

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

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
26 November 2009 (26.11.2009)

(10) International Publication Number  
**WO 2009/143288 A1**

PCT

(51) International Patent Classification:

*A61K 9/00* (2006.01)      *A61K 31/57* (2006.01)  
*A61K 9/16* (2006.01)      *A61K 31/519* (2006.01)  
*A61K 31/58* (2006.01)

(21) International Application Number:

PCT/US2009/044732

(22) International Filing Date:

20 May 2009 (20.05.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

|            |                          |    |
|------------|--------------------------|----|
| 61/054,511 | 20 May 2008 (20.05.2008) | US |
| 61/054,519 | 20 May 2008 (20.05.2008) | US |
| 61/054,506 | 20 May 2008 (20.05.2008) | US |

(71) Applicants (for all designated States except US): **YALE UNIVERSITY** [US/US]; 433 Temple Street, 1st Floor, New Haven, CT 06511 (US). **UNIVERSITY OF IOWA RESEARCH FOUNDATION** [US/US]; 2660 University Capitol Centre, Iowa City, IA 52242-5500 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LAVIK, Erin** [US/US]; 15 Cottage Street, Branford, CT 06405 (US). **BERTRAM, James** [US/US]; 99 Mansfield Street, New Haven, CT 06511 (US). **SALUJA, Sandeep** [IN/US]; 1906 Greenwood Drive, Fair Lawn, NJ 07410 (US). **KEUHN, Markus** [DE/US]; 827 32nd Avenue, Amana, IA 52203 (US). **KWON, Young, H.** [US/US]; 875 Camp Cardinal Road, Iowa City, IA 52246 (US). **ROBINSON,**

**Rebecca** [US/US]; 2739 Waleska Way, East Point, GA 30344 (US). **HUANG, John, J.** [US/US]; 515 Aspen Glen Drive, Hamden, CT 06518 (US).

(74) Agents: **VORNDRAN, Charles** et al.; Pabst Patent Group Llp, 1545 Peachtree Street, N.e., Suite 320, Atlanta, GA 30309 (US).

(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, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: BIODEGRADABLE SUSTAINED-RELEASE POLYMERIC MICROPARTICULATES COMPRISING A HYDROPHOBIC DRUG AND DETERMINED FOR OPHTHALMIC USE

(57) Abstract: Biodegradable polymeric microparticle compositions containing one or more active agents, especially those useful for treating or preventing or one or more diseases or disorders of the eye, and methods of making and uses for the manufacture of a medicament, are described. The microsphere compositions release an effective amount of the one or more active agents for a period greater than 14 days in vivo, preferably greater than 60 days in vivo, more preferably up to 73 days in vivo, more preferably greater than 90 days in vivo, even more preferably over 100 days in vivo, and most preferably greater than 107 days in vivo. In a preferred embodiment, the microparticle compositions contain one or more active agents useful for managing elevated intraocular pressure (IOP) in the eye.



WO 2009/143288 A1

BIODEGRADABLE SUSTAINED-RELEASE POLYMERIC MICROPARTICULATES COMPRISING A HYDROPHOBIC DRUG AND DETERMINED FOR OPHTHALMIC USE

### FIELD OF THE INVENTION

This invention is in the field of pharmaceutical compositions comprising biodegradable microparticles encapsulating high weight percent drug and providing sustained release over a prolonged period of time of drug levels bioequivalent to direct administration of drug.

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S.S.N. 61/054,519 entitled "*Sustained Delivery of Ofloxacin, Prednisolone Acetate, and Methotrexate from Poly(lactic-co-glycolic acid) Microspheres*", filed on May 20, 2008, U.S.S.N. 61/054,511 entitled "*Sustained Delivery of Travoprost to Lower Intraocular Pressure*", filed on May 20, 2008, and U.S.S.N. 61/054,506 entitled "*Sustained Delivery of AG 1478, An Inhibitor of the Epidermal Growth Factor Receptor (EGFR), for Antitumor Therapy and Neural Regeneration*", filed on May 20, 2008. The disclosures in the applications listed above are herein incorporated by reference.

### BACKGROUND OF THE INVENTION

Polymeric microparticles have been used for drug delivery for decades. Numerous methods to increase the amount of drug which can be delivered, and to manipulate rate of release, and release profile, have been described. Methods have included altering microparticle size, shape, polymer composition, inclusion of additives such as surfactants and pore forming agents, and inclusion of ligands and bioadhesive agents.

Glaucoma is an ophthalmic disease characterized by the gradual degeneration of retinal ganglion cells (RGCs). RGCs synapse with bipolar cells and transmit visual inputs to the brain along the optic nerve. Degeneration of these cells leads to gradual vision loss and ultimately blindness if untreated. Glaucoma is the second leading cause of blindness (Biomdahl *et al.*, *Acta. Opth. Scan.*, 75, 310-319 (1997)). Glaucoma will affect approximately 60.5 million people in 2010, increasing to 796 million people in 2020 (Quigley *et al.*, *Brit. J. Opth.*, 90, 262-267 (2006)). This

includes peoples suffering from both open angle (OAG) and angle closure glaucoma (ACG).

Although a normal tension variant does exist, the development of glaucoma is most often associated with elevated intraocular pressure (IOP) (Migdal *et al.*, *Ophthalmol.*, 101, 1651-1656 (1994)). This elevated pressure is caused by an excess accumulation of aqueous humor in the eye due to blockage of the trabecular network (Alward *et al.*, *Amer. J. Ophthalmol.*, 126, 498-505 (1998)). With a majority of glaucoma cases associated with elevated IOP, reduction of this pressure has been found to greatly mitigate degeneration in approximately 90% of the cases, including cases in which IOP is in the normal range but optic neuropathy occurs (*Id.*).

Eye drops containing one more active agents that lower IOP are typically prescribed to treat glaucoma. are currently the primary means of delivery for this drug. However, eye drop typically deliver very small amounts of drug, requiring large numbers of doses per day for IOP management. Compliance with this treatment regime is poor with more than half of patients unable to maintain consistently lowered IOP through drops (Rotchford and Murphy, *Brit. J. Ophthalmol.*, 12, 234-236 (1998)).

Drops also lead to extensive systemic absorption of the administered drug (~80%, Marquis and Whitson, *Drugs & Aging*, 22, 1-21 (2005)). This systemic absorption can result in adverse side effects. Together, these complications make topical application of IOP-lowering drugs problematic, especially in the aging population that exhibits the lowest compliance and highest degree of complications (Marquis and Whitson, *Drugs & Aging*, 22, 1-21 (2005)). There exists a need for a sustained release formulations, which overcomes the limitations of currently available eye drops.

A variety of approaches for the sustained delivery of drugs to the have been investigated

U.S. Patent No. 6,726,918 to Wong describes methods for treating inflammation-mediated conditions of the eye, the methods including implanting into the vitreous of the eye a bioerodible implant containing a steroidal anti-inflammatory and a bioerodible polymer, wherein the implant delivers an agent to the vitreous in amount sufficient to reach a concentration

equivalent to at least about 0.65 µg/ml dexamethasone within about 48 hours and maintains a concentration equivalent to at least about 0.03 µg/ml dexamethasone for at least about three weeks. Wong does not disclose administering the implants by subconjunctive injection.

5 U.S. Patent Application Publication No. 2006/0173060 to Chang *et al.* describes biocompatible microparticles containing an alpha-2-adrenergic receptor agonist and a biodegradable polymer. The microparticles can allegedly be used to treat glaucoma. Chang alleges that the microparticles release the active agent for a period of time of at least about one week, such  
10 as between two and six months. Chang discloses that the microparticles can be administered subconjunctivally.

U.S. Patent Application Publication No. 2004/0234611 to Ahlheim *et al.* describes an ophthalmic depot formulation containing an active agent embedded in a pharmacologically acceptable biocompatible polymer or a  
15 lipid encapsulating agent for periocular or subconjunctival administration. The formulation can be in the form of microparticles or nanoparticles. Ahlheim discloses that the depot formulations are adapted to release all or substantially all of the active material over an extended period of time (e.g., several weeks up to 6 months). Suitable active agents are listed in  
20 paragraphs 0033 to 0051; however, the preferred active agent is a staurosporine, a phthalazine, or a pharmaceutically salt thereof. Suitable polymers are listed in paragraphs 0014 to 0026. Ahlheim contains no examples showing *in vitro* or *in vivo* release of any active agents.

None of the references discussed above disclose optimizing the  
25 charge, hydrophilicity or hydrophobicity, and/or the molecular weight of the polymers used to prepare the microparticles in order to maximize drug loading and release of an effective amount of the drug for a desired period of time.

Therefore, it is an object of the invention to provide sustained release  
30 polymeric microparticulate compositions which have been optimized to maximize drug loading and release an effective amount of a drug (or drugs) for a desired period of time.

It is a further object of the present invention to provide such formulations useful for reducing intraocular pressure (IOP) which provide sustained release of an amount of drug comparable to that administered topically for more than 14 days *in vivo*, and methods of making and using  
5 thereof.

It is further an object of the invention to provide sustained release compositions of one or more active agents useful for reducing intraocular pressure (IOP) which provide sustained release for more than 14 days *in vivo*, and methods using thereof, wherein the compositions exhibit minimal  
10 adverse side effects and is well tolerated by patients.

It is still further an object of the invention to provide sustained release compositions of one or more active agents useful for promoting regeneration of the optic nerve and methods of making and using thereof.

#### SUMMARY OF THE INVENTION

15 Biodegradable polymeric microparticle compositions containing one or more poorly water soluble active agents, especially those useful for treating or preventing one or more diseases or disorders of the eye, and methods of making and using thereof, are described. The microparticles are optimized for the drug to be delivered, so that the hydrophobicity or  
20 hydrophilicity of the polymer and charge of the polymer maximizes loading of the drug, and the selection and molecular weight of the polymers maximize release of an effective amount of the drug for the desired period of time. For example, poorly water soluble drugs tend to interact more strongly with hydrophobic monomers or polymers.

25 In one embodiment, the microparticle compositions contain one or more water insoluble active agents useful for managing elevated intraocular pressure (IOP) in the eye. In another embodiment, the microparticle compositions contain one or more water insoluble active agents that promote regeneration of the optic nerve. In still another embodiment, the  
30 microparticle compositions contain one or more water insoluble active agents useful for treating one or more anti-inflammatory diseases, such as uveitis.

The microsphere compositions release an effective amount of the one or more active agents for a period greater than 14 days *in vivo*, preferably

greater than 30 days, preferably greater than 60 days *in vivo*, more preferably up to 73 days *in vivo*, more preferably greater than 90 days *in vivo*, even more preferably over 100 days *in vivo*, and most preferably greater than 120 days *in vivo*. The desired amount and duration of release is dependent upon  
5 several factors including the disease or disorder to be treated, the one or more active agents to be delivered, and the frequency of administration. In one embodiment, the drug is released over a shorter period of time, for example, 14-21 days for steroids such as prednisolone. Alternatively, for delivery of agents to lower IOP and/or to promote neural regeneration, the  
10 formulations preferably release drug over longer time periods, such as those listed above.

