(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 August 2019 (15.08.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/155396 A1

(51) International Patent Classification:
A61K 9/16 (2006.01) *A61K 38/08* (2019.01)

(21) International Application Number:
PCT/IB2019/050987

(22) International Filing Date:
07 February 2019 (07.02.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
201841004614 07 February 2018 (07.02.2018) IN

(71) Applicant: **DR. REDDY'S LABORATORIES LIMITED** [IN/IN]; 8-2-337, Road No. 3, Banjara Hills, Telangana, Hyderabad 500034 (IN).

(72) Inventors: **CHOPRA, Dhiraj Kumar**; House No. 701, Madison Building, Hiranandani Estate, Patlipada; Off. G.B., Road; Thane (West), Maharashtra 400607 (IN). **MAJUMDER, Arijit**; House no: 403, Shiva Enclave; Behind Gandhi statue road, Madinaguda, Telangana, Hyderabad 500050 (IN). **BHAGWATWAR, Harshal**; B-301 Fortune Towers; Madhapur, Telangana, Hyderabad 500081 (IN). **VAN VUGHT, Jeroen**; House no: 37, Koningin Emmakade, 2518 RK Den Haag (NL). **VERRIJK, Rudolf**; House No: 154, Parallel Boulevard, 2202 HS Noordwijk (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) **Title:** SUSTAINED RELEASE MICROSPHERES WITH LOW INITIAL BURST AND METHODS OF PREPARATION THEREOF

(57) **Abstract:** The present invention provides a sustained release microspheres with low initial burst of the active agent and specific process of preparation of such compositions. In particular the present invention provides sustained release microspheres of the Octreotide or pharmaceutically acceptable salts thereof with low initial burst comprises poly (D, L-lactide-co-glycolide) polymer and process of preparation thereof.

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SUSTAINED RELEASE MICROSPHERES WITH LOW INITIAL BURST AND METHODS OF PREPARATION THEREOF

FIELD OF THE INVENTION

The present invention relates to biodegradable polymer based sustained release microsphere compositions of the active agent. In particular, the present invention provides sustained release microspheres with low initial burst of the active agent and methods of preparation of such compositions.

BACKGROUND OF THE INVENTION

Compositions containing biologically active agents in combination with biodegradable and biocompatible polymers are being increasingly used as drug delivery systems to provide sustained or delayed release of drugs. The compositions are available in various injectable depot forms including liquid forms, solid implants, microspheres, microcapsules and microparticles.

Octreotide is a long acting cyclic octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. Octreotide is known chemically as L-cysteinamide, D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[2-hydroxy-1-(hydroxymethyl)propyl]-, cyclic(2→7)-disulfide; [R—(R*,R*)].

The commercially available Sandostatin LAR formulation meant for sustained release of Octreotide acetate, which is approved for the treatment of long-term maintenance therapy in acromegalic patients who have had an inadequate response to surgery and/or radiotherapy, or for whom surgery and/or radiotherapy is not an option. The goal of treatment in acromegaly is to reduce GH and IGF-1 levels to normal (Acromegaly), long-term treatment of the severe diarrhea and flushing episodes associated with metastatic carcinoid tumors and profuse watery diarrhea associated with VIP-secreting tumors (Vasoactive Intestinal Peptide Tumors - VIPomas).

Different biodegradable polymers, especially polyol esters, their preparation and use in depot formulations of the pharmaceutical active agents were described in US5922682 patent. Sustained release compositions of water soluble peptides and process of preparation of the microspheres by various techniques were described in US5538739 patent.

US20120021018A1 describes the process of preparation of sustained release Octreotide formulations with low initial burst release by means of addition of acetic acid at a certain concentration in the dispersed phase and certain concentration of the poly (D, L-lactide-co-glycolide) polymer in the dispersed phase. The process described here in this application is

preparing the dispersed phase by combining polymer, dichloromethane (DCM), Octreotide, methanol and acetic acid; dissolving Polyvinyl alcohol (PVA) in water to form continuous phase; mixing the both phases to form microspheres suspension. Removing DCM, acetic acid, methanol and PVA from microspheres suspension; removing the residual solvents by means of washing.

US8343513 patent describes sustained release formulation comprising the biodegradable polymer, where in the prevention of the molecular weight reduction of the polymer in a polymer solution containing nucleophilic compounds is by means of a polymer having an acid number of at least 13 and but not greater than 30; further with an acid additive. Further it describes the reduction or elimination of peptide polymer impurities in the polymer solutions. Further it relates to GnRH analogue and polymer containing solutions capable of forming stable, filter sterilizable and non-gelling solutions

WO20160161296A1 patent application describes an improved stable injectable composition for controlled release drug delivery with less impurities and process of preparation of the same by using a) lactate-based polymer having a weight average molecular weight between 5,000 and 50,000 Dalton, an acid number of less than 3 mgKOH/g and the content of residual lactide monomers in the lactate-based polymer of less than about 0.3% by weight; b) a pharmaceutically acceptable organic solvent; and c) a active substance or a salt thereof that contains an amino acid serine in the molecular structure that is capable of reacting with lactide monomer to form a conjugate, with the proviso that no acid additive is added in producing the composition, and the composition reduces the formation of the conjugate.

Despite the technological advances that were made in the area of injectable depot formulations to date, several quality concerns prevent their ready use in biological applications. These include initial burst, de novo formation of conjugate substances (impurities), insolubility of the biologically active agents in solvents typically used in the polymer compositions and their propensity to form gels. Among them, initial burst is one of the major challenges in drug-encapsulated or peptide-encapsulated microparticle or microspheres systems. Initial burst release of active agent is not always detrimental, excessive drug release in the burst phase may be toxic, and irregularity in the amount of drug released.

There remains a need to develop sustained release microspheres compositions with low initial burst, while meeting the required release profiles and required duration and process of preparation of such microsphere compositions. Therefore, it is still desirable to have an improved process for preparation of sustained release microspheres with low initial burst of the active agent;

particularly the process is simple, feasible, scalable to commercial scale, commercially economically viable.

SUMMARY OF THE INVENTION

The present invention relates to sustained release microspheres with low initial burst of the active agent. The present invention also provides process of preparation of such compositions

Aspects of the present invention provide microspheres for sustained release of an active agent with a low initial burst comprises biodegradable polymer having an acid number of less than or equal to 10 mgKOH/g.

In one aspect of the present invention provides an injectable microsphere composition for sustained release of active agent with a low initial burst comprises: a biodegradable polymer having an acid number of less than or equal to 10 mgKOH/g, an active agent and one or more pharmaceutically acceptable carriers.

In embodiments, the biodegradable polymer is lactate based polymer. In a preferred embodiment the lactate based polymer is poly (D, L-lactide-co-glycolide) polymer (PLGA). In most preferred embodiment PLGA polymer is PLGA glucose star polymer.

In another embodiment, preferred active agents are peptides and their pharmaceutically acceptable salts thereof and most preferred active agent is Octreotide or its pharmaceutically acceptable salts thereof. Octreotide acetate is one of the preferred active agents of the present invention.

In another embodiment, injectable microsphere composition for sustained release of Octreotide acetate with a low initial burst comprises: (poly (D, L-lactide-co-glycolide) glucose star polymer (PLGA glucose star polymer) having an acid number of less than or equal to 10 mgKOH/g.

In another embodiment, injectable microsphere compositions for sustained release of Octreotide acetate with a low initial burst comprises: (poly (D, L-lactide-co-glycolide) glucose star polymer (PLGA glucose star polymer) having an acid number of less than or equal to 10 mgKOH/g, Octreotide acetate and pharmaceutically acceptable carriers.

Another aspect of the present invention provides a method for preparing sustained release microspheres with low initial burst of the active agent comprises lactate based polymer having an acid number of less than or equal to 10 mgKOH/g. In an embodiment the microspheres of the present invention are prepared by co-acervation phase separation technique.

In an embodiment, a process for preparing microspheres for sustained release of an active agent with a low initial burst comprises:

a) preparing a solution of active agent in a suitable solvent,
b) preparing a polymer solution by dissolving a biodegradable, biocompatible polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of co-acervates by means of addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtering the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In a preferred embodiment, a process for preparing microspheres for sustained release of Octreotide acetate with a low initial burst comprises:

a) preparing a solution of Octreotide acetate in a suitable solvent,
b) preparing a polymer solution by dissolving a PLGA glucose star polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of co-acervates by means of addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtered the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another aspect of the present invention provides, a method for preparing sustained release microspheres with low initial burst of the active agent comprises:

a) preparing a solution of active agent in a polar solvent,
b) preparing a polymer solution by dissolving a biodegradable, biocompatible polymer in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of co-acervates by means of addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtered the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In embodiments, the preferred polar solvents used to dissolve the active agents are alcoholic solvents.

