surfaces and cross sections over time. Samples were lyophilized as described in Example II. The dried samples were sputter-coated with gold 200–300 A and the samples observed using JEOL-6400 SEM.

All films displayed a dense structure with a few pores scattered throughout the device prior to hydration. However. the rate of water uptake was different depending on the incorporated salt. Thus the increase in water uptake was not dominated by initial porosity of the sample but was a function of the type of salt dispersed in the polymer film.

SEM evaluation of the control films without salts demonstrated a dense and smooth structure up to 14 days of hydration. Between 14 and 22 days. large pores became visible on the film surface and throughout the sample cross section. The appearance of these pores coincides with the secondary water uptake phase associated with polymer degradation and erosion of the polymer (see Examples II–IV).

Films loaded with water insoluble salts exhibited increasing porosity after hydration times as short as 24 hours. SEM analysis of 24 hour hydration samples of films containing 2% $MgCO_3$ showed the formation of a porous network within the film sample, concentrated at the film surface. After 7 days, the film had become uniformly porous across the cross section. Pores ranged in diameter from approximately 1–20 μm. No further increase in porosity was observed between 7 days and 22 days. Similar behavior was observed with films that contained higher $MgCO_3$ percentages.

Films that contained 10% $ZnCO_3$ were also observed to become highly porous within 3 days of hydration. Three day hydration samples showed the presence of a porous network extending throughout the entire film cross section. The morphology of hydrated $ZnCO_3$ containing films was similar to hydrated films with $MgCO_3$.

Films that contained water soluble magnesium salts also exhibited the formation of internal and surface pores and voids well before pore formation occurred in control films. Pores ranging in diameter from approximately 1–50 μm were visible in samples that had been hydrated for five days.

There was some difference between the morphology of the films loaded with soluble and insoluble salts that were hydrated for 5 to 7 days. The films loaded with $Mg(OAc)_2$ seemed to display a lower porosity and a tendency toward large voids (approximately 50 microns) compared to films that contained insoluble salts. $MgCO_3$ and $ZnCO_3$ films showed a higher porosity; a majority of the pore volume was composed of pores of less than ten microns in diameter.

EXAMPLE VI

Effect of Salts on Polymer Weight Loss

The effects of insoluble salts on polymer degradation in hydrated film samples were also assessed by monitoring the time course of polymer weight loss during incubation. The films prepared in Example I were hydrated as described in Example II. Samples were recovered at the indicated time intervals and freeze-dried as described in Example II. The weights of the dried film samples were measured after lyophilization. Percent weight loss at different times was computed according to the equation:

$$\%\ \text{Weight Loss}\ (t)=100 \times (W_{initial}-W_t)/W_{initial}$$

where $W_{initial}$ is the initial weight of the polymer film and W_t is the weight of the sample at time point t.

The time course of weight loss in the control film is shown in FIG. 15. There is little weight loss until 14 days, after which rapid weight loss takes place. This phase of weight loss is associated with degradation and erosion of the polymer, as evidenced by increased water uptake, decreased molecular weight and Tg and the appearance of pores and voids in SEMs of film samples (see Examples II, III, V and VI). Also shown in FIG. 15 are weight loss profiles for polymer films that contain 5, 10 and 15% $MgCO_3$. Instead, weight loss in these films was more gradual and of a lesser magnitude.

A portion of the weight loss occurring in $MgCO_3$-containing films was due to dissolution of the encapsulated salt particles. To assess how closely total weight loss measurements approximate polymer weight loss in salt-containing film samples, the polymer weight loss was estimated according to the following two extreme scenarios: (1) all of the encapsulated salt dissolved between the initial hydration and the first time point, and (2) no salt dissolved throughout the entire study. Regardless of which salt dissolution scenario was selected, polymer weight loss in control films exceeded that of $MgCO_3$-containing films, indicating that incorporation of the insoluble salt prevented or delayed erosion of the polymeric matrix.