In one embodiment, the microspheres are formed from polylactide-co-glycolide ("PLGA"); in another embodiment, the microspheres are formed from a blend of PLGA and polylactic acid ("PLA"). Higher  
15 molecular weight polymers, having different ratios of LA (which has a longer degradation time, up to one to two years) to GA (which has a short degradation time, as short as a few days to a week), are used to provide release over a longer period of time. The combination of drug loading and release rate, as well as the minimization of initial burst release, result in  
20 prolonged release of a higher amount of drug. As demonstrated by the examples, the microsphere compositions release a water insoluble drug for at least 35 days, preferably for at least 50 days, more preferably for at least 75 days, most preferably for at least 100 days. The sustained release of drug, in combination with the ability to administer the drug in a minimally invasive  
25 manner, should increase patient compliance.

The percent loading of the drug in the microspheres is from about 1% to about 25% by weight, preferably from about 1% to about 20% by weight, more preferably from about 1% to about 10% by weight. The percent loading is dependent on the drug to be encapsulated, the polymer or  
30 polymers used to form the microparticles, and/or the procedure used to prepare the microparticles.

The microsphere compositions can be administered to the eye using a variety of techniques in the art. In one embodiment, the compositions are

administered to the eye by injection. In a preferred embodiment, the microsphere composition is administered subconjunctivally. Subconjunctival administration is minimally invasive, and minimizes systemic absorption of the active agents.

## 5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the cumulative prednisolone acetate release ( $\mu\text{g}/\text{mg}$  of polymer) *in vitro* as a function of time (days) for microparticles prepared from PLGA 502H (■), PLGA 503 H (x), and a blend of PLGA 502H and PLA (▼).

10 Figure 2 is a graph showing the cumulative prednisolone acetate release ( $\mu\text{g}/\text{mg}$  of polymer) *in vitro* as a function of time (days) for microparticles prepared from PLGA 502H (■), PLGA 502H with PEG-1500 (▲), PLGA 502H with sonication (15% amp) (▼), PLGA 502H with sonication (30% amp) (◆), PLGA 502H with sonication (38% amp) (●), and  
15 PLGA 502H with homogenizer (□).

Figure 3 is a graph showing the *in vitro* cumulative methotrexate release ( $\mu\text{g}/\text{mg}$ ) as a function of time (days) from microspheres prepared from PLGA 503H.

Figure 4 are graphs showing the *in vitro* and *in vivo* cumulative  
20 methotrexate release ( $\mu\text{g}/\text{mg}$ ) as a function of time (days) from microspheres prepared from PLGA 502H.

Figure 5 is a graph showing the release of triamcinolone ( $\mu\text{g}/\text{mg}$ ) *in vivo* and *in vivo* as a function of time (days) from PLGA 502H microparticles.

25 Figure 6 is a graph showing the *in vitro* release profile of travoprost ( $\mu\text{g}$  drug/ $\text{mg}$  polymer) as a function of time (days) for travoprost-loaded PLGA 503H microparticles.

Figure 7 is a graph showing the *in vitro* release of AG1478 ( $\mu\text{g}$  drug/ $\text{mg}$  polymer) as a function of time (days) from PLGA 503H  
30 microparticles.

Figure 8 is a graph showing the cumulative release of AG1478 ( $\mu\text{g}/\text{mg}$  polymer) *in vitro* from microspheres prepared from PLGA 503H via

an oil-in-water emulsion technique, wherein percent drug loading is 2.5% (□) and 5.0% (▲).

Figure 9 is a graph showing the *in vitro* release profile of AG1478 (µg drug/mg polymer) as a function of time (days) for AG1478-loaded PLGA 503H microparticles prepared using a S/O/W emulsion process (x), a O/W emulsion process (□), and a O/W co-solvent emulsion process (●).

Figure 10 is a graph showing the cumulative release of AG1478 (µg/mg polymer) *in vitro* as a function of time (days) from microspheres prepared from PLGA 503H (●), PLGA 504 (◇), and PLGA 504H (□) using an oil-in-water emulsion cosolvent technique.

Figure 11 is a graph showing the cumulative release of AG1478 (µg/mg polymer) *in vitro* as a function of time (days) from microspheres prepared from PLGA 504 (◇) and PLGA 504H (□) prepared using an oil-in-water cosolvent technique.

## DETAILED DESCRIPTION OF THE INVENTION

### I. Definitions

“Nanoparticle”, as used herein, refers to particle or a structure in the nanometer (nm) range, typically from about 1 nm to about 1000 nm in diameter, which is encapsulated within the polymer.

“Microparticle”, as used herein, unless otherwise specified, generally refers to a particle of a relatively small size, but not necessarily in the micron size range; the term is used in reference to particles of sizes that can be, for example, administered to the eye subconjunctivally, and thus can be less than 50 nm to 100 microns or greater. Microparticles specifically refers to particles having a diameter from about 5 to about 25 microns, preferably from about 10 to about 25 microns, more preferably from about 10 to about 20 microns. As used herein, the microparticle encompasses microspheres, microcapsules and microparticles, unless specified otherwise. The relative sizes of microparticles and nanoparticles in the context of the present invention are such that the latter can be incorporated into the former. A micro- or nanoparticle may be of composite construction and is not necessarily a pure substance; it may be spherical or any other shape.

Formulations can be prepared using a pharmaceutically acceptable “carrier” composed of materials that are considered safe and effective and may be administered to an individual without causing undesirable biological side effects or unwanted interactions. As used herein, the “carrier” is all components present in the pharmaceutical formulation other than the active ingredient or ingredients. The term “carrier” includes, but is not limited to, solvents, suspending agents, dispersants, buffers, pH modifying agents, isotonicity modifying agents, preservatives, antimicrobial agents, and combinations thereof.

“Poorly water soluble drug”, as used herein, refers to a drug having a solubility of less than 10 mg/ml at 25°C, preferably less than 5 mg/ml at 25°C, more preferably less than 1 mg/ml at 25°C, most preferably less than 0.5 mg/ml at 25°C.

“Water-soluble drug”, as used herein, refers to a drug having a solubility of greater than 10 mg/ml at 25°C, preferably greater than 25 mg/ml at 25°C, more preferably greater than 50 mg/ml at 25°C, most preferably greater than 100 mg/ml at 25°C.

“Hydrophilic polymer”, as used herein, refers to polymers that have an affinity for water, though are not water soluble.

“Hydrophobic polymer”, as used herein, refers to polymers that tend to repel water.

## **II. Compositions**

### **A. Active Agents**

The microparticle compositions described herein contain one or more poorly water soluble active agents. In one embodiment, the one or more active agents are useful for treating diseases or disorders of the eye. Suitable classes of active agents include, but are not limited to, active agents that lower intraocular pressure, antibiotics, anti-inflammatory agents, chemotherapeutic agents, and steroids. The active agents described above can be administered alone or in combination to treat diseases or disorders of the eyes.

Alternatively, the poorly water soluble drug can be co-administered with a water-soluble drug, either in the same microspheres or in different

microspheres or microparticles. The water-soluble drugs can be formulated in polymeric microparticles in which the hydrophilicity, molecular weight, and/or monomer composition has been optimized to maximize loading of the drug in the microparticles and to provide sustained release for a period  
5 greater than 14 days *in vivo*, preferably greater than 30 days, preferably greater than 60 days *in vivo*, more preferably up to 73 days *in vivo*, more preferably greater than 90 days *in vivo*, even more preferably over 100 days *in vivo*, and most preferably greater than 120 days *in vivo*, most preferably at least 175 days *in vivo*. Microparticles containing water-soluble active  
10 agents, and methods of making and using thereof, are described in WO 2008/157614.

*Active agents that lower IOP*

In one embodiment, the microparticles contain one or more active agents that manage (e.g., reduce) elevated IOP in the eye. Suitable active  
15 agents include, but are not limited to, prostaglandins analogs, such as travoprost, bimatoprost, latanoprost, unoprostone, and combinations thereof; and carbonic anhydrase inhibitors (CAI), such as methazolamide, and 5-acylimino- and related imino-substituted analogs of methazolamide; and combinations thereof. The microparticles can be administered alone or in  
20 combination with microparticles containing a second drug that lowers IOP. The second drug can be poorly water soluble or water-soluble and can be formulated in the same microparticles or different microparticles as described above.

*Antibiotics*

25 The microparticles compositions can contain one or more poorly water soluble antibiotics. Exemplary antibiotics include, but are not limited to, cephaloridine, cefamandole, cefamandole nafate, cefazolin, cefoxitin, cephacetrile sodium, cephalixin, cephaloglycin, cephalosporin C, cephalothin, cefcillin, cephamycins, cephapirin sodium, cephradine,  
30 penicillin BT, penicillin N, penicillin O, phenethicillin potassium, pivampiculin, amoxicillin, ampicillin, cefatoxin, cefotaxime, moxalactam, cefoperazone, cefsulodin, ceflizoxime, ceforanide, cefixone, ceftazidime

thienamycin, N-formimidoyl thienamycin, clavulanic acid, penemcarboxylic acid, piperacillin, sulbactam, cyclosporins, and combinations thereof.

#### *Inhibitors of Growth Factor Receptors*

In another embodiment, the poorly water soluble active agent is an  
5 inhibitor of a growth factor receptor. Suitable inhibitors include, but are not limited to, inhibitors of Epidermal Growth Factor Receptor (EGFR), such as AG1478, and EGFR kinase inhibitors, such as BIBW 2992, erlotinib, gefitinib, lapatinib, and vandetanib.

AG1478 is a potent inhibitor of the epidermal growth factor receptor  
10 (EGFR). It was developed initially as a small-molecule tyrosine kinase antagonist to treat tumors, such as breast and ovarian, that have large excesses of EGFR on their surfaces. EGFR is present in many cell types in the body and is responsible for mediating basic cell behaviors such as proliferation and fate choice of cells, thus making systemic knockdown of  
15 EGFR problematic.

To treat a tumor, one would like to have a delivery technology that provides a large and sustained dose of the drug in a localized manner. By fabricating microspheres that deliver AG1478 for an extended period of time (e.g., over 3 months), one has an injectable technology that can be delivered  
20 in a minimally invasive manner, and can deliver the drug over a long time scale in a localized manner. This will allow higher concentrations of the drug at the tumor over longer time periods and limited amounts at other sites potentially augmenting the therapeutic effect of the drug and mitigating side effects.

AG1478 has also been shown to have a role in neural regeneration.  
25 By delivering AG1478 in a sustained and localized manner, it may be possible to promote regeneration following injury to the central nervous system (CNS).

#### *Chemotherapeutic Agents and Steroids*

30 The microparticle compositions can contain one or more poorly water soluble chemotherapeutic agents and/or steroids. In one embodiment, the poorly water soluble chemotherapeutic agent is methotrexate. Methotrexate is an antimetabolite which has been used to treat autoimmune disorders as

well as certain types of cancers. In the eye, methotrexate is used to treat a number of inflammatory diseases, such as uveitis. Methotrexate is known to cause adverse side effects when administered systemically. Sustained, local delivery has the potential to reduce the amount of methotrexate in serum or  
5 eliminate it completely and thus mitigate adverse side effects.