In an embodiment of the present invention provides, a method for preparing sustained release microspheres with low initial burst of the Octreotide acetate comprises:

a) preparing a solution of Octreotide acetate in alcoholic solvent,
b) preparing a polymer solution by dissolving a PLGA glucose star polymer in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of co-acervates by means of addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtering the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another aspect of the present invention provides, a method for preparing sustained release microspheres with low initial burst of the active agent comprises:

a) preparing polymer solution by dissolving a poly(D,L-lactide-co-glycolide) polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent
b) active agent is dissolved in polar solvent to form drug solution;
c) combining polymer solution and drug solution to form a combined phase of drug and polymer;
d) adding suitable phase separating agent to form co-acervates;
e) mixing with hardening agent to the co-acervates to form a microsphere suspension
f) filtering microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another embodiment of the present invention, a method for preparing sustained release microspheres with low initial burst of the Octreotide acetate comprises:

a) preparing polymer solution by dissolving the poly(D,L-lactide-co-glycolide) glucose polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent
b) Octreotide acetate is dissolved in alcoholic solvent to form drug solution;
c) combining polymer solution and drug solution to form a combined phase of drug and polymer;
d) adding suitable phase separating agent to form co-acervates;
e) mixing with hardening agent to the co-acervates to form a microsphere suspension
f) filtering microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another embodiment of the present invention, a method for preparing sustained release microspheres with low initial burst of the Octreotide acetate comprises:

a) preparing polymer solution by dissolving the poly(D,L-lactide-co-glycolide) glucose polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent
b) Octreotide acetate is dissolved in alocholic solvent to form Octreotide solution;
c) combining polymer solution and Octreotide solution to form a combined phase of drug and polymer, where in the concentration of the alcoholic solvent in the drug polymer combined phase is less than about 10% by weight ;
d) adding suitable phase separating agent to form co-acervates;
e) mixing with hardening agent to the co-acervates to form a microsphere suspension
f) filtering microspheres followed by washing and drying of microspheres to get the finished microspheres.

In one embodiment, the concentration (in % weight) of the alcoholic solvent in the drug polymer combined phase is less than about 10%. In a preferred embodiment, the concentration of the alcoholic solvent is less than about 9% or less than about 8% or less than about 7% or less than about 6% or less than about 5% or less than about 4 % or less than about 3% or less than about 2% or less than about 1%.

According to another embodiment of present invention, the acid number of the polymer is less than or equal to 10 mgKOH/g. According to another embodiment of present invention, the acid number of the polymer is less than or equal to 5 mgKOH/g. According to another embodiment of present invention, the acid number of the polymer is less than or equal to 4 mgKOH/g. In other embodiment, the acid number of the polymer is less than or equal to 3 mgKOH/g. In other embodiment the acid number of the polymer is less than or equal to 2 mgKOH/g.

According to any embodiment of the present invention, the PLGA glucose star polymer having a molar ratio of lactide to glycolide ranging from 90:10 to 10:90, preferably from 80:20 to 20:80, more preferably 75:25 to 25:75 and most preferably 40:60 to 60:40. In another preferred embodiment, the lactide to glycolide molar ratio of the polymer is 50:50.

In an embodiment, the lactate based polymer having a weight average molecular weight range is between 5,000 - 500,000 Daltons. In a preferred embodiment, the weight average molecular weight of the polymer ranges between 30,000 - 60,000 Daltons

In an embodiment, the lactate based polymer having a residual monomer content (in % weight) of less than or equal to about 2.0 %. In a preferred embodiment, the residual monomer

content is less than or equal to about 1.0%. In a most preferred embodiment, the residual monomer content of the polymer is less than or equal to 0.1%

In another embodiment, the lacate based polymer having an inherent viscosity (IV) in the range of 0.3 - 0.7 dL/g in chloroform. In a preferred embodiment, the IV of the polymer is 0.40 - 0.60 dL/g in chloroform. In another embodiment, the IV of the polymer is 0.42 - 0.52 dL/g in chloroform.

According to any embodiment of the present invention, the drug loading in the microspheres is less than or equal to 10% by weight. In a preferred embodiment, the drug loading in the microspheres is ranging from 3% to 5% by weight.

According to any embodiment of the present invention, the drug: polymer ratio in the finished microspheres is ranges about 1:10 to 1:30. In a preferred embodiment, the drug: polymer ration is about 1:15 to 1:20.

According to any embodiment of the present invention, the weight % of the polymer in the finished microspheres is about 50% to 90%. In a preferred embodiment, the % of the polymer is about 60% to 80%

According to any embodiment of the present invention, the particle size of the microspheres ranges from 1 to 250 microns, preferably 10 to 200 microns, most preferably 10 to 130 microns in diameter. In another embodiment, the D_{50} of the microspheres ranges from 10-200 microns, preferably 20-150 microns.

According to any embodiment of the present invention, the concentration (in % weight) of the said polymer in said drug polymer combined phase ranges from about 2% to about 15%. In a preferred embodiment, the said concentration is about 3% to about 10%. According to any embodiment of the present invention, the concentration of said Active agent in the drug polymer combined phase ranges from about 0.1% to about 5%. In a preferred embodiment, the said concentration of the drug ranges from about 0.1% to 1%.

According to any embodiment of the present invention, the drug: polymer ratio in the drug polymer combined phase is ranges about 1:5 to about 1:50. In a preferred embodiment, the ratio is about 1:10 to 1:20.

According to one embodiment of the present invention, the acid number of the PLGA glucose star polymer is less than or equal to 10 mgKOH/g.

According to any embodiment of the present invention, the pharmaceutically acceptable carrier comprises an anti-agglomerating agent, viscosity enhancing agent, surfactant and diluent

According to any embodiment of the present invention, the pharmaceutically acceptable carrier comprises mannitol, sodium CMC, Poloxamer 188 and water for injection.

Sustained release microspheres of the present invention are administered by IM or SC administration. Preferably microspheres of the present invention are administered by IM administration.

Microspheres of the present invention are filled in to the vials or ampoules, and these microspheres are to be added to vehicle before administration. These microspheres when injected, delivers active agent for a period of at least one month.

Sustained release Octreotide microspheres is used for the treatment of acromegaly, long-term treatment of the severe diarrhea and flushing episodes associated with metastatic carcinoid tumors and profuse watery diarrhea associated with VIP-secreting tumors (Vasoactive Intestinal Peptide Tumors - VIPomas).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a graphical representation of the effect of acid number on the initial burst release, represented as percentage of Octreotide released from the sustained release microspheres in a dissolution medium with respect to time

Figure 2 provides the graphical representation of the one batch full IVR data represented as percentage of Octreotide release from the microspheres with respect to time

DETAILED DESCRIPTION OF THE INVENTION

Aspects of the present invention relate to sustained release microspheres with low initial burst of the active agent. The present invention also provides methods of preparation of such compositions

The polymeric compositions of the present invention comprise an active agent, a biodegradable polymer and one or more pharmaceutically acceptable carriers. The polymeric compositions may be in the forms of microparticles, microspheres, microcapsules, microgranules or solid implants by removing the organic solvent prepared in vitro. These compositions can be administered by methods known in the art, examples include but not limited to injection or surgical intervention or oral or transdermal or topical etc. The words, microsphere, microparticle and microcapsule can be used interchangeably regarding the invention and encapsulation of the active agent by the polymer. Active agent is dispersed in a matrix of the PLGA polymer. In particular, the term microsphere is used throughout this disclosure. Injectable compositions of the present invention can be administered by different routes and is very well known in the art, for example subcutaneous, Intravenous, intramuscular, intra-arterial, intrathecal etc. Alternatively, it may be in the forms of solutions, emulsions, suspensions, paste, cream, or gel which moves as a fluid so that it may be injected through a needle, cannula, tube, laproscope, probe, or other delivery device. When administered to a subject, such injectable composition forms a depot in-situ from which the controlled release of the active agent can be sustained for a desired period of time depending upon the composition. The depot or implant may be a solid, a gel, a paste, a semisolid, or a viscous liquid. With the selections of the biodegradable polymer and other components, the duration of the sustained release of the active substance can be controlled over a period of time from several weeks to one year. In a preferred embodiment of the present invention, the sustained release microspheres can control the active agent over a period of time from several hours to days and several days to weeks and several weeks to months, i.e. till 6 months and meant for at least once weekly or bi-weekly administration. In a preferred embodiment, the sustained release microspheres are meant for once monthly administration. In another embodiment, the sustained release microspheres are meant for once in two months or once in 4 months or once in 6 months administration.