EXAMPLE VII

Effect of Salts on the Release of RNase-A or ACTH from PLGA Microspheres

RNase-A was microencapsulated into 5000 Dalton PLGA (I.V. 0.15 dL/g Birmingham Polymers, Birmingham, Ala.) with either $ZnCO_3$ or $Mg(OH)_2$. Adrenocorticotropin hormone (ACTH) was microencapsulated into the same type PLGA with $MgCO_3$. The method described in U.S. Pat. No. 5,019,400, issued to Gombotz et al. was used to encapsulate the RNase-A and ACTH proteins. Each protein (10% w/w) and the salts (0%, 5%, 10% or 15% w/w) were added separately as particulates to a solution of PLGA in methylene chloride which was sonicated at 4° C. for 30 seconds. The suspension was then sprayed into liquid nitrogen which was overlaying frozen ethanol. The methylene chloride was extracted into the ethanol at −80° C. The microspheres were filtered and lyophilized to produce a dry powder.

The effect of the salts upon the in vitro release kinetics of RNase-A and ACTH was assessed. Release studies were conducted by suspending 20 mg of microspheres in 1 mL of 10 mM HEPES buffer at 37° C. Assays were done in 2 mL polypropylene Eppendorf tubes. Release studies of ACTH were conducted in the same manner with the exception of using PBS in lieu of HEPES buffer. At the specified time points, the buffer was removed in full and replaced with fresh buffer. The concentration of RNase-A in buffer was measured using the BCA Protein Assay (Pierce, Rockford, Ill.) and the concentration of ACTH was measured using the Biorad Protein assay (Biorad, Richmond, Calif.).

The effects of $ZnCO_3$ or $Mg(OH)_2$ on the release kinetics of RNase-A are shown in FIGS. 16 and 17. RNase-A encapsulated into PLGA alone exhibited release of the protein over the first 24 hours after which no further release was observed until day twenty one. $Mg(OH)_2$ resulted in continuous release of the protein over days. $ZnCO_3$ resulted in continuous release of the protein over thirty five days.

The effect of $MgCO_3$ on the release kinetics of ACTH is shown in FIG. 18. ACTH encapsulated into PLGA alone exhibited approximately 40% release of the protein over the first 24 hours after which no further release was observed. $MgCO_3$ resulted in continuous release of the protein over the same period.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

The invention claimed is:

1. A pharmaceutical composition for the modulated release of a biologically active pharmaceutical agent, comprising:

a) a biocompatible and biodegradable polymeric matrix;

b) an effective amount of a biologically active pharmaceutical agent, the biologically active pharmaceutical agent being dispersed within the polymeric matrix; and

c) a metal cation component for modulating release of the biologically active agent from the polymeric matrix, wherein the metal cation component comprises a cation selected from the group consisting of Zn(II). Mg(II) and a combination of at least two different multivalent metal cations. and wherein the metal cation component is separately dispersed within the polymeric matrix.

2. A modulated release composition of claim 1 wherein said metal cation component is selected from the group consisting of magnesium hydroxide. magnesium carbonate. zinc carbonate. magnesium acetate. zinc acetate. magnesium chloride. zinc chloride. magnesium sulfate. zinc sulfate. magnesium citrate and zinc citrate.

3. A composition for the modulated release of a biologically active pharmaceutical agent. comprising:

a) a biocompatible and biodegradable polymeric matrix;

b) an effective amount of a biologically active pharmaceutical agent. the biologically active pharmaceutical agent being dispersed within the polymeric matrix; and

c) a metal cation component for modulating the release of the biologically active agent from the polymeric matrix. wherein said metal cation component is selected from the group consisting of magnesium hydroxide. magnesium carbonate. calcium carbonate. zinc carbonate. magnesium acetate. zinc acetate. magnesium chloride. zinc chloride. magnesium sulfate. zinc sulfate. magnesium citrate and zinc citrate. and wherein the metal cation component is separately dispersed within the polymeric matrix.