In another embodiment, the drug is a poorly water soluble steroid, such as prednisolone acetate, triamcinolone, prednisolone, hydrocortisone, hydrocortisone acetate, hydrocortisone valerate, vidarabine, fluorometholone, fluocinolone acetonide, triamcinolone acetonide,  
10 dexamethasone, dexamethasone acetate, and combinations thereof.

The side effects most associate with ophthalmic surgery are post-operative ocular inflammation and/or infection. Topical administration of eye drops containing a steroid and an antibiotic has typically been used for controlling inflammation and preventing infection. However, poor patient  
15 compliance and/or the risk of re-opening the stitched wound due to continuous touching of the wound when applying the drops are limitations of such formulations. Therefore, it is preferable to use sustained release formulations that release the steroid and/or antibiotic over extended periods of time (e.g., 2-3 weeks) to minimize dosing frequency, improve compliance,  
20 reduce side effects, and keep the stitched wound intact. Triamcinolone is a steroid used to treat macular oedema, a complication of diabetes and retinal vein occlusion.

#### *Pharmaceutically Acceptable Salts*

The one or more active agents can be administered as the free acid or  
25 base or as a pharmaceutically acceptable acid addition or base addition salt.

Examples of pharmaceutically acceptable salts include but are not limited to mineral or organic acid salts of basic residues such as amines; and alkali or organic salts of acidic residues such as carboxylic acids. The pharmaceutically acceptable salts include the conventional non-toxic salts or  
30 the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. Such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric acids; and the salts

prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, tolunesulfonic, naphthalenesulfonic, methanesulfonic, ethane disulfonic, oxalic, and isethionic salts.

The pharmaceutically acceptable salts of the compounds can be synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 20th ed., Lippincott Williams & Wilkins, Baltimore, MD, 2000, p. 704; and "Handbook of Pharmaceutical Salts: Properties, Selection, and Use," P. Heinrich Stahl and Camille G. Wermuth, Eds., Wiley-VCH, Weinheim, 2002.

As generally used herein "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

## **B. Polymers**

The microparticles described here can be formed from natural and/or synthetic polymeric materials. "Polymer" or "polymeric", as used herein, refers to oligomers, adducts, homopolymers, random copolymers, pseudo-copolymers, statistical copolymers, alternating copolymers, periodic copolymer, bipolymers, terpolymers, quaterpolymers, other forms of copolymers, substituted derivatives thereof, and combinations of two or more thereof (i.e., polymer blends). The polymers can be linear, branched, block, graft, monodisperse, polydisperse, regular, irregular, tactic, isotactic,

syndiotactic, stereoregular, atactic, stereoblock, single-strand, double-strand, star, comb, dendritic, and/or ionomeric.

Bioerodible polymers may be used, so long as they are biocompatible. Preferred bio-erodible polymers are polyhydroxyacids such as polylactic acid and copolymers thereof. These are approved for implantation into humans. Another class of approved biodegradable polymers are the polyhydroxyalkanoates.

Other suitable polymers have been described in great detail in the prior art. They include, but are not limited to: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethylmethacrylate), poly(butylmethacrylate), poly(isobutylmethacrylate), poly(hexylmethacrylate), poly(isodecylmethacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), poly(vinyl acetate), poly vinyl chloride polystyrene and polyvinylpyrrolidone.

The bioerodable polymers may be used to provide delayed or extended release of nanoparticles comprising a diagnostic, therapeutic, or prophylactic agent.

As demonstrated by the examples, the percent loading is increased by "matching" the hydrophilicity or hydrophobicity of the polymer to the agent to be encapsulated. In some cases, such as PLGA, this can be achieved by

selecting the monomer ratios so that the copolymer is more hydrophilic for hydrophilic drugs or less hydrophilic for hydrophobic drugs. Alternatively, the polymer can be made more hydrophilic, for example, by introducing carboxyl groups onto the polymer. A combination of a hydrophilic drug and  
5 a hydrophobic drug can be encapsulated in microparticles prepared from a blend of a more hydrophilic PLGA and a hydrophobic polymer, such as PLA.

The percent loading of the one or more active agents in the microparticles is from about 1 to 50 weight percent, most preferably 5 to 30  
10 weight percent, more preferably 10 to 20 weight percent.

The preferred polymer is a PLGA copolymer or a blend of PLGA and PLA. The molecular weight of PLGA is from about 10 kD to about 80 kD, more preferably from about 10 kD to about 35 kD. The molecular weight range of PLA is from about 20 to about 30 kDa. The ratio of lactide to  
15 glycolide is from about 75:25 to about 50:50. In one embodiment, the ratio is 50:50.

Exemplary polymers include, but are not limited to, poly(D,L-lactic-co-glycolic acid) (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 10$  kDa, referred to as 502H); poly(D,L-lactic-co-glycolic acid) (PLGA, 50:50  
20 lactic acid to glycolic acid ratio,  $M_n = 25$  kDa, referred to as 503H); poly(D,L-lactic-co-glycolic acid) (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 30$  kDa, referred to as 504H); poly(D,L-lactic-co-glycolic acid) (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 35$  kDa, referred to as 504); and poly(D,L-lactic-co-glycolic acid) (PLGA, 75:25 lactic acid to  
25 glycolic acid ratio,  $M_n = 10$  kDa, referred to as 752).

The microsphere compositions described herein can release an effective amount of one or more active agents suitable for managing elevated IOP, for a period greater than 14 days *in vivo*, preferably greater than 60 days  
30 *in vivo*, more preferably up to 73 days *in vivo*, more preferably greater than 90 days *in vivo*, even more preferably over 100 days *in vivo*, and most preferably greater than 107 days *in vivo*. Release for a period of 90 days or greater corresponds to the typical time period between ophthalmologist visits for patients suffering from glaucoma. The sustained release of drug, in

combination with the ability to administer the drug in a minimally invasive manner, should increase patient compliance.

In other embodiments, the drug is released over a period of greater than 150 days, more preferably over 200 days, more preferably over 250  
5 days.

The examples show that release is influenced by a variety of factors, including molecular weight of the polymer, hydrophilicity or hydrophobicity of the polymer, percent loading of the drug, and/or methods of manufacturing the microspheres. For example, release of prednisolone  
10 acetate was less at a given time period for microspheres prepared from PLGA 502H compared to microspheres prepared from PLGA 503H and a blend of PLGA 502H and PLA. Release of prednisolone acetate is also influenced by the method in which the microspheres are prepared. PLGA 502H microspheres prepared using sonication or homogenization exhibited  
15 greater release than PLGA 502H microspheres prepared without sonication or homogenization or prepared with PEG 1500.

With respect to AG1478, release was greater for PLGA 503H microspheres prepared using an oil-in-water emulsion technique having a drug loading of 5.0% compared to a loading of 2.5%. The microspheres  
20 exhibited a more rapid release of drug over the first 50 days, followed by a more linear release over the next 125 days.

Hydrophilicity of the polymer influences the release profile of AG 1478. For example, release of AG1478 was greater from microspheres prepared from PLGA having carboxylic end groups, such as PLGA 503H  
25 and 504H, compared to the non-carboxylated polymer, PLGA 504, using the oil-in-water cosolvent technique. This is likely due to the fact that AG1478 interacts more strongly with the less hydrophilic polymer PLGA 504, thus slowing the rate of release.

In one embodiment, the microparticles are formed from poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n$  =  
30 10 kDa, referred to as 502H). In another embodiment, the microparticles are formed from poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n$  = 25 kDa, referred to as 503H). In still another

embodiment, the microparticles are formed from poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 30$  kDa, referred to as 504H). In yet another embodiment, the microparticles are formed from poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 35$  kDa, referred to as 504). In still another embodiment, the microparticles are formed from poly(D,L-lactic-co-glycolic acid (PLGA, 75:25 lactic acid to glycolic acid ratio,  $M_n = 10$  kDa, referred to as 752). In yet another embodiment, the microparticles are prepared from a blend of PLGA and PL (referred to as PLGA:PL). The designation "H" means the polymer is terminated with a carboxylic acid group. Microparticles can also be prepared from polylactic acid. As demonstrated by the examples, the H polymers are preferred for loading of hydrophilic drug.

### C. Solvents and Surfactants for preparation of microparticles

Typical solvents are organic solvents such as methylene chloride, which leave low levels of residue that are generally accepted as safe. Suitable water-insoluble solvents include methylene chloride, chloroform, carbon tetrachloride, dichloroethane, ethyl acetate and cyclohexane. Additional solvents include, but are not limited to, alcohols such as methanol (methyl alcohol), ethanol, (ethyl alcohol), 1-propanol (n-propyl alcohol), 2-propanol (isopropyl alcohol), 1-butanol (n-butyl alcohol), 2-butanol (sec-butyl alcohol), 2-methyl-1-propanol (isobutyl alcohol), 2-methyl-2-propanol (t-butyl alcohol), 1-pentanol (n-pentyl alcohol), 3-methyl-1-butanol (isopentyl alcohol), 2,2-dimethyl-1-propanol (neopentyl alcohol), cyclopentanol (cyclopentyl alcohol), 1-hexanol (n-hexanol), cyclohexanol (cyclohexyl alcohol), 1-heptanol (n-heptyl alcohol), 1-octanol (n-octyl alcohol), 1-nonanol (n-nonyl alcohol), 1-decanol (n-decyl alcohol), 2-propen-1-ol (allyl alcohol), phenylmethanol (benzyl alcohol), diphenylmethanol (diphenylcarbinol), triphenylmethanol (triphenylcarbinol), glycerin, phenol, 2-methoxyethanol, 2-ethoxyethanol, 3-ethoxy-1,2-propanediol, Di(ethylene glycol) methyl ether, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 2,3-butanediol, 1,4-butanediol, 1,2-pentanediol, 1,3-pentanediol, 1,4-pentanediol, 1,5-pentanediol, 2,3-pentanediol, 2,4-pentanediol, 2,5-pentanediol, 3,4-pentanediol, 3,5-pentanediol, and combinations thereof.

#### D. Excipients for Administration to the Eye

Considerations in the formulation of the microsphere compositions include, but are not limited to, sterility, preservation, isotonicity, and buffering. The preparation of ophthalmic solutions and suspensions are described in Ansel *et al.*, Pharmaceutical Dosage Forms and Drug Delivery Systems 6<sup>th</sup> Ed., pp. 396-408, Williams and Wilkins (1995). Suspensions are often more advantageous than solution as they typically have increased corneal contact time and thus can provide higher efficacy. Ophthalmic suspensions must contain particles of appropriate chemical characteristics and size to be non-irritating to the eyes. The suspension must also not agglomerate upon administration. Excipients, such as dispersants, can be included to prevent aggregation of the particles.

The microspheres are typically suspended in sterile saline, phosphate buffered saline, or other pharmaceutically acceptable carriers for administration to the eye.