A typical release mechanism for these types of microspheres compositions includes three phases that can be generally represented as the initial lag phase (including initial burst release), the erosion phase and plateau phase. The initial burst release typically occurs as soon as the formulation comes in contact with the dissolution medium, till 24 - 72 hours. Thereafter, there is a

lag phase where there is further negligible release of drug from the polymer matrix. This phase is primarily governed by diffusion. Erosion phase commences once the polymer molecular weight falls beyond a threshold value due to hydrolysis by dissolution media. The bulk of the drug in the polymer matrix is released during this period. Erosion phase and its onset is very important for any sustained release microspheres as this is governing the time required to get the maximum concentration of the drug, i.e. t_{max} and total time period of drug release. Erosion is also influences the maxim concentration of the drug in the body, i.e. C_{max}. 80% - 90% weight of the drug is released during this phase. Plateau phase is the final phase wherein the remaining of the drugs gets released from the polymer matrix. The duration of each phase can be modified as per the need of the required release profile, treatment duration and type of the active agent etc.

In the field of sustained release microspheres, it is very well known that various factors or parameters affecting the release rate from the sustained release microspheres. These are factors are type of polymer, concentration of the polymer and its molecular weight, copolymer composition, the nature of the excipients added to the microspheres composition, method of manufacturing, type of the drug etc

In one aspect of the present invention provides an injectable microsphere composition for sustained release of active agent with a low initial burst comprises: a biodegradable polymer having an acid number of less than or equal to 10 mgKOH/g, an active agent and one or more pharmaceutically acceptable carriers.

In embodiments, the biodegradable polymer is lactate based polymer. In a preferred embodiment the lactate based polymer is poly (D, L-lactide-co-glycolide) polymer (PLGA). In most preferred embodiment PLGA polymer is PLGA glucose star polymer.

“Acid number” of the polymer is the "mg" amount of potassium hydroxide required to neutralize the acid present in one gram of the polymer. Polymers with acid ended groups will have some acid number. Lower molecular weight polymers will have more acid ended groups, and will have higher acid numbers. Extractable oligomer acids in polymers may also contribute to the acid number.

The acid number of the lactate-based polymers of the present invention is from 0 to 30 mgKOH/g. The lactate-based polymers of the present invention are having an acid number of less than or equal to 20 mgKOH/g, preferably less than or equal to 10 mgKOH/g, more preferably less than or equal to 5 mgKOH/g.

According to one embodiment of present invention, the acid number of the polymer is less than or equal to 5 mgKOH/g. According to another embodiment of present invention, the acid number of the polymer is less than or equal to 4 mgKOH/g. In other embodiment, the acid number of the polymer is less than or equal to 3 mgKOH/g. In other embodiment the acid number of the polymer is less than or equal to 2 mgKOH/g.

In an embodiment, the polymer acid number is in between 1 mgKOH/g and 10 mgKOH/g. In another embodiment, the polymer acid number is in between 1 mgKOH/g and 5 mgKOH/g. In another embodiment, the polymer acid number is in between 5 mgKOH/g and 10 mgKOH/g. In preferred embodiment, the polymer acid number is in between 2 mgKOH/g and 5 mgKOH/g. In other preferred embodiment, the polymer acid number is in between 2.5 and 5 mgKOH/g. In other preferred embodiment, the polymer acid number is in between 2.5 and 4.5 mgKOH/g. In other preferred embodiment, the polymer acid number is in between 3 mgKOH/g and 5 mgKOH/g. In other preferred embodiment, the polymer acid number is in between 3 and 4.5 mgKOH/g. In other preferred embodiment, the polymer acid number is in between 4 and 5 mgKOH/g. In other preferred embodiment, the polymer acid number is in between 2 and 3 mgKOH/g. In other preferred embodiment, the polymer acid number is in between 2 and 4 mgKOH/g.

The term “low initial burst” is defined to mean the microspheres release less than or equal to 1% by weight of a total amount of the active agent within 1 hour at 37° C and pH 4.0 and preferably less than or equal to 0.5% by weight of a total amount of active agent within 1 hr and most preferably less than or equal to 0.2% by weight of a total amount of active agent within 1 hr. The low initial burst can also be characterized by release of less than or equal to 10% by weight of a total amount of the active agent within 1 day at 37° C and pH of 4.0 and preferably less than or equal to 5% by weight of a total amount of the active agent within 1 day at 37° C and pH of 4.0 and most preferably less than or equal to 2% by weight of a total amount of the active agent within 1 day at 37° C and pH of 4.0.

In vitro release of the microspheres according to the present invention is performed in shaking water batch having a temperature of 37° C in 50mM acetate buffer in a pH of 4.

The term “about” as described in the specification and appended claims indicate that values slightly outside the cited values, i.e., plus or minus 0.1 to 10%.

The term "active agent" is meant to include any materials having diagnostic and/or therapeutic properties including, but not limited to, organic small molecules, inorganic small molecules, macromolecules, peptides, oligopeptides, proteins, or enzymes, nucleotides,

nucleosides, oligonucleotides, oligonucleosides, polynucleotides, polynucleotides, polynucleic acids or similar molecules constitute such chemical compounds. Peptides are one of preferred active agents. Non-limiting examples of therapeutic properties are antimetabolic, antifungal, anti-inflammatory, antitumoral, antiinfectious, antibiotics, nutrient, agonist, and antagonist properties. These active agents of the present invention may be in the form of a free molecule, an organic or inorganic salt of the free molecule, or it may be complexed or covalently conjugated with a carrier agent, may be a pro-drug, or may be a multiform active agent (multiple units of the active agent either complexed or covalently bonded together).

The term "peptide" as used herein is in a generic sense to include poly(amino acids) that are normally generally referred to as "peptides", "oligopeptides", and "polypeptides" or "proteins" which are used interchangeably herein. The term also includes active peptide analogs, derivatives, acylated derivatives, glycosylated derivatives, pegylated derivatives, fusion proteins and the like. The term "peptide" is meant to include any active peptides having diagnostic and/or therapeutic properties including, but not limited to, antimetabolic, antifungal, anti-inflammatory, antitumoral, antiinfectious, antibiotics, nutrient, agonist, and antagonist properties. The term also includes synthetic analogues of peptides, unnatural amino acids having basic functionality, or any other form of introduced basicity. The peptide of the present invention contains at least one nucleophilic group. The phrase "at least one" means that the peptide may also contain a multiple number of nucleophilic groups.

Specifically, the active peptides of the invention may include, but are not limited to, oxytocin, vasopressin, adrenocorticotropic hormone (ACTH), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), prolactin, luteinising hormone, luteinizing hormone releasing hormone (LHRH), LHRH agonists such as euprorelin, buserelin, gonadorelin, deslorelin, fertirelin, histrelin, lutrelin, goserelin, nafarelin, triptorelin, cetrorelix, enfuvirtide, thymosin a abarelix, LHRH antagonists, growth hormones (including human, porcine, and bovine), growth hormone releasing factor, insulin, erythropoietin (including all proteins with erythropoietic activity), somatostatin, glucagon, interleukin (which includes IL-2, IL-1 1 , IL-12, etc.), interferon-alpha, interferon-beta, interferon-gamma, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, calcitonin, enkephalins, endorphins, angiotensins, thyrotropin releasing hormone (TRH), tumor necrosis factor (TNF), parathyroid hormone (PTH), nerve growth factor (NGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF), heparinase, vascular endothelial growth factor

(VEG-F), bone morphogenic protein (BMP), hANP, glucagon-like peptide (GLP-1), exenatide, peptide YY (PYY), renin, bradykinin, bacitracins, polymyxins, colistins, tyrocidine, gramicidins, cyclosporins, enzymes, cytokines, antibodies, vaccines, antibiotics, antibodies, glycoproteins, follicle stimulating hormone, kyotorphin, taftsin, thymopoietin, thymosin, thymostimulin, thymic humoral factor, serum thymic factor, colony stimulating factors, motilin, bombesin, dinorphin, neurotensin, cerulein, urokinase, kallikrein, substance P analogues and antagonists, angiotensin II, blood coagulation factor VII and IX, gramicidines, melanocyte stimulating hormone, thyroid hormone releasing hormone, thyroid stimulating hormone, pancreozymin, cholecystokinin, human placental lactogen, human chorionic gonadotrophin, protein synthesis stimulating peptide, gastric inhibitory peptide, vasoactive intestinal peptide, platelet derived growth factor, and synthetic analogues and modifications and pharmacologically-active fragments thereof. In another embodiment, the active agent is Risperidone or Exenatide.