4. A method for significantly stabilizing the glass transition temperature for a polymeric matrix during hydration. comprising the steps of:

a) forming a solution of a polymer;

b) dispersing a metal cation component into said polymer solution; and

c) solidifying said polymer from said polymer solution to form a polymeric matrix. containing the metal cation component dispersed therein. thereby stabilizing the glass transition temperature for a polymeric matrix during hydration.

5. A method for enhancing the initial hydration capacity of a polymeric matrix without significant polymer degradation. comprising the steps of:

a) forming a solution of a biodegradable polymer in a solvent;

b) dispersing a metal cation component within said polymer solution; and

c) solidifying said polymer from said polymer solution to form a polymeric matrix. containing the metal cation component dispersed therein. thereby enhancing the initial hydration capacity of a polymeric matrix without significant polymer degradation.

6. A method for increasing the porosity of a polymeric matrix. comprising the steps of:

a) forming a solution of a polymer and a solvent;

b) dispersing a metal cation component within said polymer solution;

c) solidifying said polymer from said polymer solution. to form a polymeric matrix containing the metal cation component dispersed therein; and

d) hydrating said polymeric matrix to thereby form at least one gap within said polymeric matrix. thereby increasing the porosity of said polymeric matrix.

7. A modulated release composition of claim 3 wherein said biodegradable and biocompatible polymer is selected from the group consisting of poly(lactide) s. poly(glycolide) s. poly(lactide-co-glycolide)s. polyanhydrides. polyorthoesters. polyetheresters. polycaprolactone. polyesteramides. blends and copolymers thereof.

8. A composition for the modulated release of a biologically active protein. comprising:

a) a biocompatible polymeric matrix;

b) an effective amount of a biologically active protein. the biologically active protein being dispersed within the polymeric matrix; and

c) a metal cation component for modulating release of the biologically active protein from the polymeric matrix. wherein the metal cation component comprises a cation selected from the group consisting of Zn(II). Mg(II) and a combination of at least two different multivalent metal cations. and wherein the metal cation component is separately dispersed within the polymeric matrix.

9. A modulated release composition of claim 8 wherein said polymer is biodegradable.

10. A modulated release composition of claim 7 wherein said biologically active agent comprises a protein.

11. A modulated release composition of claim 10 wherein said protein is selected from the group consisting of nucleases. erythropoietin. human growth hormone. interferons. interleukins. growth factors. tumor necrosis factor. adrenocorticotropic hormone. and colony-stimulating factors.

12. A method for modulating the release of a biologically active agent from a polymeric matrix. comprising:

a) dissolving a biocompatible polymer in a solvent to form a polymer solution;

b) dispersing a metal cation component in said solvent. wherein the metal cation component comprises a metal cation selected from the group consisting of Zn(II). Mg(II) and a combination of at least two different multivalent metal cations;

c) separately dispersing a biologically active agent in said polymer solution; and

d) solidifying said polymer from said polymer solution to form a polymeric matrix. whereby the metal cation component modulates the release of the biologically active agent from the polymeric matrix.

13. A composition for the modulated release of a biologically active agent. comprising:

a) a biocompatible polymeric matrix of a polymer selected from the group consisting of poly(lactide)s. poly(glycolide)s. poly(lactide-co-glycolide)s and blends thereof;

b) an effective amount of a biologically active protein. said biologically active protein being dispersed within the polymeric matrix; and

c) a metal cation component for modulating release of the biologically active agent from the polymeric matrix. wherein said metal cation component is selected from the group consisting of magnesium hydroxide. magnesium carbonate. calcium carbonate. zinc carbonate. magnesium acetate. zinc acetate. magnesium sulfate. zinc sulfate. magnesium chloride. zinc chloride. zinc citrate and magnesium citrate. and wherein the metal cation component is separately dispersed within the polymeric matrix.