Materials that may be used to formulate or prepare the microparticles include anionic, cationic, amphoteric, and non-ionic surfactants. Anionic surfactants include di-(2 ethylhexyl) sodium sulfosuccinate; non-ionic surfactants include the fatty acids and the esters thereof; surfactants in the amphoteric group include (1) substances classified as simple, conjugated and derived proteins such as the albumins, gelatins, and glycoproteins, and (2) substances contained within the phospholipid classification, for example, lecithin. The amine salts and the quaternary ammonium salts within the cationic group also comprise useful surfactants. Other surfactant compounds useful to form coacervates include polysaccharides and their derivatives, the mucopolysaccharides and the polysorbates and their derivatives. Synthetic polymers that may be used as surfactants include compositions such as polyethylene glycol and polypropylene glycol. Further examples of suitable compounds that may be utilized to prepare coacervate systems include glycoproteins, glycolipids, galactose, gelatins, modified fluid gelatins and galacturonic acid.

Hydrophobic surfactants such as fatty acids and cholesterol are added during processes to improve the resulting distribution of hydrophobic drugs

in hydrophobic polymeric microparticles. Examples of fatty acids include butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, caprylic acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, heptadecylic acid, stearic acid, nonadecanoic acid, arachic acid, isocrotonic acid, undecylenic acid, oleic acid, elaidic acid, sorbic acid, linoleic acid, linolenic acid and arachidonic acid.

In one embodiment, the surfactant polyvinyl alcohol is used to prepare the microparticles. Studies indicate that percent loading is dependent on the nature of surfactant used in the double emulsion methods described above. For example, using the PLGA/PLA blend and 20% timolol maleate by weight, a loading of 18.76  $\mu\text{g}$  of timolol/mg of spheres was obtained when a 5% PVA solution was used. In contrast, the load of timolol was 2.3  $\mu\text{g}$  per mg of spheres when the spheres were prepared using a 5% poly(ethylene-alt-maleic anhydride) (PEMA) solution.

### III. Methods of Making

There are several processes whereby microparticles can be made, including, but not limited to, spray drying, interfacial polymerization, hot melt encapsulation, phase separation encapsulation, spontaneous emulsion, solvent evaporation microencapsulation, solvent removal microencapsulation, coacervation, low temperature microsphere formation, and phase inversion nanoencapsulation ("PIN").

The dispersion of the one or more active agents within the polymer matrix can be enhanced by varying: (1) the solvent used to solvate the polymer; (2) the ratio of the polymer to the solvent; (3) the particle size of the material to be encapsulated; (4) the percentage of the active agent(s) relative to the polymer (e.g., drug loading); and/or the polymer concentration.

The following are representative methods for forming microparticles.

#### Spray Drying

In spray drying, the core material to be encapsulated is dispersed or dissolved in a solution. Typically, the solution is aqueous and preferably the solution includes a polymer. The solution or dispersion is pumped through a

micronizing nozzle driven by a flow of compressed gas, and the resulting aerosol is suspended in a heated cyclone of air, allowing the solvent to evaporate from the microdroplets. The solidified microparticles pass into a second chamber and are trapped in a collection flask.

5           **Interfacial polycondensation**

Interfacial polycondensation is used to microencapsulate a core material in the following manner. One monomer and the core material are dissolved in a solvent. A second monomer is dissolved in a second solvent (typically aqueous) which is immiscible with the first. An emulsion is  
10       formed by suspending the first solution through stirring in the second solution. Once the emulsion is stabilized, an initiator is added to the aqueous phase causing interfacial polymerization at the interface of each droplet of emulsion.

**Hot Melt Encapsulation**

15       In hot melt microencapsulation, the core material (to be encapsulated) is added to molten polymer. This mixture is suspended as molten droplets in a nonsolvent for the polymer (often oil-based) which has been heated to approximately 10°C above the melting point of the polymer. The emulsion is maintained through vigorous stirring while the nonsolvent bath is quickly  
20       cooled below the glass transition of the polymer, causing the molten droplets to solidify and entrap the core material.

**Solvent Evaporation Microencapsulation**

In solvent evaporation microencapsulation, the polymer is typically dissolved in a water immiscible organic solvent and the material to be  
25       encapsulated is added to the polymer solution as a suspension or solution in an organic solvent. An emulsion is formed by adding this suspension or solution to a beaker of vigorously stirring water (often containing a surface active agent, for example, polyethylene glycol or polyvinyl alcohol, to stabilize the emulsion). The organic solvent is evaporated while continuing  
30       to stir. Evaporation results in precipitation of the polymer, forming solid microcapsules containing core material.

The solvent evaporation process can be used to entrap a liquid core material in a polymer such as PLA, PLA/PGA copolymer, or PLA/PCL

copolymer microcapsules. The polymer or copolymer is dissolved in a miscible mixture of solvent and nonsolvent, at a nonsolvent concentration which is immediately below the concentration which would produce phase separation (i.e., cloud point). The liquid core material is added to the  
5 solution while agitating to form an emulsion and disperse the material as droplets. Solvent and nonsolvent are vaporized, with the solvent being vaporized at a faster rate, causing the polymer or copolymer to phase separate and migrate towards the surface of the core material droplets. This phase-separated solution is then transferred into an agitated volume of  
10 nonsolvent, causing any remaining dissolved polymer or copolymer to precipitate and extracting any residual solvent from the formed membrane. The result is a microcapsule composed of polymer or copolymer shell with a core of liquid material.

Solvent evaporation microencapsulation can result in the stabilization  
15 of insoluble or poorly soluble drug particles in a polymeric solution for a period of time ranging from 0.5 hours to several months. .

The stabilization of insoluble or poorly soluble drug particles within the polymeric solution could be critical during scale-up. By stabilizing suspended drug particles within the dispersed phase, said particles can  
20 remain homogeneously dispersed throughout the polymeric solution as well as the resulting polymer matrix that forms during the process of microencapsulation. The homogeneous distribution of drug particles can be achieved in any kind of device, including microparticles, nanoparticles, rods, films, and other device.

25 Solvent evaporation microencapsulation (SEM) has several advantages. SEM allows for the determination of the best polymer-solvent-insoluble particle mixture that will aid in the formation of a homogeneous suspension that can be used to encapsulate the particles. SEM stabilizes the insoluble particles or within the polymeric solution, which will help during  
30 scale-up because one will be able to let suspensions of insoluble particles sit for long periods of time, making the process less time-dependent and less labor intensive. SEM allows for the encapsulated particles to remain suspended within a polymeric solution for up to 30 days, which may increase

the amount of insoluble material entrapped within the polymeric matrix, potentially improving the physical properties of the drug delivery vehicle. SEM allows for the creation of microparticles or nanoparticles that have a more optimized release of the encapsulated material. For example, if the insoluble particle is localized to the surface of the microparticle or nanoparticle, the system will have a large 'burst' effect. In contrast, creating a homogeneous dispersion of the insoluble particle within the polymeric matrix will help to create a system with release kinetics that begin to approach the classical 'zero-ordered' release kinetics that are often perceived as being ideal in the field of drug delivery).

In a preferred embodiment, the microspheres are prepared using an oil-in-water emulsion cosolvent technique, in which an organic cosolvent, such as DMSO, is used to prepare the microspheres.

#### **Solvent Removal Microencapsulation**

In solvent removal microencapsulation, the polymer is typically dissolved in an oil miscible organic solvent and the material to be encapsulated is added to the polymer solution as a suspension or solution in organic solvent. Surface active agents can be added to improve the dispersion of the material to be encapsulated. An emulsion is formed by adding this suspension or solution to vigorously stirring oil, in which the oil is a nonsolvent for the polymer and the polymer/solvent solution is immiscible in the oil. The organic solvent is removed by diffusion into the oil phase while continuing to stir. Solvent removal results in precipitation of the polymer, forming solid microcapsules containing core material.

#### **Phase Separation Microencapsulation**

In phase separation microencapsulation, the material to be encapsulated is dispersed in a polymer solution with stirring. While continually stirring to uniformly suspend the material, a nonsolvent for the polymer is slowly added to the solution to decrease the polymer's solubility. Depending on the solubility of the polymer in the solvent and nonsolvent, the polymer either precipitates or phase separates into a polymer rich and a polymer poor phase. Under proper conditions, the polymer in the polymer

rich phase will migrate to the interface with the continuous phase, encapsulating the core material in a droplet with an outer polymer shell.

### **Spontaneous Emulsification**

Spontaneous emulsification involves solidifying emulsified liquid polymer droplets by changing temperature, evaporating solvent, or adding chemical cross-linking agents. The physical and chemical properties of the encapsulant, and the material to be encapsulated, dictates the suitable methods of encapsulation. Factors such as hydrophobicity, molecular weight, chemical stability, and thermal stability affect encapsulation.

### **Coacervation**

Encapsulation procedures for various substances using coacervation techniques have been described in the prior art, for example, in GB-B-929 406; GB-B-929 401; U.S. Patent nos. 3,266,987; 4,794,000 and 4,460,563. Coacervation is a process involving separation of colloidal solutions into two or more immiscible liquid layers (Ref. Dowben, R. General Physiology, Harper & Row, New York, 1969, pp. 142-143.). Through the process of coacervation compositions comprised of two or more phases and known as coacervates may be produced. The ingredients that comprise the two phase coacervate system are present in both phases; however, the colloid rich phase has a greater concentration of the components than the colloid poor phase.

### **Phase Inversion Nanoencapsulation ("PIN")**

A preferred process is PIN. In PIN, a polymer is dissolved in an effective amount of a solvent. The agent to be encapsulated is also dissolved or dispersed in the effective amount of the solvent. The polymer, the agent and the solvent together form a mixture having a continuous phase, wherein the solvent is the continuous phase. The mixture is introduced into an effective amount of a nonsolvent to cause the spontaneous formation of the microencapsulated product, wherein the solvent and the nonsolvent are miscible. PIN has been described by Mathiowitz *et al.* in U.S. Patent Nos. 6,131,211 and 6,235,224. A hydrophobic agent is dissolved in an effective amount of a first solvent that is free of polymer. The hydrophobic agent and the solvent form a mixture having a continuous phase. A second solvent and then an aqueous solution are introduced into the mixture. The introduction

of the aqueous solution causes precipitation of the hydrophobic agent and produces a composition of micronized hydrophobic agent having an average particle size of 1 micron or less.

An improved process is demonstrated in the examples. The process  
5 uses a mixed solvent including at least one water-insoluble solvent and water that contains a surfactant, such as PVA. The drug is either dissolved or dispersed together with a substance that has a high molecular weight (such as a polymer) into an organic solvent composition, optionally containing non-ionic surfactants of various hydrophilic-lipophilic ratios. The composition is  
10 then introduced into an aqueous solution that contains a surfactant like PVA. The water-insoluble solvent forms an oil phase (inner phase) and is stirred into the aqueous solution as a water phase (outer phase). The O/W emulsion is combined with fresh water that contains surfactant such as PVA and is stirred to help aid the solvent evaporation. The aqueous solution contains an  
15 activator such as polyvinyl alcohol, whereby the oil phase is enclosed as small droplets within the aqueous solution as shells. The proportion of the water-miscible solvent in the oil phase is from 5% to 95%. An important aspect of this improved method is the use of high shear during the initial mixing phase, which is achievable, for example, using sonication for a period  
20 of one hour, with stirring, to uniformly mix in high amounts of drug particles in the polymer liquefied by dissolution or by melting.