The preferred peptide used herein also includes peptides such as somatostatin, Octreotide, pasireotide, SOM230, and lanreotide. The term "somatostatin" includes its analogues or derivatives thereof. The term Octreotide includes its analogues or derivatives thereof. The terms derivatives and analogues mean branched, straight chain or cyclic polypeptides in which at least one of the amino acids has been omitted or substituted by at least one other amino acid radical(s); and also include at least one functional group being substituted for at least one other functional group(s); and at least one group being substituted by at least one other isosteric group(s). In a broad sense, the terms mean all modified derivatives of Octreotide that are biologically active and have a similar effect as unmodified Octreotide. The invention further provides the use of a pharmaceutical composition according to the present invention for inter alia long-term maintenance therapy in acromegalic patients, and treatment of severe diarrhea and flushing associated with malignant carcinoid tumors and vasoactive intestinal peptide tumors (VIPoma tumors).

The active agent used in the present invention may be in the form of free base or a pharmaceutically acceptable salt. The acid used to form the pharmaceutically acceptable salt of the active agent preferably has a pKa less than 5. The acids suitable for the present invention may be selected from, but not limited to, the group consisting of hydrochloric acid, hydrobromic acid, nitric acid, chromic acid, sulfuric acid, methanesulfonic acid, trifluromethane sulfonic acid, trichloroacetic acid, dichloroacetic acid, bromoacetic acid, chloroacetic acid, cyanoacetic acid, 2-chloropropanoic acid, 2-oxobutanoic acid, 2-chlorobutanoic acid, 4-cyanobutanoic acid, pamoic acid, perchloric acid, phosphoric acid, hydrogen iodide, acetic acid, 2,2- dichloroacetic acid, adipic

acid, alginic acid, L-ascorbic acid, L-aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamido benzoic acid, (+)-camphoric acid, (+)-camphor-10-sulfonic acid, capric acid, (decanoic acid), caproic acid (hexanoic acid), caprilic acid (octanoic acid)carbonic acid, cinnamic acid, citric acid, cyclamic acid, decanoic acid, dodecylsulfuric acid, ethane-1 ,2-disufonic acid, ethanesulfonic acid, 2-hydroxy-ethanesulfonic acid, formic acid, fumaric acid, galactic acid, gentisic acid, D-glucoheptonic acid, D-gluconic acid, D-glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, DL-lactic acid, lactobionic acid, lauric acid, maleic acid, (-)-L-malic acid, malonic acid, DL-mandelic acid, muric acid, naphthalene-1 ,5-disulfonic acid, naphthalene-2-sulfonic acid, 1 -hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, embonic acid, proprionic acid, (-)-L- pyroglutamic acid, salicyclic acid, 4-amino-salicylic acid, sebacic acid, stearic acid, succinic acid, (+)-L-tartaric acid, thiocyanic acid, p-toluenesulfonic acid, undecylenic acid. The selection of the suitable acids is well-known to those of skill in the art.

In a preferred embodiment, the active agent of the present invention is Octreotide free base or suitable salts or complex thereof. Suitable salts include inorganic or organic acids or polymeric acids. This includes Octreotide acetate. Complexes might be formed by addition of Octreotide and inorganic compounds.

According to one embodiment of the present invention, the sustained release microspheres of Octreotide acetate is used for the treatment of acromegaly, long-term treatment of the severe diarrhea and flushing episodes associated with metastatic carcinoid tumors and profuse watery diarrhea associated with VIP-secreting tumors (Vasoactive Intestinal Peptide Tumors - VIPomas).

In another embodiment, the amount of the octreotide present in the finished microspheres is 10mg or 20mg or 30 mg.

Sustained release microspheres of the present invention comprises biodegradable polymer. The "biodegradable polymers" herein are polymers that are hydrolyzable, and/or bioerode in situ primarily through hydrolysis and/or enzymolysis. The term "biodegradable polymer" as used herein is meant to include any biocompatible and/or biodegradable synthetic and natural polymers that can be used in vivo. Generally, the biodegradable polymer of the present invention is polyester. These polyesters may be a linear polymer, or a branched or star polymer, or a mixture of a linear polymer and a branched and/or star polymer. Preferably, the biodegradable polymer of the present invention is lactate-based polymer.

These polymers are available in a variety of molecular weights, and the appropriate molecular weight to provide the desired release rate for the active agent is readily determined by one of skill in the art. Thus, for instance, polylactide-co-glycolides which are preferably used according to the invention having a molecular weight between 2,000 and 500,000 Daltons. Preferably, suitable molecular weights generally ranges from about 10,000 to about 200,000 Daltons, more specifically from about 15,000 to about 150,000 Daltons, and most specifically from about 30,000 to about 60,000 Daltons.

In a PLGA polymer a variety of lactic acid: glycolic acid ratios are applicable herein, and the ratio is largely a matter of choice, depending in part on the rate of degradation desired. For example, a 50:50 PLGA polymer, containing 50% D,L-lactide and 50% glycolide, is a fast resorbing polymer while 75:25 PLGA degrades more slowly, and 85:15 and 90:10, even more slowly, due to the increased lactide component. It is readily apparent that a suitable ratio of lactide: glycolide is easily determined by one of skill in the art based on the nature of the disorder to be treated. Moreover, mixtures of microspheres with varying lactide: glycolide ratios can be employed in the compositions of the disclosure to achieve the desired release kinetics.

In an embodiment, the biodegradable polymer as per the present invention having the molar ratios of lactide: glycolide is ranges from about 90:10 to 10:90. In another embodiment the said ratio is from 80:20 to 20:80. In a preferred embodiment the molar ratios of lactide: glycolide is ranges from about 75:25 to 25:75. In other preferred embodiment the molar ratios of lactide: glycolide is ranges from about, 60:40 to 40:60. The most preferred molar ratio of lactide: glycolide as per the present invention is 55:45 to 45:55. Another preferred molar ratio of lactide: glycolide as per the present invention is 50:50.

The selection of particular monomer ratios of lactic acid to glycolic acid in the polymer can be readily modified by one of ordinary skill in the art as discussed above. The disclosure advantageously use sugar modified PLGA. That is, there are sugar moieties such as glucose bonded to the polymer chain, as there are in the "star polymer" disclosed in U.S. Pat. No. 5,538,739 & GB 2,145,422B and used in Sandostatin LAR. The star polymers preferably have a polydispersity of from 1.7 to 3.0, preferably from 2.0 to 2.5. The intrinsic viscosities of star polymers of M_w 35,000 and M_w 60,000 are 0.36 and 0.51 dL/g in chloroform respectively. A star polymer having a M_w 52,000 has a viscosity of 0.475 dL/g in chloroform.

The lactate-based polymer of the present invention includes homopolymers of lactic acid or lactide monomers (poly(lactic acid) or polylactide, PLA), and copolymers of lactic acid (or lactide)

with other monomers (for example, glycolic acid (or glycolide) (poly(lactide-co-glycolide), PLG or PLGA) and the like). The lactate-based polymer may have the same end groups, i.e., all the end groups are the same, such as ester, or hydroxyl or carboxylic acid. The lactate-based polymer may have mixed end groups of ester, hydroxyl, and/or carboxylic acid. The lactate-based polymer can have a diol core with end hydroxyl groups. Similarly, the lactate-based polymer may have a triol or polyol core, such as glucose, with end hydroxyl groups. The lactate-based polymer may have one end group as an ester and the other end with a hydroxyl group or carboxylic acid group. The lactate-based polymer may also have one end hydroxyl group and the other end with a carboxylic acid or an ester, or vice versa.