14. A modulated release composition of claim 13 wherein said biologically active protein is selected from the group consisting of nucleases. erythropoietin. human growth hormone. interferons. interleukins. growth factors. adrenocorticotropic hormone. tumor necrosis factor and colony-stimulating factors.

15. A method of claim 12. further comprising the step of suspending particles of said metal cation component in a

second solvent before dispersing the metal cation component in the polymer solution. wherein the second solvent is miscible with said first solvent. and wherein said polymer is soluble in the second solvent.

16. A method for modulating the release of a biologically active agent from a polymeric matrix. comprising:

a) dissolving a biocompatible polymer in a solvent to form a polymer solution;

d) dispersing a metal cation component in said solvent wherein said metal cation component is selected from the group consisting of magnesium hydroxide. magnesium carbonate. calcium carbonate. zinc carbonate. magnesium acetate. zinc acetate. magnesium sulfate. zinc sulfate. magnesium chloride. zinc chloride. zinc citrate and magnesium citrate;

c) separately dispersing a biologically active agent in said polymer solution; and

d) solidifying said polymer from said polymer solution to form a polymeric matrix. whereby the metal cation component modulates the release of the biologically active agent from the polymeric matrix.

17. A method of claim 16 wherein said biologically active agent comprises a protein.

18. A method of claim 17 wherein said protein is selected from the group consisting of nucleases. erythropoietin. human growth hormone. interferons. interleukins. growth factors. adrenocorticotropic hormone. tumor necrosis factor and colony-stimulating factors.

19. A method of claim 18 wherein said polymer is biodegradable.

20. A method of claim 19 wherein said biodegradable polymer is selected from the group consisting of poly (lactide)s. poly(glycolide) s. poly(lactide-co-glycolide)s. polyanhydrides. polyorthoesters. polyetheresters. polycaprolactone. polyesteramides. blends and copolymers thereof.

21. A method of claim 18 wherein said polymer is non-biodegradable.

22. A method of claim 21 wherein said non-biodegradable polymer is selected from the group consisting of polyacrylates. polymers of ethylene-vinyl acetates. and acyl substituted cellulose acetates. non-degradable polyurethanes. polystyrenes. polyvinyl chloride. polyvinyl fluoride. poly(vinyl imidazole). chlorosulphonate polyolefins. polyethylene oxide. blends and copolymers thereof.

23. A method of claim 15. further comprising the step of dissolving said metal cation component in a second solvent before dispersing the metal cation component in the polymer solution. wherein the second solvent is miscible with the first solvent. and wherein said polymer is soluble in the second solvent.

* * * * *

14. PATENT CERTIFICATION WITH RESPECT TO ANY PATENT WHICH CLAIMS THE DRUG

All investigations in this application were conducted by or for the applicant; hence, this section is not applicable.

BEST POSSIBLE COPY

PEDIATRIC PAGE

(Complete for all original applications and all efficacy supplements)

NOTE: A new Pediatric Page must be completed at the time of each action even though one was prepared at the time of the last action.

NDA LA # 21-075 Supplement # N/A Circle one [✓ SE1] SE2 SE3 SE4 SE5 SE6

HFD 510 Trade and generic names/dosage form: Nutropin [somatropin [rDNA origin] for injectable suspension) Depot Action: [✓ AP] AE NA

Applicant Genentech Inc Therapeutic Class growth hormone

Indication(s) previously approved SIMILAR TO THE REQUESTED IN THIS APPLICATION

Pediatric information in labeling of approved indication(s) is adequate X inadequate __

Proposed indication in this application TREATMENT OF GROWTH HORMONE DEFICIENCY IN CHILDREN

FOR SUPPLEMENTS, ANSWER THE FOLLOWING QUESTIONS IN RELATION TO THE PROPOSED INDICATION. N/A

IS THE DRUG NEEDED IN ANY PEDIATRIC AGE GROUPS? ___Yes (Continue with questions) ___No (Sign and return the form)