#### **Melt-Solvent Evaporation Method**

In the melt-solvent evaporation method, the polymer is heated to a point of sufficient fluidity to allow ease of manipulation (for example,  
25 stirring with a spatula). The temperature required to do this is dependent on the intrinsic properties of the polymer. For example, for crystalline polymers, the temperature will be above the melting point of the polymer. After reaching the desired temperature, the agent is added to the molten polymer and physically mixed while maintaining the temperature. The  
30 molten polymer and agent are mixed until the mixture reaches the maximum level of homogeneity for that particular system. The mixture is allowed to cool to room temperature and harden. This may result in melting of the agent in the polymer and/or dispersion of the agent in the polymer. This can

result in an increase in solubility of the drug when the mixture is dissolved in organic solvent. The process is easy to scale up since it occurs prior to encapsulation. High shear turbines may be used to stir the dispersion, complemented by gradual addition of the agent into the polymer solution  
5 until the desired high loading is achieved. Alternatively the density of the polymer solution may be adjusted to prevent agent settling during stirring.

This method increases microparticle loading as well as uniformity of the resulting microparticles and of the agent within the microparticles. When an agent is formed into microspheres by double-emulsion solvent  
10 evaporation, transfer of the agent from the inner phase to the outer water phase can be prevented. This makes it possible to increase the percentage of agent entrapped within the microspheres, resulting in an increased amount of the drug in the microspheres.

The distribution of the agent in particles can also be made more  
15 uniform. This can improve the release kinetics of the agent. Generally, the agent is dissolved or dispersed together with a substance that has a high molecular weight in an organic solvent composition; with or without non-ionic surfactants of various hydrophilic-lipophilic ratios. The composition is introduced into an aqueous solution that contains a surfactant like PVA. The  
20 water-insoluble solvent forms an oil phase (inner phase) and is stirred into the aqueous solution as a water phase (outer phase). The O/W emulsion is combined with fresh water that contains PVA and is stirred to help aid the solvent evaporation. The aqueous solution contains an activator such as polyvinyl alcohol, whereby the oil phase is enclosed as small droplets within  
25 the aqueous solution as shells.

In one embodiment, the microparticles are formed using a water-in-oil double emulsion (w/o/w) solvent evaporation technique. For example, the one or more active agents are dissolved in deionized water. The polymer is dissolved in an organic solvent or cosolvent. The aqueous and organic  
30 phases are emulsified via vortexing to obtain the desired active agent to polymer ratio (e.g., 10%, 20%, or greater). The emulsion is then added dropwise to an aqueous solution of a surfactant (such as polyvinyl alcohol) and allowed to stir/harden for 3 hours. The resulting microparticles are

collected, such as by centrifugation, washed with deionized water, and dried (e.g., freeze drying). As demonstrated by the examples, the percent loading is increased by “matching” the hydrophilicity or hydrophobicity of the polymer to the agent to be encapsulated. In some cases, such as PLGA, this can be achieved by selecting the monomer ratios so that the copolymer is more hydrophilic. Alternatively, the polymer can be made more hydrophilic, for example, by treating the polymer with a carboxyl solution.

The percent loading of the one or more active agents in the microparticles is from about 1 to about 50 wt%, preferably from about 1 to about 20 wt%, more preferably from about 10 to about 20 wt%. In one embodiment, the percent loading of the drug is 10 wt% or 20 wt%. As demonstrated by the examples, the percent loading is increased by “matching” the hydrophilicity or hydrophobicity of the polymer to the poorly water soluble agent to be encapsulated. Alternatively, the polymer can be made more hydrophilic, for example, by introducing carboxyl groups on to the polymer. A combination of a hydrophilic drug and a hydrophobic drug can be encapsulated in microparticles prepared from a blend of a more hydrophilic PLGA and a hydrophobic polymer, such as PLA.

#### **IV. Methods of Use**

##### **A. Disorders to be Treated**

The microsphere compositions described herein can be administered to treat or prevent diseases or disorders, most preferably of the eye. The dosage of the drug which is released at the site of administration should be bioequivalent as defined by the Food and Drug Administration for the drug when administered in solution, suspension or enterally, in the absence of the microparticles.

##### *Glaucoma*

In one embodiment, the microsphere compositions can be administered to manage (e.g., reduce) IOP in patients needing such treatment, for example, patients suffering from glaucoma. Glaucoma is an ophthalmic disease characterized by the gradual degeneration of retinal ganglion cells (RGCs). RGCs synapse with bipolar cells and transmit visual

inputs to the brain along the optic nerve. Degeneration of these cells leads to gradual vision loss and ultimately blindness if untreated.

The microspheres described herein can also be used to deliver one or more active agents that promote neural regeneration, for example, in patients suffering from glaucoma. AG1478 has been shown to promote neural regeneration. AG1478 is an inhibitor of EGFR.

The neural degeneration in glaucoma is accompanied by extensive remodeling of the extracellular matrix (ECM) including the production of chondroitin sulfate proteoglycans (CSPGs) which inhibit regeneration. Administration of an EGFR inhibitor, such as AG1478, has been shown to lead to a reduction in activated astrocytes, a reduction in the production of CSPGs, and regeneration in the optic nerve.

AG1478 can be co-administered with neural progenitor cells to replace lost retinal ganglion cells (RGCs) along with sustained delivery of AG1478 to promote regeneration. The optic nerve crush model is an excellent first model for studying methods to promote regeneration in glaucoma as well as in the CNS more broadly.

Recent work suggests that the EGFR plays an important role in regulating the production of CSPGs and maintaining specific astrocyte phenotype. EGFR, also known as human EGF receptor (HER) and ErbB1, is a member of a family of transmembrane proteins with tyrosine kinase activity. EGFR has seven different but structurally similar ligands, including EGF, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), and transforming growth factor- $\alpha$  (TGF- $\alpha$ ). EGFR activation controls cell migration, apoptosis, protein secretion and differentiation. Activation of EGFR has been shown to affect the behavior of astrocytes. Ligands of EGFR stimulate astrocyte proliferation and differentiation, induce morphological changes and process formation, and enhance their mobility in vitro. In glaucomatous optic neuropathy, EGFR activation is increased in astrocytes and their activation in the cribriform plates to the damaged optic nerve bundles creates compression, backward bowing, and disorganization of the optic nerve head-characteristic features of glaucomatous eyes with high or normal intraocular pressure.

The EGFR ligands EGF and TGF- $\beta$ 1 greatly increase CSPG production after injury, including neurocan and phosphacan, while upregulation of CSPGs by astrocytes is mediated specifically by the EGFR receptor. In addition, activation of EGFR causes optic nerve astrocytes and brain astrocytes to form cribriform structures with cavernous spaces, similar to the structures that reactive astrocytes form in the glial scar. EGFR also plays a role in astrocyte phenotype. In normal tissue astrocytes are quiescent, producing only a moderate amount of CSPGs and retaining a stellate morphology. After injury, these quiescent astrocytes are activated and become reactive, with elongated processes and increased motility. Astrocytes upregulate and activate EGFR in three different optic nerve injury models: transient eye ischemia, chronic glaucoma, and optic nerve transection. However, application of a commercially available EGFR tyrosine kinase inhibitor, AG1478-a potent, reversible antagonist of EGFR-in a rodent model of glaucomatous optic neuropathy and an optic nerve crush model, reverses this upregulation and activation of astrocytes and increases the survival of RGCs. Further, evidence shows that EGFR activation mediates inhibition of axon regeneration in retinal explants by production of CSPGs and myelin. These studies provide evidence for the idea that modulating the behavior of astrocytes via EGFR signaling is an attractive candidate for treatment of CNS disorders.

### *Uveitis*

Uveitis specifically refers to inflammation of the middle layer of the eye, termed the "uvea" but in common usage may refer to any inflammatory process involving the interior of the eye. Uveitis is estimated to be responsible for approximately 10% of the blindness in the United States. Uveitis requires an urgent referral and thorough examination by an ophthalmologist, along with urgent treatment to control the inflammation.

Uveitis is usually categorized anatomically into anterior, intermediate, posterior and panuveitic forms. Anywhere from two-thirds to 90% of uveitis cases are anterior in location (anterior uveitis), frequently termed iritis - or inflammation of the iris and anterior chamber. This condition can occur as a single episode and subside with proper treatment or

may take on a recurrent or chronic nature. Symptoms include red eye, injected conjunctiva, pain and decreased vision. Signs include dilated ciliary vessels, presence of cells and flare in the anterior chamber, and keratic precipitates ("KP") on the posterior surface of the cornea. Intermediate  
5 uveitis consists of vitritis - inflammatory cells in the vitreous cavity, sometimes with snowbanking, or deposition of inflammatory material on the pars plana. Posterior uveitis is the inflammation of the retina and choroid. Pan-uveitis is the inflammation of all the layers of the uvea.

Myriad conditions can lead to the development of uveitis, including  
10 systemic diseases as well as syndromes confined to the eye. In anterior uveitis, no specific diagnosis is made in approximately one-half of cases. However, anterior uveitis is often one of the syndromes associated with HLA-B27.

The prognosis is generally good for those who receive prompt  
15 diagnosis and treatment, but serious complication (including cataracts, glaucoma, band keratopathy, retinal edema and permanent vision loss) may result if left untreated. The type of uveitis, as well as its severity, duration, and responsiveness to treatment or any associated illnesses, all factor in to the long term prognosis. Uveitis can be treated using steroids, such as  
20 prednisolone, and chemotherapeutic agents, such as methotrexate. In a preferred embodiment, the microspheres are loaded with ofloxacin, prednisolone, or a combination thereof. The microspheres preferably provide release of the one or more active agents for a period of between 14 and 21 days. In another embodiment, the microspheres provide sustained  
25 release of the one or more active agents over a period greater than three weeks, preferably over a period of greater than 49 days, more preferably over a period of two months, most preferably over a period of three months.

#### *Post Surgical Ocular Inflammation/Infection*

Most surgeries involving the eye are followed by ocular inflammation  
30 and/or infection. Topical administration of eye drops containing a combination of a steroid and an antibiotic is the predominant treatment for controlling inflammation as well as infection. Although such eye drops have been shown to be effective, poor compliance and the risk of re-opening of

the stitched wound due to continuous touching of the wound when applying the eye drops remain fundamental issues. Therefore, it is desirable to provide a long-term ocular delivery system that provides release of the active agents for approximately 2-3 weeks in order to minimize dosing frequency, improve patient compliance, reduce side effects due to systemic absorption of the active agents, and keep the stitched wound intact. In one embodiment, microspheres loaded with an antibiotic, a steroid, or combinations thereof are administered to a patient post eye surgery. In a preferred embodiment, the microspheres are loaded with ofloxacin, prednisolone, or a combination thereof. The microspheres preferably provide release of the one or more active agents for a period of between 14 and 21 days. In another embodiment, the microspheres provide sustained release of the one or more active agents over a period greater than three weeks, preferably over a period of greater than 49 days, more preferably over a period of two months, most preferably over a period of three months.

#### *Dry Eye Syndrome*

Dry eye syndrome (Keratoconjunctivitis sicca (KCS)) is one of the most common problems treated by eye physicians. Over ten million Americans suffer from dry eyes. It is usually caused by a problem with the quality of the tear film that lubricates the eyes.