In an embodiment, the lactate based polymer having a weight average molecular weight range is between 5,000 - 500,000 Daltons. In a preferred embodiment, the weight average molecular weight of the polymer ranges between 30,000 - 60,000 Daltons. In most preferred embodiment of the present invention, the weight average molecular weight of the polymer is 40,000 - 60,000 Daltons.

In an embodiment, the lactate based polymer having a residual monomer content of less than or equal to 2.0% by weight. In a preferred embodiment, the residual monomer content is less than or equal to 1.0% by weight. In a most preferred embodiment, the residual monomer content of the polymer is less than or equal to 0.1% by weight

In another embodiment, the lacate based polymer having an inherent viscosity (IV) in the range of 0.3 - 0.7 dL/g in chloroform. In a preferred embodiment, the IV of the polymer is 0.40 - 0.60 dL/g in chloroform. In another preferred embodiment, the IV of the polymer is 0.42 - 0.52 dL/g in chloroform.

In another embodiment, injectable microsphere composition for sustained release of Octreotide acetate with a low initial burst comprises: (poly (D, L-lactide-co-glycolide) glucose star polymer (PLGA glucose star polymer) having an acid number of less than or equal to 10 mgKOH/g.

In another embodiment, injectable microsphere compositions for sustained release of Octreotide acetate with a low initial burst comprises: (poly (D, L-lactide-co-glycolide) glucose star polymer (PLGA glucose star polymer) having an acid number of less than or equal to 10 mgKOH/g, Octreotide acetate and pharmaceutically acceptable carriers.

The microspheres compositions of the present invention may contain drug loading in a range of 0.01 to 40% by weight. Preferably the drug loading is about 1 to 10% by weight. Most

preferably the drug loading is ranges from 3% to 5% by weight. In general, the optimal drug loading depends upon the period of release desired and the potency of the active substance

According to any embodiment of the present invention, the drug: polymer ratio in the finished microspheres is ranges about 1:10 to 1:30. In a preferred embodiment, the drug: polymer ration is about 1:15 to 1:20.

According to any embodiment of the present invention, the weight % of the polymer in the finished microspheres is about 50% to 90%. In a preferred embodiment, the % of the polymer is about 60% to 80%

Another aspect of the present invention describes the process of preparation of the sustained release microspheres with low initial burst of the active agent comprises lactate based polymer with an acid number of less than or equal to 10 mgKOH/g. This process of preparation of sustained release microspheres with low initial burst of the active agent of the present invention is simple, improved, feasible, scalable to commercial scale, commercially economically viable.

There are number of techniques for the microencapsulation of peptides in PLGA microspheres. The most widely used techniques both in lab scale and for commercial productions include phase separation/coacervation technique, spray drying and single or double emulsion/solvent evaporation technique (PDA J Pharm Sci and Tech 2008, 62 125-154; Microencapsulation Methods and Industrial Applications Second Edition).

1. In phase-separation or coacervation technique, an aqueous solution of peptide/protein is emulsified or alternatively the peptide/protein is dispersed in solid form into solution containing dichloromethane and PLGA. Silicone oil is added to this dispersion at a defined rate, reducing solubility of polymer in its solvent. The polymer-rich liquid phase (coacervate) encapsulates the dispersed peptide/protein molecules, and embryonic microspheres are subjected to hardening process by means of hardening agent to get the hardened microspheres. Filtering the microspheres and washing the microspheres with suitable solvent to remove the residual solvent followed by drying the microspheres to get the finished microspheres.

2. In spray-drying technique a polymer is dissolved in a volatile organic solvent such as dichloromethane or acetone. The protein is suspended as solid or emulsified as aqueous solution in this organic solution by homogenization. After that, the resulting dispersion is atomized through a (heated) nozzle into a heated air flow. The organic solvent evaporates, thereby forming microspheres with dimensions of typically 1-100 m. The microspheres are collected in a cyclone separator. For the complete removal of the organic solvent, a vacuum- drying or Lyophilization

step can follow downstream. The internal structure of the resulting polymeric microspheres depends on the solubility of the peptide/protein in the polymer before being spray-dried leading to the formation of reservoir or matrix type products. When the initial dispersion is solution, the final product obtained following spray drying is matrix or monolithic type, that is, polymer particles with dissolved or dispersed nature of the active ingredient (defined as microspheres). Conversely, when the initial dispersion is in suspension, the product obtained is reservoir type, that is, a distinct polymeric envelope/shell encapsulating a liquid core of dissolved active ingredient (defined as microcapsules)

3. Oil-in-water (o/w) and water-in-oil-water (w/o/w) are the two hydrous techniques representing, respectively the single and double emulsion formation during microspheres preparation. In these processes, peptides/proteins are dissolved in an organic solvent (e.g., alcohol) or in an aqueous solution and then mixed or emulsified with an organic solution (non-miscible with water) of the polymer to form a solution or water-in-oil (w/o) emulsion, respectively. Dichloromethane serves as organic solvent for the PLGA and the o/w primary emulsion is formed using either high-shear homogenization or ultrasonication. This primary emulsion is then rapidly transferred to an excess of aqueous medium containing a stabilizer, usually polyvinyl alcohol (PVA). Again homogenization or intensive stirring is necessary to initially form a double emulsion of w/o/w. Subsequent removal (by evaporation) of organic solvent by heat, vacuum, or both results in phase separation of polymer and core to produce microspheres. Instead of solvent evaporation, solvent extraction with large quantity of water with or without a stabilizer can also be undertaken to yield microspheres containing peptide/protein.

4. In the solvent extraction method wherein a physiologically active agent is dissolved or suspended into a polymer solution in an organic solvent, the resulting fluid is sprayed into a liquid of very low temperature, such as liquid argon, nitrogen or oxygen, and the organic solvents is extracted by cold ethanol from the frozen products. This method provides high loading efficiency of the drug, and is applicable for peptide or protein drugs that lose their biological activity easily at high temperatures.

In one of the preferred embodiment, the microspheres of the present invention are prepared by Phase separation technique (Co-acervation phase separation).

In an embodiment, a process for preparing microspheres for sustained release of an active agent with a low initial burst comprises:

a) preparing a solution of active agent in a suitable solvent,

b) preparing a polymer solution by dissolving a biodegradable, biocompatible polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of Co-acervates by means of addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtering the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In a preferred embodiment, a process for preparing microspheres for sustained release of Octreotide acetate with a low initial burst comprises:

a) preparing a solution of Octreotide acetate in a suitable solvent,
b) preparing a polymer solution by dissolving a PLGA glucose star polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of co-acervates by means of addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtered the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another embodiment, the suitable solvent for the active agent dissolution is polar solvent. In preferred embodiment the polar solvent is an alcoholic solvent. In preferred embodiment, the alcoholic solvents are methanol, ethanol or combination thereof. According to further embodiment of the present invention, the concentration (in % weight) of the alcoholic solvent in the drug polymer combined phase is less than about 10%.

In another aspect of the present invention provides, a method for preparing sustained release microspheres with low initial burst of the active agent comprises:

a) preparing a solution of active agent in a polar solvent,
b) preparing a polymer solution by dissolving a biodegradable, biocompatible polymer in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of co-acervates by addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtered the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another embodiment of the present invention provides, a method for preparing sustained release microspheres with low initial burst of the Octreotide acetate comprises:

a) preparing a solution of Octreotide acetate in alcoholic solvent,
b) preparing a polymer solution by dissolving a PLGA glucose star polymer in a suitable solvent,
c) combining these two solutions to form drug polymer combined phase,
d) formation of co-acervates by means of addition of suitable phase separating agent,
e) addition of the suitable hardening solvent to get microsphere suspension,
f) filtering the microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another aspect of the present invention provides, a method for preparing sustained release microspheres with low initial burst of the active agent comprises:

a) preparing polymer solution by dissolving a poly(D,L-lactide-co-glycolide) polymer having an acid number of less than or equal to 10 mgKOH/g in a suitable solvent
b) active agent is dissolved in alcoholic solvent to form drug solution;
c) combining polymer solution and drug solution to form a combined phase of drug and polymer;
d) adding suitable phase separating agent to form co-acervates;
e) mixing with hardening agent to the co-acervates to form a microsphere suspension
f) filtering microspheres followed by washing and drying of microspheres to get the finished microspheres.