WHAT PEDIATRIC AGE GROUPS IS THE DRUG NEEDED? (Check all that apply)

__Neonates (Birth-1month) __Infants (1month-2yrs) __Children (2-12yrs) __Adolecents(12-16yrs)

✓ 1. **PEDIATRIC LABELING IS ADEQUATE FOR <u>ALL</u> PEDIATRIC AGE GROUPS.** Appropriate information has been submitted in this or previous applications and has been adequately summarized in the labeling to permit satisfactory labeling for all pediatric age groups. Further information is not required.

__ 2. **PEDIATRIC LABELING IS ADEQUATE FOR CERTAIN AGE GROUPS.** Appropriate information has been submitted in this or previous applications and has been adequately summarized in the labeling to permit satisfactory labeling for certain pediatric age groups (e.g., infants, children, and adolescents but not neonates). Further information is not required. N/A

__ 3. **PEDIATRIC STUDIES ARE NEEDED.** There is potential for use in children, and further information is required to permit adequate labeling for this use. N/A

- __ a. A new dosing formulation is needed, and applicant has agreed to provide the appropriate formulation.
- __ b. A new dosing formulation is needed, however the sponsor is either not willing to provide it or is in negotiations with FDA.
- __ c. The applicant has committed to doing such studies as will be required.
  - __ (1) Studies are ongoing.
  - __ (2) Protocols were submitted and approved.
  - __ (3) Protocols were submitted and are under review.
  - __ (4) If no protocol has been submitted, attach memo describing status of discussions.
- __ d. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

__ 4. **PEDIATRIC STUDIES ARE NOT NEEDED.** The drug/biologic product has little potential for use in pediatric patients. Attach memo explaining why pediatric studies are not needed. N/A

__ 5. **If none of the above apply, attach an explanation, as necessary.**

ARE THERE ANY PEDIATRIC PHASE IV COMMITMENTS IN THE ACTION LETTER? ___ Yes X No

ATTACH AN EXPLANATION FOR ANY OF THE FOREGOING ITEMS, AS NECESSARY.

This page was completed based on information from /S/ (e.g., [✓ medical review], [✓ medical officer], [✓ team leader])

/S/ Team Leader 11/23/99

Signature of Preparer and Title Date

orig NDA/~~BLA~~ # NDA 21-075

HFD 510 /Div File

NDA/BLA Action Package

HFD-006/ KRoberts

(revised 10/20/97)

FOR QUESTIONS ON COMPLETING THIS FORM CONTACT, KHYATI ROBERTS, HFD-6 (ROBERTSK)

NEW DRUG APPLICATION: **ITEM 16**

NUTROPIN DEPOT™ [somatropin (rDNA origin) for injectable suspension]

16. DEBARMENT CERTIFICATION (Section 306[k[1] of the Act 21 U.S.C. 335a[k][1])

This is to certify that Genentech, Inc. has not and will not use, in any capacity, the services of any person debarred under subsections (a) or (b) (Section 306[a] or [b]), in connection with this New Drug Application (NDA).

Signed by:
Robert L. Garnick, Ph.D.

Title: Vice President, Regulatory Affairs

Date: 6/1/99

APPEARS THIS WAY
ON ORIGINAL

Exclusivity Checklist

NDA: 21-075
Trade Name: Nutropin Depot
Generic Name: somatropin (rDNA origin) for injectable suspension
Applicant Name: Genentech, Inc.
Division: HFD-510 Metabolic
Project Manager: Crystal King
Approval Date: 12/22/99

PART I: IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete Parts II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following questions about the submission.

a. Is it an original NDA?	Yes	✓	No	
b. Is it an effectiveness supplement?	Yes		No	✓
c. If yes, what type? (SE1, SE2, etc.)	N/A			
Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")	Yes	✓	No	

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

Explanation:
N/A

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

Explanation:
N/A

d. Did the applicant request exclusivity?	Yes	✓	No	
If the answer to (d) is "yes," how many years of exclusivity did the applicant request?	7 years			

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule previously been approved by FDA for the same use?	Yes		No	✓
If yes, NDA #				
Drug Name:				

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE

BLOCKS.