Dry eye syndrome has many causes. One of the most common reasons for dryness is simply the normal aging process. As we grow older, bodies produce less oil – 60% less at age 65 than at age 18. This is more pronounced in women, who tend to have drier skin than men. The oil deficiency also affects the tear film. Without as much oil to seal the watery layer, the tear film evaporates much faster, leaving dry areas on the cornea.

Many other factors, such as hot, dry or windy climates, high altitudes, air-conditioning and cigarette smoke also cause dry eyes. Contact lens wearers may also suffer from dryness because the contacts absorb the tear film, causing proteins to form on the surface of the lens. Certain medications, thyroid conditions, vitamin A deficiency, and diseases such as Parkinson's and Sjogren's can also cause dryness.

Inflammation occurring in response to tears film hypertonicity can be treated by administering the microspheres described herein loaded with poorly water soluble steroids and/or with poorly water soluble immunosuppressants.

5                    *Macular Degeneration*

Macular degeneration is a medical condition predominantly found in elderly adults in which the center of the inner lining of the eye, known as the macula area of the retina, suffers thinning, atrophy, and in some cases, bleeding. This can result in loss of central vision, which entails inability to see fine details, to read, or to recognize faces. According to the American Academy of Ophthalmology, it is the leading cause of central vision loss (blindness) in the United States today for those over the age of fifty years. Although some macular dystrophies that affect younger individuals are sometimes referred to as macular degeneration, the term generally refers to age-related macular degeneration (AMD or ARMD). Macular degeneration can be treated using anti-angiogenesis inhibitors. In one embodiment, the microspheres are loaded with a poorly water soluble anti-angiogenesis inhibitor or growth factor for the treatment of macular degeneration.

**B.        Methods of Administration**

20            The composition can be administered using a variety of techniques well known in the art including, but not limited to, topically and by injection. Suitable dosage forms include but are not limited to, ointments and solutions and suspensions, such as eye drops. In one embodiment, the compositions are administered to the eye by injection. In a preferred embodiment, the  
25    microsphere composition is administered subconjunctivally. "Subconjunctival" or "subconjunctivally", as used herein, refers to administration under the conjunctiva of the eye. The conjunctiva is the clear membrane that coats the inner aspect of the eyelids and the outer surface of the eye. The microsphere compositions are generally administered as  
30    suspensions in a pharmaceutically acceptable carrier, such as phosphate buffered saline (PBS). Subconjunctival administration of drugs, typically by injection, has shown minimal concentration of drug in the plasma and notable concentrations in the eye, including the aqueous humor.

## V. Kits

The kits contain the microsphere compositions and optionally one or more pharmaceutically acceptable excipients or carriers. In one embodiment, the kit can contain the microspheres in dry powder form in one container, such as a vial, jar, or ampule, and the pharmaceutically acceptable carrier in another container, such as a vial, jar, or ampule. The kit typically contains instructions for resuspending the microparticles in the carrier and for administering the composition. If excipients are present, they can be in one or both containers.

In another embodiment, the kit can contain the microparticles resuspended in the carrier and optionally one or more pharmaceutically excipients. The kit would typically contain instructions for administering the composition. The kit can also contain one or more apparatus for preparing and/or administering the compositions, such as a needle and syringe. The container(s) containing the microspheres and the carrier can be packaged using techniques well known in the art. Suitable package materials include, but are not limited to, boxes

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs.

## Examples

### Materials

poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 10$  kDa, referred to as 502H); poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 25$  kDa, referred to as 503H); poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 30$  kDa, referred to as 504H); and poly(D,L-lactic-co-glycolic acid (PLGA, 50:50 lactic acid to glycolic acid ratio,  $M_n = 35$  kDa, referred to as 504) were purchased from Boehringer Ingelheim (Ingelheim, Germany). The designation "H" means the polymer is terminated with a carboxylic acid group.

Poly(D,L-lactic acid) (PLA,  $M_n = \sim 20\text{-}30$  kDa) and poly(vinyl alcohol) (PVA, 88mol% hydrolyzed) were purchased from Polysciences (Warrington, PA, USA).

Methotrexate and Prednisolone acetate were purchased from Sigma (St. Louis, MO, USA).

All other chemicals were A.C.S. reagent grade from Sigma (St. Louis, MO, USA).

#### **Example 1. Preparation of Microspheres**

The microspheres were prepared by phase separation using a single emulsion solvent evaporation method. Two hundred milligrams of the specific polymer was dissolved in 1 mL of dichloromethane (DCM) and 4 mL of trifluoroethanol (TFE). 40 mg of prednisolone acetate or 20 mg of methotrexate was added to the polymer solution and vortexed to obtain the desired drug to polymer ratio: 20% (40 mg drug/200 mg polymer) for prednisolone acetate and 10% (20 mg drug/200 mg polymer) for methotrexate. The organic phase was added dropwise to 200 mL of 5% (w/v) PVA aqueous solution. The aqueous and organic phases were emulsified via stirring/hardening for three hours. Microspheres were collected by centrifugation, washed three times with deionized water, and freeze dried for three days. Blank microspheres were made at the same time under identical conditions except no drug was added.

#### *Particle sizing and scanning electron microscopy*

The volume-weighted mean diameter of the microparticles from each batch can be determined using a Beckman Coulter Multisizer 3 (Fullerton, CA, USA) with a 100  $\mu\text{m}$  diameter aperture based on a sample size of at least 80,000 microspheres. Scanning electron microscopy (SEM) analysis can be used to examine the morphology of the spheres. Microspheres can be sputter coated with gold for 30 seconds at 25 mA and SEM micrographs can be taken on a FEI XL-30 environmental SEM operating at 4 kV.

#### **Example 2. *In vitro* Release Studies of Prednisolone acetate-Loaded Microspheres**

In 1.5 mL eppendorf tubes, 10 mg prednisolone acetate-loaded microspheres or blank microspheres were suspended in 1 mL of phosphate

buffered saline (PBS). Samples were prepared in triplicate. The mixtures were incubated at 37°C on a labquake rotating shaker. At specific time points, 1, 3, 5, and 8 hours and 1, 3, and 7 days, and once every 7 days thereafter until no pellets were present, the mixture was centrifuged and the supernatant and the supernatant was collected. One milliliter of phosphate buffered saline was then added to replace the withdrawn supernatant and the microspheres were resuspended and returned to the shaker. Supernatants for each of the sets of microspheres was frozen and stored at -80°C for subsequent analysis using UV spectroscopy at 245 nm and 303 nm for prednisolone acetate and methotrexate respectively. The concentration of dissolved prednisolone acetate or methotrexate was determined as a function of from their respective standard curves. Plotting prednisolone acetate or methotrexate concentration versus UV absorbance produced a calibration curve for quantification of prednisolone acetate or methotrexate. A linear fit was established from - 0.09 - 25 µg/mL of prednisolone acetate ( $Y = 26.798x + 0.185$  ;  $r^2 = 0.9997$ ) or methotrexate ( $Y = 19.763x + 0.0372$ ;  $r^2 = 1$ ) in phosphate buffered saline.

#### *In vitro release of prednisolone acetate*

Figure 1 shows the *in vitro* release profile for 20% prednisolone acetate-loaded microspheres. Prednisolone acetate was released less rapidly from PLGA 502H microspheres than from microspheres prepared from PLGA 503H or a blend of PLGA 502H and PLA.

Such long sustained release of prednisolone acetate can cause adverse side effects. Therefore the techniques for preparing the microparticles were modified in an attempt to reduce the period of release to 14-21 days. Three different modifications were made during preparation of the microspheres:

- (a) Addition of 20 mg of PEG 1500 to the solution of PLGA 502H;
- (b) Dropwise addition of the organic phase to 4 mL of 5% PVA solution, sonication of the mixture three times for 10 seconds each time, followed by the dropwise addition of the polymer/PVA solution to 196 mL of a 5% PVA solution in water; and

(c) dropwise addition of the organic phase to 4 mL of 5% PVA solution while using a homogenizer at 18000 rpm, followed by dropwise addition of the polymer/PVA solution to 196 mL of 5% PVA.

In procedure (b), the amplitude of the sonicator was varied between 5 batches to observe the effect of amplitude on the release profiles. The amplitude was set at 15%, 30%, and 38%.

Figure 2 shows the *in vitro* release profiles from the microparticles made using the modified procedures describes in methodologies (a)-(c) above. As is shown in the graph, the modified procedures had little effect on 10 the duration of release of prednisolone acetate.

**Example 3. *In Vitro* and *In Vivo* Release Studies of Methotrexate-Loaded Microspheres**

10% methotrexate-loaded microspheres were prepared as described in Example 1. The *in vitro* release study was conducted as described in 15 Example 2. The results are shown in Figure 3. Figure 3 shows that the microspheres were still releasing methotrexate after 7 days from PLGA 503H microspheres.

Figure 4 shows the *in vivo* release profile of methotrexate from PLGA 502H microspheres. The graph shows that an effective amount of 20 methotrexate was released *in vivo* over at least 35 days.

**Example 4. Preparation and *In Vivo* Release Studies of Triamcinolone-Loaded Microparticles**

25 Triamcinolone microspheres were prepared using a water-in-oil-in-water (w/o/w) double emulsion solvent evaporation technique. Figure 5 shows the *in vitro* release profile for triamcinolone-loaded microspheres. The microspheres provide a burst release over the first one to two days followed by linear release of an effective amount of triamcinolone over a period of about 60 days.

**Example 5. Preparation and *In Vitro* Release Studies of Travoprost-Loaded Microspheres**

*Preparation of Microspheres*

100 mg of PLGA 503H was dissolved in a 4:1 ratio of trifluoroethanol (TFE) and dichloromethane (DCM) (2.5 ml total).

Travoprost is a liquid at room temperature. 1 mg of Travoprost was mixed with 50 µl of ethanol. The travoprost solution was added dropwise to the polymer solution and vortexed. The resulting solution was added dropwise to 100 ml of a 5% PVA solution while stirring. The solution was stirred for 3 hours to harden the microspheres, and the microspheres were collected, washed, and lyophilized.

#### *In Vitro Release Studies*

The *in vitro* release profile was measured using the procedure in Example 2. The results are shown in Figure 6. The PLGA 503H-loaded microspheres release drug over a period of at least 7 days in a linear manner. At day 7, approximately 22% of the drug had been released.

#### **Example 6. Preparation and *In Vitro* Release Studies of AG1478-Loaded Microspheres**

##### *Single Emulsion Technique*

5 mg of AG1478 was dissolved in 500 µL of dimethyl sulfoxide (DMSO). 200 mg of PLGA 503H was dissolved in 2 mL of dichloromethane (DCM). The two solutions are vortexed together (setting 5 on the vortex genie). The resulting solution was added to 4 mL of 5% PVA (polyvinyl alcohol) while vortexing (set at 10). The resulting emulsion was added dropwise to 100 mL of 5% PVA and stirred for 4 hours. The spheres were collected by centrifugation and washed three times in MilliQ water. The microspheres were flash frozen and lyophilized. The resulting powder was stored at -20°C until needed.

The *in vitro* release profile for microspheres prepared using the single emulsion technique is shown in Figure 7. The microspheres released an effective amount of AG1478 in a linear manner for at least 126 days.