In another embodiment of the present invention, a method for preparing sustained release microspheres with low initial burst of the Octreotide acetate comprises:

a) preparing polymer solution by dissolving the poly(D,L-lactide-co-glycolide) glucose polymer having an acid number of less than or equal to 10 mgKOH/g in a DCM solvent
b) Octreotide acetate is dissolved in alcoholic solvent to form drug solution;
c) combining polymer solution and drug solution to form a combined phase of drug and polymer, where in the concentration of the alcoholic solvent in the drug polymer combined phase is less than about 10% by weight;
d) adding silicon oil to form co-acervates;
e) mixing with heptane to the co-acervates to form a microsphere suspension

f) filtering microspheres followed by washing and drying of microspheres to get the finished microspheres.

In one embodiment the said Octreotide solution is prepared by dissolving the Octreotide acetate in Methanol solvent. In another embodiment the said Octreotide solution is prepared by dissolving the Octreotide acetate in Ethanol solvent. In another embodiment the said Octreotide solution is prepared by dissolving the Octreotide acetate in combination of ethanol and methanol solvent.

In further embodiments, the concentration of alcoholic solvent is less than about 9% or less than about 8% or less than about 7% or less than about 6 % or less than about 5% or less than about 4% or less than about 3% or less than about 2% or less than about 1%. In most preferred embodiment the concentration of ethanol or methanol is less than about 5%. In further embodiment the ethanol or methanol concentration in the drug-polymer combined phase is less than about 4%, preferably less than about 3%, more preferably less than about 2%, most preferably less than about 1%.

Examples for the suitable solvents for the polymer matrix material include but not limited to Dichloromethane, Chloroform, Benzene, Ethyl acetate, Benzyl alcohol, THF, Acetone, dimethyl sulfoxide (DMSO), dimethylformamide, dimethyl acetamide, dioxane, tetrahydrofuran (THF), acetonitrile, methylene chloride, ethylene chloride, carbon tetrachloride, chloroform, lower alkyl ethers such as diethyl ether and methyl ethyl ether, hexane, cyclohexane, benzene, acetone, ethyl acetate, and the like. One of the preferred solvent for the polymer is dichloromethane (DCM). The weight % of the polymer in the polymer solution is less than 10%. The % weight of active agent in the drug solution is less than 20%. The concentration of the said polymer in the drug polymer combined phase ranges from about 2% to about 15% by weight, in a preferred embodiment, the said concentration ranges about 3 % to about 10% by weight. According to one embodiment, the concentration of said Active agent in the drug-polymer combined phase ranges from 0.1% to about 5% by weight and with a preferable range of from 0.1% to 1% by weight. According to any embodiment of the present invention, the drug: polymer ratio in the drug polymer combined phase is ranges about 1:5 to about 1:50. In a preferred embodiment, the ratio is about 1:10 to 1:20

The phase inducers (coacervation agents) are solvents which are miscible with the polymer-drug mixture, and cause the embryonic microcapsules to form prior to hardening; silicon oils, natural and mineral oils & triglycerides are used as a phase inducer. Silicone oils are the

preferred phase inducers. The ratio of the polymer solvent (DCM): phase inducers (silicon oil) are 1:5 to 5:1.

A suitable hardening agent, which is a non-solvent for the polymer, while it is miscible with polymer solvent and silicone oil, it extracts both, eventually hardening the microspheres. Suitable hardening agents of the present invention are single solvent system or combination of solvents. Heptane, hexane or higher alkanes are preferred hardening solvents. Combination of the solvents, i.e. heptane with alcoholic solvents like methanol or ethanol may be used as hardening agents. The ratio of the polymer to hardening solvent ranges from 1:50 to 1:250, a preferred range of the same are 1:200. Separating the solvents from the microspheres suspension by means of filtering the wet suspension with a required micron size filter to collect the microspheres

Residual solvents are removed from the microspheres by washing step. Multiple washings were performed until to get the desired limit of the residual solvent level. Single solvent system or combination of the solvent systems used for the washing. Different washing solvents used in the present invention includes but not limited to Heptane, Hexane, ethanol, water or buffered heptane, or any combinations thereof. A little quantity of the surfactant for example span-80 may be used along with washing solvent to aid the dispersibility or wetting of the suspension and to avoid the aggregation of the microspheres. The weight % of the surfactant in the washing solvent ranges from 0.01% to 1%.

Method of drying of the microspheres and suitable drying techniques were described in detail in many of the references which incorporated here as a reference (US 6080429, US6194006, US20050064039 etc). In another embodiment, the microspheres of the invention may be dried, e.g. to remove residual solvents, especially volatiles like dichloromethane etc. Dryers used according to the present inventions are vacuum dryer or freeze dryer. In another aspect, in the drying step, the microspheres of the present invention may be subjected to 1) a freeze-drying process or 2) vacuum drying at 50 to 10^{-2} millibars, e.g. 30 millibars or 3) addition of mannitol in powder to the filtered microparticles in a tumbler or under vacuum as in the drying process and heating of 45° C. to 55° C., preferably 48 to 54° C., most preferably 50 to 52° C. In preferred embodiment, drying is done under vacuum in vacuum dryer at a loading temperature of -5°C to 5°C for a period of 12-24hrs and ramping to a temperature of 25°C to 50°C for a period of 12 – 24 hrs. and plateau drying at a temperature of up to about 50°C for a period of 12 - 48 hrs. One of the other aspects, the microspheres of the invention may be purged with nitrogen or another inert gas. If desired the microparticles of the invention may be heated, e.g. from 25 to 55° C., preferably from 48 to 54° C.

Duration of the drying period may be, e.g. from 2 hours to 5 days. The resultant microspheres may be free-flowing powders of spherical particles.

According to any embodiment of the present invention, the microspheres contain less than 2000 ppm of DCM. According to another embodiment of the present invention, the microspheres contain less than 2000 ppm of ethanol. According to other embodiment of the present invention, the microspheres contain less than 20,000 ppm of heptane.

The microparticles according to the present invention may be mixed or coated with an anti-agglomerating agent or covered by a layer of an anti-agglomerating agent, e.g. in a prefilled syringe or vial. Suitable anti-agglomerating agents include, e.g. mannitol, glucose, dextrose, sucrose, lactose, trehalose, glycine, sodium chloride, or water-soluble polymers such as polyvinylpyrrolidone or polyethylene glycol. For microparticles according to the present invention in dry state preferably an anti-agglomerating agent is present in an amount of about 0.1 to about 20%, preferentially about 10% to 20%, e.g. about 15-18% by weight of the microparticles. A preferred anti-agglomerating agent in this respect is mannitol. Alternatively, an anti-agglomerating agent can be applied to the microparticles during their manufacturing process. For example, at the step of filtering/washing the microparticles they can be additionally rinsed with an aqueous solution of an anti-agglomerating agent. Thus, a layer of the anti-agglomerating agent is formed on the surface of the microparticles. Preferably, the anti-agglomerating agent is present in the microparticles at an amount of less than 20%, more preferably it is less than 18% by weight of the microparticles. A preferred anti-agglomerating agent in this respect is mannitol.

Sifting was done to obtain the microspheres with desired particle size distribution. Sifting can be done by using mechanical or manual sifting. According to any embodiment of the present invention, the particle size of the microspheres ranges from 1 to 250 microns, preferably 10 to 200 microns, most preferably 10 to 130 microns in diameter. In another embodiment, the D_{50} of the microspheres ranges from 10 to 200 microns, preferably 20 to 150 microns.

According to yet another aspect of the present disclosure, the microspheres of the present invention can be administered as a suitable pharmaceutical composition. The microspheres can be administered alone, or in appropriate combination with other active agents or drug therapies, as part of a pharmaceutical composition. These pharmaceutical compositions may include the microspheres in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the microsphere in a unit of weight or volume suitable for

administration to a patient. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human or other mammal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient-containing microspheres are combined to facilitate the application. The components of the pharmaceutical composition are capable of being co-mingled with the components of the present disclosure (e.g., the active agent, the biodegradable polymer), and with each other, in a manner such that there is no interaction that substantially impairs the desired pharmaceutical efficacy. Pharmaceutically acceptable carrier further means a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, desiccants, bulking agents, propellants, acidifying agents, coating agents, solubilizers, and other materials which are well known in the art. Pharmaceutically acceptable carriers suitable for oral, subcutaneous, intravenous, intramuscular, or other type of administrations also are well known, and can be found, e.g., in Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, Pa.), as well as in other sources. The "pharmaceutically-acceptable carrier" according to the disclosure can be bulking agents and wetting agents, for example, sodium carboxymethylcellulose and mannitol. The amount of mannitol in the microspheres composition ranges from 10% to 50%, even more preferably about 18% to about 21% by weight of the microsphere composition.