3. Is this drug product or indication a DESI upgrade?	Yes		No	✓

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS (even if a study was required for the upgrade).

PART II: FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2, as appropriate) N/A

1. Single active ingredient product.	Yes		No	
Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.	Yes		No	

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

Drug Product	
NDA #	
Drug Product	
NDA #	
Drug Product	
NDA #	

2. Combination product.	Yes		No	
If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing <u>any one</u> of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)	Yes		No	

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

Drug Product	
NDA #	
Drug Product	
NDA #	
Drug Product	
NDA #	

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY

TO THE SIGNATURE BLOCKS. IF "YES," GO TO PART III.

PART III: THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2, was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.	Yes	✓	No	

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application. For the purposes of this section, studies comparing two products with the same ingredient(s) are considered to be bioavailability studies.

a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?	Yes	✓	No	

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval **AND GO DIRECTLY TO SIGNATURE BLOCKS.**

Basis for conclusion: N/A

b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?	Yes		No	✓
1) If the answer to 2 b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? <u>If not applicable</u>, answer NO.	Yes		No	✓

If yes, explain:

2) If the answer to 2 b) is "no," are you aware of published

studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?	Yes		No	✓

If yes, explain:

c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

Investigation #1, Study #: 03-004	
Investigation #2, Study #: 03-003	
Investigation #3, Study #:	

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1 03-004	Yes		No	✓
Investigation #2 03-003	Yes		No	✓
Investigation #3	Yes		No	

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

Investigation #1 -- NDA Number	
Investigation #2 -- NDA Number	
Investigation #3 -- NDA Number	

b) For each investigation identified as "essential to the approval," does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 03-004	Yes		No	✓
Investigation #2 03-003	Yes		No	✓
Investigation #3	Yes		No	

If you have answered "yes" for one or more investigations, identify the NDA in which a similar investigation was relied on:

Investigation #1 -- NDA Number	
Investigation #2 -- NDA Number	
Investigation #3 -- NDA Number	

If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

Investigation #1 03-004	
Investigation #2 03-003	

Investigation #3				

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a. For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1 [redacted] 03-004	Yes		No	✓
IND#: [redacted]				
Explain:				
Investigation #2 [redacted] 03-003	Yes		No	✓
IND#: [redacted]				
Explain:				
Investigation #3	Yes		No	
IND#:				
Explain:				

b. For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1 [redacted] 03-004	Yes	✓	No	
IND#: [redacted]				
Explain:				
Investigation #2 [redacted] 03-003	Yes	✓	No	
IND#: [redacted]				
Explain:				
Investigation #3	Yes		No	
IND#:				
Explain:				

c. Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may				

not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)	Yes		No	✓
If yes, explain: [redacted] is a contract manufacturer for Genentech.				

BACK TO TOP

/S/

Signature of PM/CSO

Date: 12/3/99

/S/

Signature of Division Director

Date: 12/21/99

ATTACHMENT

cc:

Original NDA

Division File

HFD-93 Mary Ann Holovac

BACK TO TOP

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration

CERTIFICATION: FINANCIAL INTERESTS AND ARRANGEMENTS OF CLINICAL INVESTIGATORS

Form Approved: OMB No. 0910-0396
Expiration Date: 3/31/02

TO BE COMPLETED BY APPLICANT

With respect to all covered clinical studies (or specific clinical studies listed below (if appropriate)) submitted in support of this application, I certify to one of the statements below as appropriate. I understand that this certification is made in compliance with 21 CFR part 54 and that for the purposes of this statement, a clinical investigator includes the spouse and each dependent child of the investigator as defined in 21 CFR 54.2(d).