The rate of release is also influenced the percent loading of drug. Figure 8 shows the release profile from microspheres prepared from PLGA 503H using via oil-in-water emulsion containing 2.5% and 5% AG1478. The microspheres loaded with 5% AG1478 released a greater amount of drug at a given time point than microspheres loaded with 2.5% AG1478.

*Cosolvent Techniques*

5 mg of AG1478 was dissolved in 500  $\mu$ L dimethyl sulfoxide (DMSO). 200 mg of PLGA 503H was dissolved in 2 mL of trifluoroethanol (TFE) and 500  $\mu$ L of dichloromethane (DCM) at a ratio of 1:5 (DCM:TFE).

5 The AG1478 solution was added to the polymer solution while vortexing and the combined solutions were added to 4 mL of 5% PVA (polyvinyl alcohol) while vortexing. The resulting emulsion was added dropwise to 100 mL of 5% PVA and stirred for 3 hours to harden the spheres. The spheres were collected, washed with deionized water, and stored at -20°C.

10 The *in vitro* release profiles for the microspheres prepared by three different processes, s/o/w, o/w, and o/w cosolvent are shown in Figure 9. The use of a co-solvent results in a higher loading of drug and release of a great amount of drug at a given time point compared to microspheres prepared using oil-in-water emulsion techniques. Further, microspheres  
15 prepared using the cosolvent technique released an effective amount of drug over at least 250 days.

Figure 10 shows the release profile of AG1478 from microspheres prepared from PLGA 503H, PLGA 504, and PLGA 504H. The microspheres exhibit similar release profiles over the first 20 days.

20 However, over the next 60 days, release of AG1478 was greater from the microspheres prepared from PLGA 503H and PLGA 504H. This likely due to the fact that the poorly water soluble AG1478 associates more strongly with the less hydrophilic PLGA 504 than with the more hydrophilic PLGA 503H and 504H.

25 Figure 11 shows the release profile of AG1478 from microspheres prepared from PLGA 504 and PLGA 504 H. The microspheres exhibited similar release profiles over the first 20 days. However, over the next 60 days, release was greater from the PLGA 504H microspheres. This likely due to the fact that the poorly water soluble AG1478 associates more  
30 strongly with the less hydrophilic PLGA 504 than with the more hydrophilic PLGA 504H.

We claim:

1. A biodegradable polymeric microparticulate pharmaceutical composition for delivery of a poorly water-soluble active agent,  
wherein the biodegradable polymeric microparticles comprise a biodegradable polymer and between one and 50 weight percent active agent dispersed therein,  
wherein the hydrophobicity of the polymer forming the microparticles corresponds to the hydrophobicity of the active agent to be released, the hydrophobicity and charge of the polymer are selected to optimize percent loading of the active agent relative to a particle where the hydrophobicity is not optimized, and the molecular weight and monomer composition result in release of active agent over a period of time of at least 60 days equivalent to administration of the active agent via the same route of administration in the absence of microparticles.
2. The composition of claim 1, wherein the microparticles are formed from one or more polymers selected from the group consisting of poly(lactic-co-glycolic) acid (PLGA), a blend of PLGA and polylactic acid (PLA).
3. The composition of claim 1, wherein the one or more active agents are selected from the group consisting of active agents that lower intraocular pressure, antibiotics, steroids, growth factors, chemotherapeutic agents, and combinations thereof.
4. The composition of claim 3, wherein the active agent that lowers intraocular pressures is selected from the group consisting travoprost, bimatoprost, latanoprost, and combinations thereof.
5. The composition of claim 3, wherein the antibiotic is selected from the group consisting of cephaloridine, cefamandole, cefamandole nafate, cefazolin, cefoxitin, cephacetrile sodium, cephalixin, cephaloglycin, cephalosporin C, cephalothin, cefcillin, cephamycins, cephapirin sodium, cephradine, penicillin BT, penicillin N, penicillin O, phenethicillin potassium, pivampic ulin, amoxicillin, ampicillin, cefatoxin, cefotaxime, moxalactam, cefoperazone, cefsulodin, ceflizoxime, ceforanide, cefiaxone, ceftazidime, thienamycin, N-formimidoyl thienamycin, clavulanic acid,

penemcarboxylic acid, piperacillin, sulbactam, cyclosporins, and combinations thereof.

6. The composition of claim 3, wherein the active agent is the growth factor inhibitor AG1478.

7. The composition of claim 3, wherein the steroid is selected from the group consisting of prednisolone acetate, triamcinolone, prednisolone, hydrocortisone, hydrocortisone acetate, hydrocortisone valerate, vidarabine, fluorometholone, fluocinolone acetonide, triamcinolone acetonide, dexamethasone, dexamethasone acetate, and combinations thereof.

8. The composition of claim 4, wherein the one or more active agents is travoprost or a pharmaceutically acceptable salt thereof.

9. The composition of any one of claims 1-8, wherein the percent loading of active agent is between 5 and 30 weight percent.

10. The composition of claim 1, wherein the polymer is PLGA having a molecular weight in the range from about 10 kD to about 80 kD.

11. The composition of claim 1 wherein the period of release is 90 days or greater *in vivo*.

12. The composition of claim 1 wherein the polymer is treated to increase the number of carboxyl groups.

13. The composition of claim 12 wherein the polymer is PLGA.

14. The composition of claim 1, wherein the composition further comprises one or more pharmaceutically acceptable excipients.

15. A method for administering a poorly water soluble active agent, comprising administering to a site in an individual a biodegradable polymeric microparticulate pharmaceutical composition for delivery of the active agent,

wherein the biodegradable polymeric microparticles comprise a biodegradable polymer and between one and 50 weight percent active agent dispersed therein,

wherein the hydrophobicity of the polymer forming the microparticles corresponds to the hydrophobicity of the active agent to be released, the hydrophobic and charge of the polymer are selected to optimize percent loading of the active agent relative to a particle where the

hydrophobicity is not optimized, and the molecular weight and monomer composition result in release of active agent over a period of time of at least 60 days equivalent to administration of the active agent via the same route of administration in the absence of microparticles

16. The method of claim 15 for delivering drug to the eye, comprising administering the microparticles to the eye.

17. The method of claim 15, wherein the microparticles are formed from poly(lactic-co-glycolic) acid (PLGA) or a blend of PLGA and polylactic acid (PLA).

18. The method of claim 15, wherein the one or more active agents are selected from the group consisting of active agents that lower intraocular pressure, antibiotics, steroids, growth factors, chemotherapeutic agents, and combinations thereof.

19. The method of claim 18, wherein the active agent that lowers intraocular pressures is selected from the group consisting of travoprost, bimatoprost, latanoprost, and combinations thereof.

20. The method of claim 18, wherein the antibiotic is selected from the group consisting of cephaloridine, cefamandole, cefamandole nafate, cefazolin, cefoxitin, cephacetrile sodium, cephalexin, cephaloglycin, cephalosporin C, cephalothin, cefaclor, cephalexins, cephalixin sodium, cephadrine, penicillin BT, penicillin N, penicillin O, phenethicillin potassium, pivampic ulin, amoxicillin, ampicillin, cefatoxin, cefotaxime, moxalactam, cefoperazone, cefsulodin, ceflizoxime, ceforanide, cefiaxone, ceftazidime, thienamycin, N-formimidoyl thienamycin, clavulanic acid, penemcarboxylic acid, piperacillin, sulbactam, cyclosporins, and combinations thereof.

21. The method of claim 18, wherein the active agent is the growth factor inhibitor AG1478.

22. The method of claim 18, wherein the steroid is selected from the group consisting of prednisolone acetate, triamcinolone, prednisolone, hydrocortisone, hydrocortisone acetate, hydrocortisone valerate, vidarabine, fluorometholone, fluocinolone acetonide, triamcinolone acetonide, dexamethasone, dexamethasone acetate, and combinations thereof.

23. The method of claim 19, wherein the one or more active agents is travoprost or a pharmaceutically acceptable salt thereof.
24. The composition of claim 15 wherein the polymer is a carboxylated PLGA and the percent loading of drug is from 5 to 30% by weight
25. The method of claim 24, wherein the polymer is a PLGA with a molecular weight from about 10 kD to about 80 kD.
26. The method of claim 15, wherein the composition is administered by injection.
27. The method of claim 26, wherein the composition is administered subconjunctivally.
28. A kit comprising the compositions of any one of claims 1-14.
29. The kit of claim 28, wherein the kit further comprises instructions for preparing and/or administering the composition, optionally comprising a needle and syringe for administering the composition.
30. The kit of claim 28, wherein the microparticles and the carrier are stored in the same container or in separate containers.
31. The kit of claim 30, wherein the container is selected from the group consisting of sterile vials, jars, sealed ampules, and combinations thereof.