The pharmaceutical composition according to the present invention containing microparticles may also contain a vehicle to facilitate reconstitution. Prior to administration, the microparticles are suspended in a suitable vehicle for injection. The suitable quantity of vehicle used for reconstitution is ranges from 1ml to 5ml, preferably 2 ml to 2.5 ml. Preferably, said vehicle is water based containing pharmaceutical excipients such as mannitol, sodium chloride, glucose, dextrose, sucrose, or glycerins, non-ionic surfactants (e.g. poloxamers, for example poloxamer 188, poly(oxyethylene)-sorbitan-fatty acid esters), carboxymethylcellulose sodium (CMC-Na), sorbitol, poly(vinylpyrrolidone), or aluminium monostearate in order to ensure isotonicity and to improve the wettability and sedimentation properties of the microparticles. The wetting and viscosity enhancing agents may be present in an amount of 0.01 to 10% preferably about 0.5% to about 5% by weight in the pharmaceutical composition; the isotonicity agents are added in a suitable amount to ensure an isotonic injectable suspension. The viscosity of the diluent ranges from 1cps to 20,000 cps, preferably 1cps to 20cps. The invention further provides a kit

comprising the depot formulation in a vial, optionally equipped with a transfer set, together with a water-based vehicle in an ampoule, vial or prefilled syringe or as microparticles and vehicle separated in a double chamber syringe.

According to one embodiment of the present invention, the pharmaceutically acceptable carrier comprises an anti-agglomerating agent, viscosity enhancing agent, surfactant and diluent

According to any embodiment of the present invention, the pharmaceutically acceptable carrier comprises mannitol, sodium CMC, Poloxamer 188 and water for injection.

Sustained release microspheres of the present invention are administered by IM or SC administration. Preferably microspheres of the present invention are administered by IM administration.

Microspheres of the present invention are filled in to the vials or ampoules, and these microspheres are to be added to vehicle before administration. These microspheres when injected, delivers active agent for a period of one day or a week or one month or 6 months.

In one embodiment, the microspheres of the present invention deliver the active agent for a period of one month. In further embodiments, less than or equal to 10% of the active agent is released within 3 days. In another embodiment, less than or equal to 20% of the active agent is released within 15 days. In another embodiment, greater than or equal to 60% of the active agent is released within 23 days. In another embodiment, greater than or equal to 80% of the active agent is released within 30 days. In further embodiment, the onset of the erosion phase is approx 15^{th} day to 23^{th} day. Still further embodiment of the present invention, onset of the erosion phase is approx. 17^{th} day to 20^{th} day.

Sustained release Octreotide microspheres is used for the treatment of acromegaly, long-term treatment of the severe diarrhea and flushing episodes associated with metastatic carcinoid tumors and profuse watery diarrhea associated with VIP-secreting tumors (Vasoactive Intestinal Peptide Tumors - VIPomas).

The following examples illustrates the present invention

EXAMPLES

Example 1

Octreotide Loaded PLGA microspheres with low Initial burst release and brief process of preparation

Octreotide acetate solution is prepared by addition of approx. 450 mg of Octreotide acetate in suitable quantity of ethanol or methanol. Polymer solution is prepared by addition of 6gm of poly(D,L-lactide-co-glycolide)glucose (PLGA glucose polymer) in approx. 100 gm of DCM. (55:45 molar ratio of lactide to glycolide, Mw approx. 45,000 to 55,000 Daltons, acid number of 2-5). Adding the Octreotide solution to the polymer solution to form drug-polymer combined phase. Phase separation was affected by adding approx. 150 ml of silicon oil (350 cs) to the drug-polymer combined phase to form co-acervates. The resultant mixture was added to a stirred solution of approx.1000 gm of n-heptane, after stirring for 10 to 30 minutes, the microspheres were collected by vacuum filtration.

The resultant microspheres were washed with sequence of solvent systems, i.e. stirring with 500 ml of n-heptane, followed by approx. 500 ml of ethanol, optionally containing 1 ml of Span 80 and final washing done with 500 ml of n-heptane. Finally, the microspheres were collected by vacuum filtration and dried at suitable temperature conditions of -5^{0}C to 45^{0}C for 3-5 days. The residual DCM and ethanol level of these microspheres was less than 2000 ppm

Example 2

Prepare the Octreotide acetate solution by addition of approx. 500 mg of Octreotide acetate in suitable quantity of ethanol or methanol. Prepare the polymer solution is by addition of 6gm of poly(D,L-lactide-co-glycolide)glucose (PLGA glucose polymer) in approx. 150 gm of DCM. (55:45 molar ratio of lactide to glycolide, Mw approx. 45,000 to 55,000 Daltons, acid number of 6-10). Add the Octreotide solution to the polymer solution to form drug-polymer combined phase. Add the approx. 150 ml of silicon oil (350 cs) to the Octreotide-polymer combined phase to form co-acervates. Add the resultant mixture to a stirred solution of approx.1000 gm of n-heptane, Stirr for 10 to 30 minutes, filter the microspheres and collect the resultant microspheres. Wash the microspheres with ethanol and / or heptane or combination and dry the microspheres at suitable temperature conditions of -5^{0}C to 45^{0}C for 3-5 days to get the finished microspheres.

Example 3 - 12

Microspheres were prepared in the same manner as described in example 1, with a difference in the acid number, type pf solvent which are given below

Table 1

Examples	Batch number	Acid number in mgKOH/g	Type of polar solvent for drug dissolution
Example 3	OCT-1-30-F044	2.3	Ethanol
Example 4	OCT-1-30-F-117	3.7	Ethanol
Example 5	OCT-1-30-F-113	2.7	Ethanol
Example 6	OCT-1-30-F041	5	Methanol
Example 7	OCT-1-30-F35	2.4	Methanol
Example 8	OCT-1-30-F066	2.4	Methanol
Example 9	OCT-1-30-F39	2.4	Methanol
Example 10	OCT-1-30-F-062	5	Ethanol
Example 11	OCT-1-30-F-151	4.1	Ethanol
Example 12	OCT-1-30-F-173	3.2	Ethanol

Example 13-18

Microspheres were prepared in the same manner as described in example 1, with a difference in concentration of polar solvent and acid number

Examples	Batch number	Acid number in mgKOH/g	Type of polar solvent for drug dissolution	Weight % of Polar solvent
Example 13	OCT-1-30-F-030	2.3	Ethanol	3
Example 14	OCT-1-30-F-043	2.3	Ethanol	3
Example 15	OCT-1-30-F-035	2.3	Ethanol	5
Example 16	OCT-1-30-F-047	2.9	Ethanol	6
Example 17	OCT-1-30-F-049	2.9	Ethanol	6
Example 18	OCT-1-30-F-096	2.6	Ethanol	2

In-vitro release data:

From the above examples, In-vitro release (IVR) was estimated in few batches. IVR is performed in shaking water bath having a temperature of 37°C in 50mM acetate buffer media in pH of 4. Below table provides the cumulative % release of the Octreotide with time approx. in 30 days for the batch OCT-1-30-F-096.

Table 2

Time point (Days)	Weight % of Octreotide release

0.042 (i.e. 1hr)	0.2
1	1.2
3	1.9
16	2.7
18	11.9
19	36.8
20	73.1
21	83.0
22	88.7
25	94.1
28	97.4
31	98.3

Effect of polymer acid number on the initial burst release of microspheres.

Table 3

Example / Batch Number	**Acid number in mg KOH/g.**	**Initial IVR (weight % of drug released) with time (in days)**	
		0.042 day (1 hr)	1 day
OCT-1-30-F044	2.3	0.4	2.8
OCT-1-30-F-113	2.7	0.3	2.1
OCT-1-30-F-173	3.2	0.1	0.4
OCT-1-30-F-117	3.7	0.1	0.4
OCT-1-30-F-151	4.1	0.1	0.3
OCT-1-30-F-062	5	0.3	0.7

From the figure 1 and above table, it is observed that the initial burst release of Octreotide is well controlled by selection of suitable polymer having an acid number less than 10 mg//KOH/g.