Please mark the applicable checkbox.

☐ (1) As the sponsor of the submitted studies, I certify that I have not entered into any financial arrangement with the listed clinical investigators (enter names of clinical investigators below or attach list of names to this form) whereby the value of compensation to the investigator could be affected by the outcome of the study as defined in 21 CFR 54.2(a). I also certify that each listed clinical investigator required to disclose to the sponsor whether the investigator had a proprietary interest in this product or a significant equity in the sponsor as defined in 21 CFR 54.2(b) did not disclose any such interests. I further certify that no listed investigator was the recipient of significant payments of other sorts as defined in 21 CFR 54.2(f).

Clinical Investigators		

☒ (2) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that based on information obtained from the sponsor or from participating clinical investigators, the listed clinical investigators (attach list of names to this form) did not participate in any financial arrangement with the sponsor of a covered study whereby the value of compensation to the investigator for conducting the study could be affected by the outcome of the study (as defined in 21 CFR 54.2(a)); had no proprietary interest in this product or significant equity interest in the sponsor of the covered study (as defined in 21 CFR 54.2(b)); and was not the recipient of significant payments of other sorts (as defined in 21 CFR 54.2(f)).

☐ (3) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that I have acted with due diligence to obtain from the listed clinical investigators (attach list of names) or from the sponsor the information required under 54.4 and it was not possible to do so. The reason why this information could not be obtained is attached.

NAME: Robert L. Garnick, Ph.D.
TITLE: Vice President, Regulatory Affairs
FIRM/ORGANIZATION: Genentech, Inc.
SIGNATURE:
DATE: 6/9/99

Paperwork Reduction Act Statement

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Public reporting burden for this collection of information is estimated to average 1 hour per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the necessary data, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information to the address to the right:

Department of Health and Human Services
Food and Drug Administration
5600 Fishers Lane, Room 14C-03
Rockville, MD 20857

FORM FDA 3454 (3/99)

Created by Electronic Document Services/USDHHS: (301) 443-2454 EF

[redacted]

[redacted]

May 28, 1999

Robert Garnick, Ph.D.
Vice President, Regulatory Affairs
Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080

RE: Financial Disclosure by Clinical Investigators for [redacted]03-004

Dear Dr. Garnick:

We understand that Genentech, Inc. intends to submit [redacted] Study No [redacted]03-004, "A Phase III Multicenter, Open-Label Study of the Safety and Efficacy of [redacted] hGH Administered in Children with Growth Failure Due to Growth Hormone Deficiency", in support of a New Drug Application for Nutropin Depot™ [redacted] hGH). Study [redacted]03-004 is considered a "covered clinical study" as defined under 21 CFR 54 since it will be relied upon to establish efficacy of Nutropin Depot. As such, [redacted] is required under 21 CFR 54 to supply a financial certification/disclosure statement for investigators directly involved in the treatment or evaluation of research subjects for Study [redacted]03-004.

As the sponsor of [redacted]03-004, [redacted] certifies that we have not entered into any financial arrangement with the listed clinical investigators or subinvestigators whereby the value of compensation to the investigator or subinvestigator could be affected by the outcome of the study as defined in 21 CFR 54.2(a). [redacted] also certifies that each listed clinical investigator or subinvestigator has been required to disclose to the sponsor whether the investigator or subinvestigator had a proprietary interest in Nutropin Depot or a significant equity in [redacted] as defined in 21 CFR 54.2(b) and no such interests were disclosed. [redacted] further certifies that no listed investigator or subinvestigator was the recipient of significant payments of other sorts as defined in 21 CFR 54.2(f). The certifications set forth in this letter cover each clinical investigator and subinvestigator, and their spouses and dependent children, as required by 21 CFR 54.

Attached please find a cumulative list of all investigators and subinvestigators who participated in [redacted]03-004.

Sincerely,

[redacted]

Redacted 3

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