WE CLAIM:

1. Microsphere composition for sustained release of Octreotide or its pharmaceutically acceptable salts thereof with a low initial burst comprises: a poly (D, L-lactide-co-glycolide) polymer having an acid number of less than or equal to 10 mgKOH/g, Octreotide or its pharmaceutically acceptable salts and one or more pharmaceutically acceptable carriers.
2. Microsphere composition of claim 1 wherein the said microspheres composition releases less than or equal to 1% by weight of a total amount of the Octreotide within 1 hour at 37° C and pH 4.0
3. Microsphere composition of claim 1 wherein the said microspheres composition releases less than or equal to 5% by weight of a total amount of the Octreotide within 1 day at 37° C and pH 4.0
4. Microsphere composition of claim 1 wherein poly (D, L-lactide-co-glycolide) polymer is a poly (D, L-lactide-co-glycolide) glucose star polymer with a molar ratio of lactide to glycolide ranging from 60:40 to 40:60
5. Microsphere composition of claim 4 wherein the polymer is having an inherent viscosity ranges from 0.30 - 0.70 dL/g in chloroform.
6. Microsphere composition of claim 4 wherein the polymer having a weight average molecular weight ranges from 30,000 Daltons - 60,000 Daltons
7. Microsphere composition of claim 1 wherein pharmaceutically acceptable salt of Octreotide is Octreotide acetate.
8. Microsphere composition for sustained release of Octreotide acetate with a low initial burst comprises: a poly (D, L-lactide-co-glycolide) glucose star polymer having an acid number of less than or equal to 10 mgKOH/g, Octreotide acetate and one or more pharmaceutically acceptable carriers, wherein microspheres releases less than or equal to 1% by weight of a total amount of the Octreotide within 1 hour and less than or equal to 5% by weight of total amount of Octreotide within 1 day at 37° C and pH 4.0
9. Microsphere composition of any one of the above claims 1-8 wherein the microspheres composition is prepared by a process comprising:
    a. preparing a solution of Octreotide by dissolving Octreotide or pharmaceutically acceptable salt in an alcoholic solvent

b. preparing a polymer solution by dissolving poly (D, L-lactide-co-glycolide) glucose star polymer in dichloromethane,
c. combining these two solutions to form Octreotide-polymer combined phase, wherein the concentration of the alcoholic solvent in Octreotide-polymer combined phase is less than about 10% by weight
d. formation of co-acervates by means of addition of silicon oil,
e. addition of the heptane solvent to get microsphere suspension,
f. filtered the microspheres followed by washing and drying of microspheres to get the finished microspheres.

10. Microsphere composition of claim 9 wherein an alcoholic solvent is selected from the group consisting of methanol, ethanol or combination thereof.
11. A process of preparing sustained release microspheres of the Octreotide or pharmaceutically acceptable salts thereof, with a low initial burst comprises:
    a. preparing a solution of Octreotide by dissolving Octreotide or pharmaceutically acceptable salts thereof in an alcoholic solvent
    b. preparing a polymer solution by dissolving poly (D, L-lactide-co-glycolide) glucose start polymer in dichloromethane
    c. combining these two solutions to form Octreotide-polymer combined phase, wherein the concentration of the alcoholic solvent in Octreotide-polymer combined phase is less than about 10% by weight
    d. formation of co-acervates by means of addition of silicon oil,
    e. addition of the heptane solvent to get microsphere suspension,
    f. filtered the microspheres followed by washing and drying of microspheres to get the finished microspheres.
12. A process of preparing sustained release microspheres of claim 11, wherein an alcoholic solvent is selected from the group consisting of methanol, ethanol or combination thereof.
13. A process of preparing sustained release microspheres of claim 11, wherein poly (D, L-lactide-co-glycolide) glucose start polymer having an acid number of less than or equal to 10 mgKOH/g

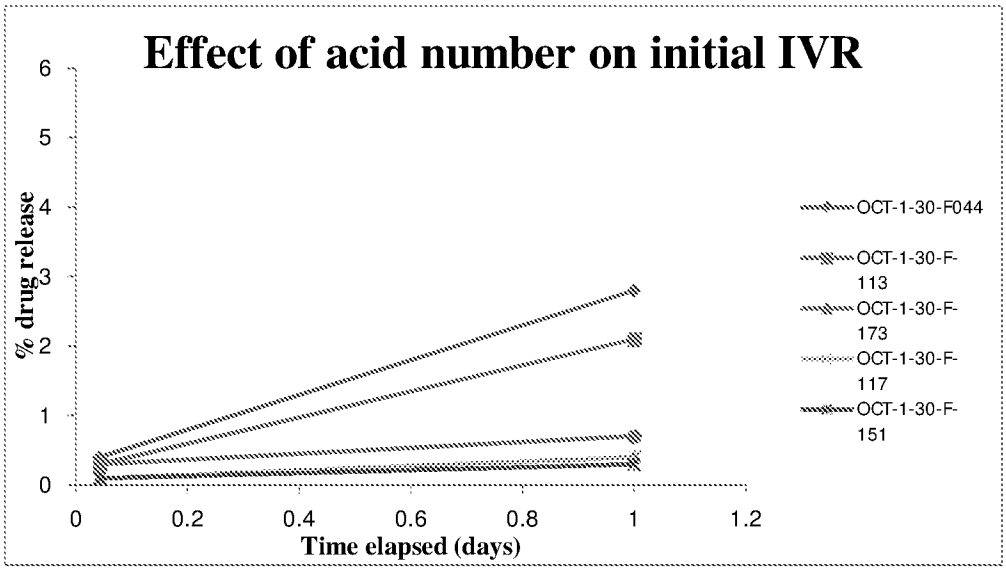

Figure 1

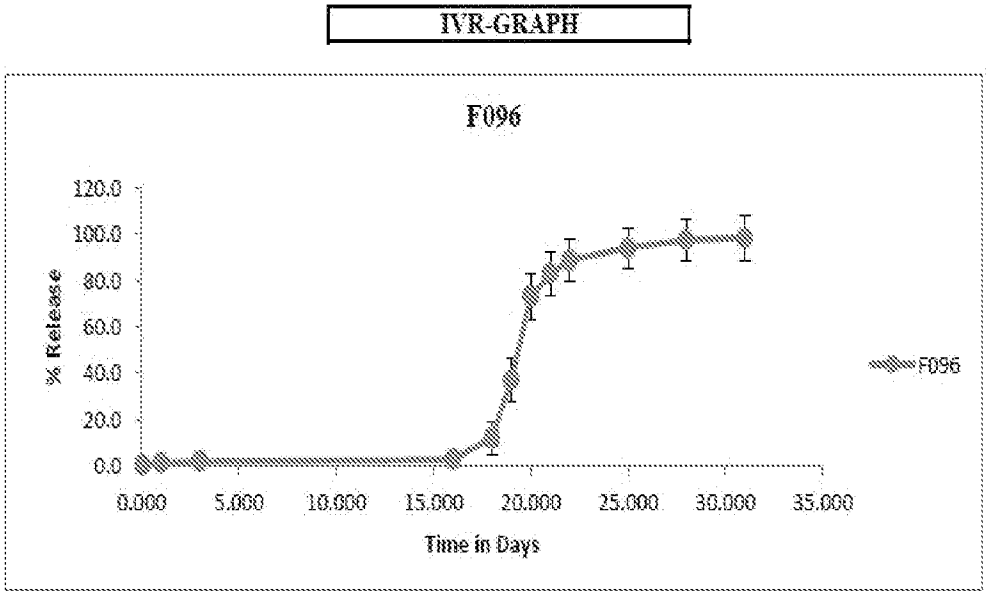

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2019/050987

A. CLASSIFICATION OF SUBJECT MATTER
A61K9/16,A61K38/08 Version=2019.01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US20100086597 A1 (OAKWOOD LAB LLC[US]) 08 April 2010 claims 1, 5, 21; para[0055]; para[0025];	1-8
Y	claim 21	9-13
Y	WO2004045633 A2 (NOVARTIS AG[CH]; NOVARTIS PHARMA GMBH[AT]) 03 June 2004 claim 1	9-13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24-06-2019	24-06-2019

Name and mailing address of the ISA/	Authorized officer
Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Abhas Kumar Bhoi Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2019/050987

Citation	Pub.Date	Family	Pub.Date
US 20100086597 A1	08-04-2010	WO 2010042432 A1	15-04-2010
WO 2004045633 A2	03-06-2004	EP 2377519 B1	23-03-2016
		US 2014323415 A1	30-10-2014
		KR 101240071 B1	06-03-2013
		JP 5784402 B2	24-09-2015