1/6

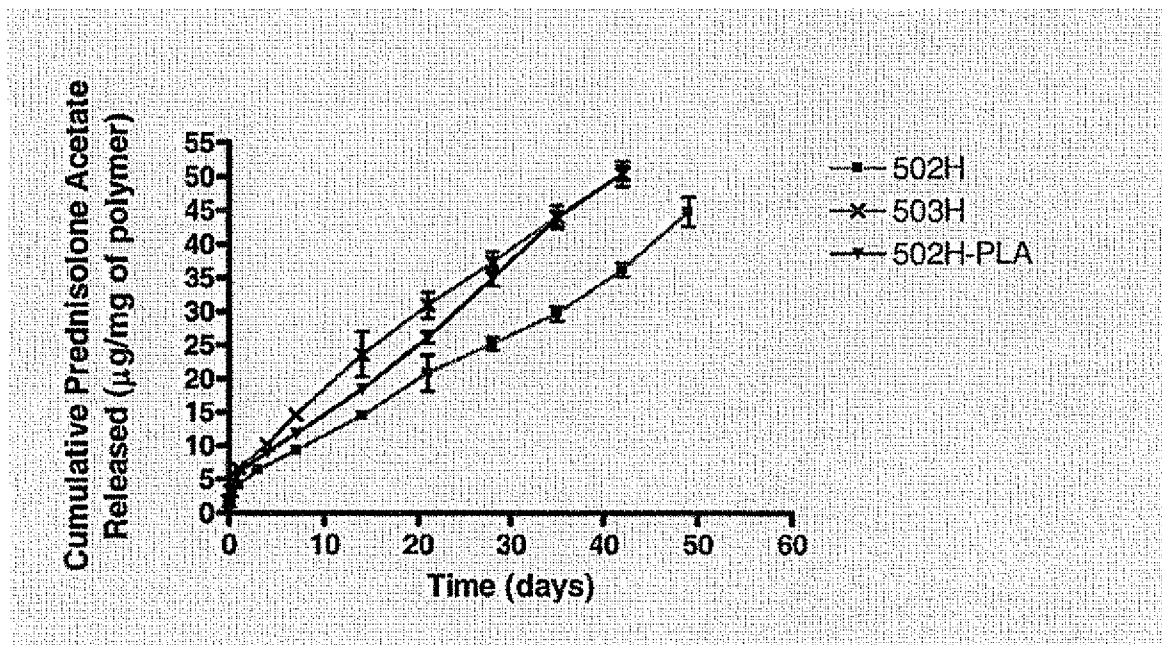


Figure 1

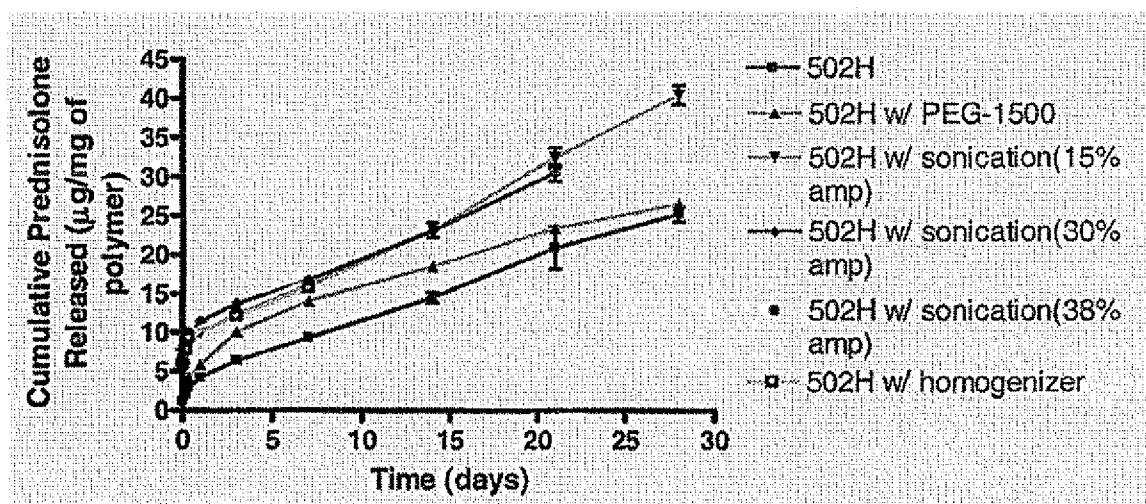


Figure 2

2/6

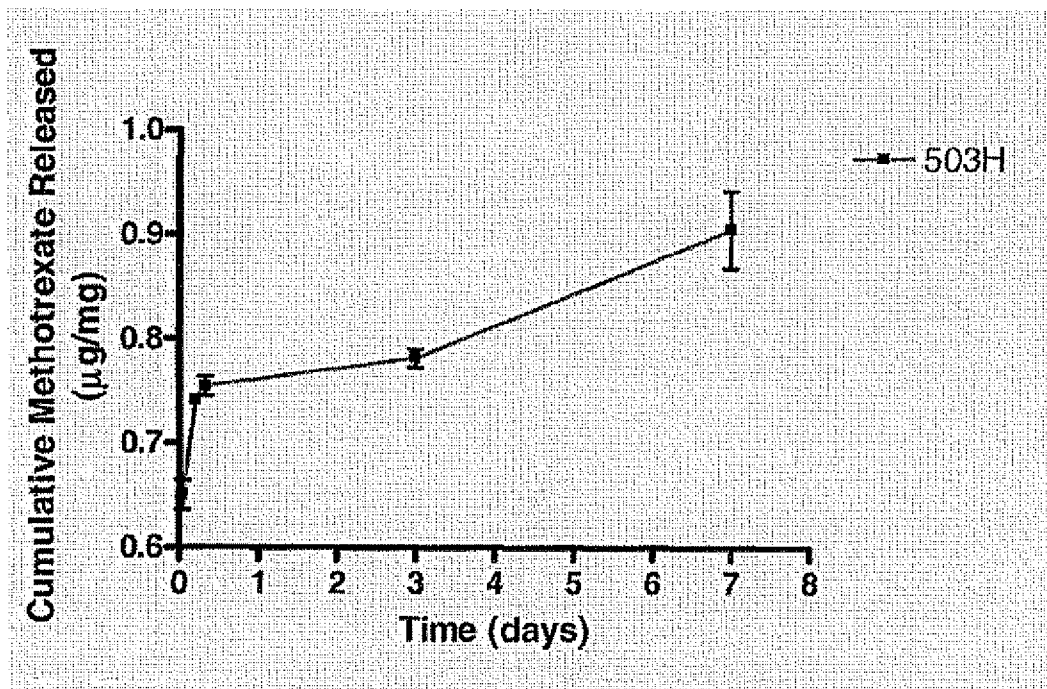


Figure 3

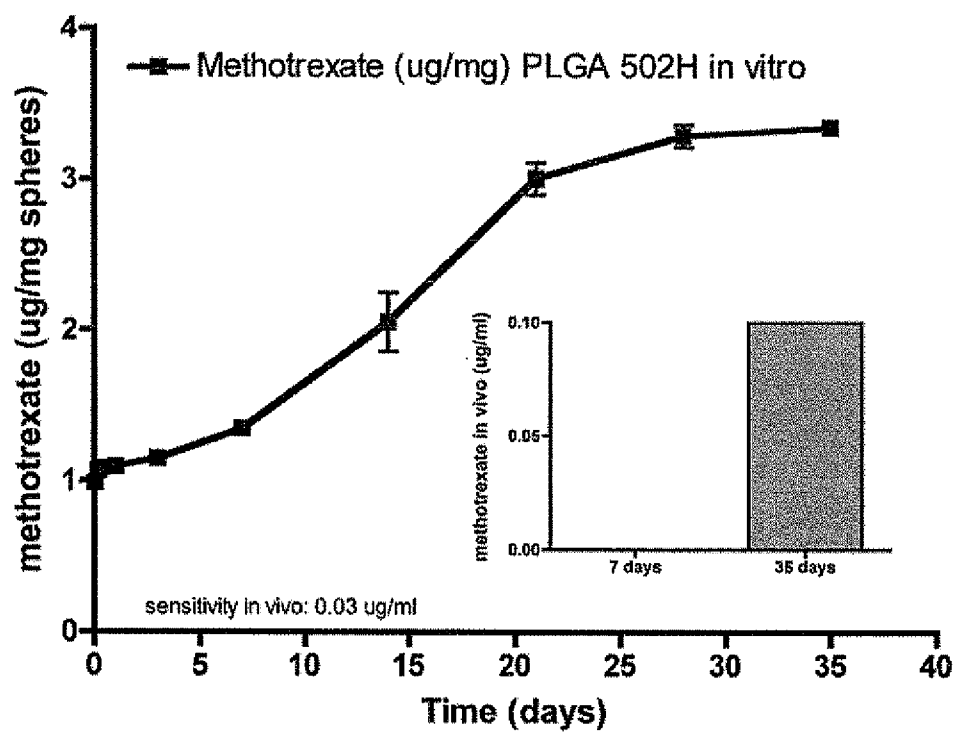


Figure 4

3/6

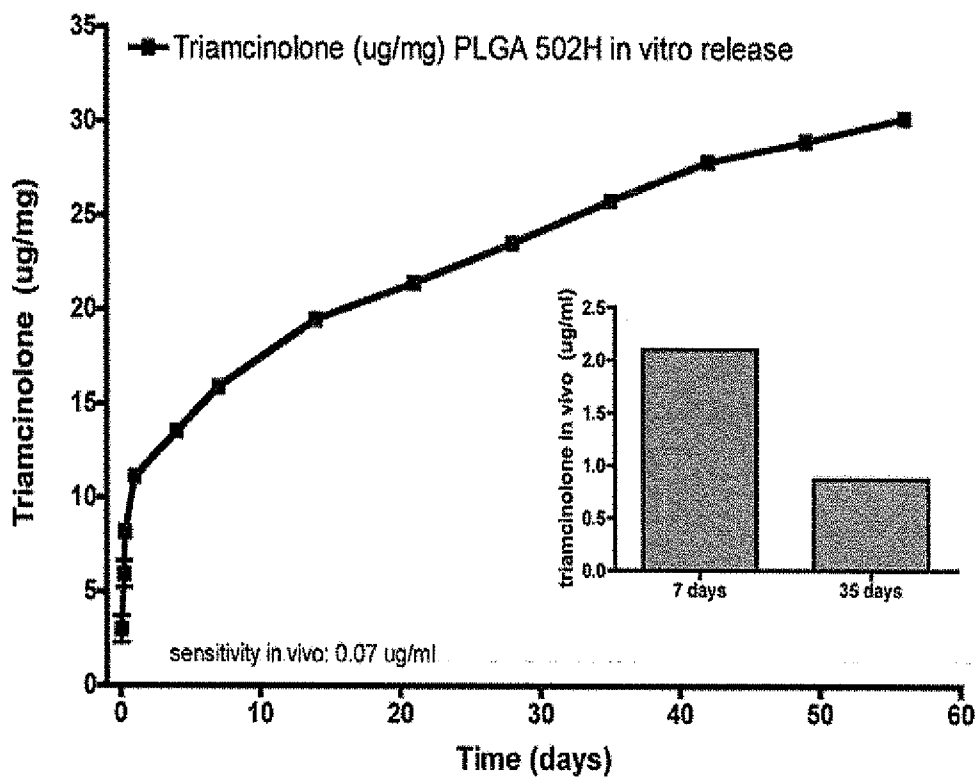


Figure 5

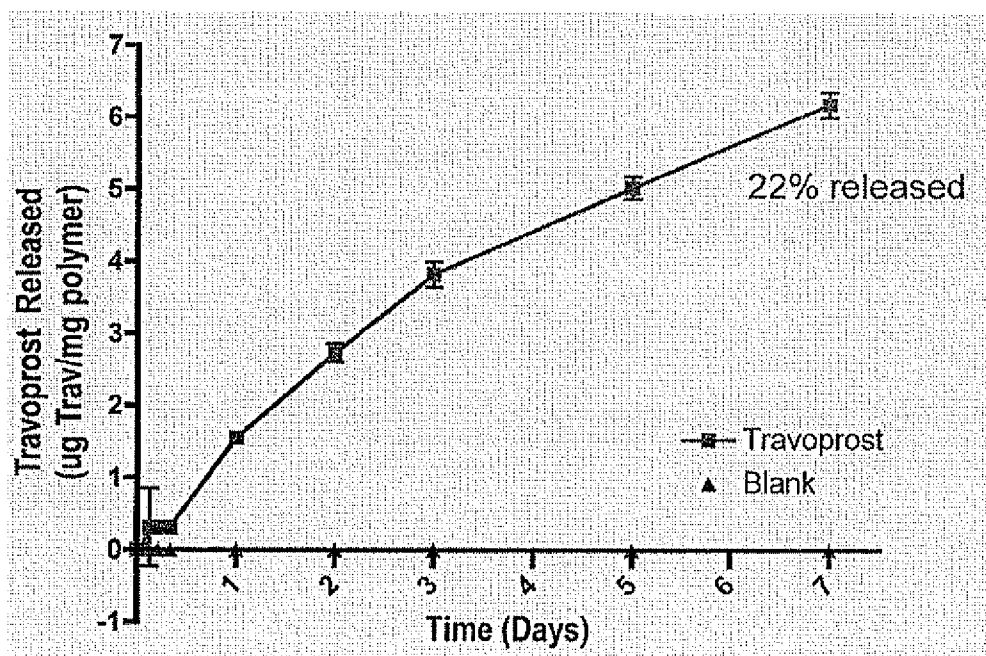


Figure 6

4/6

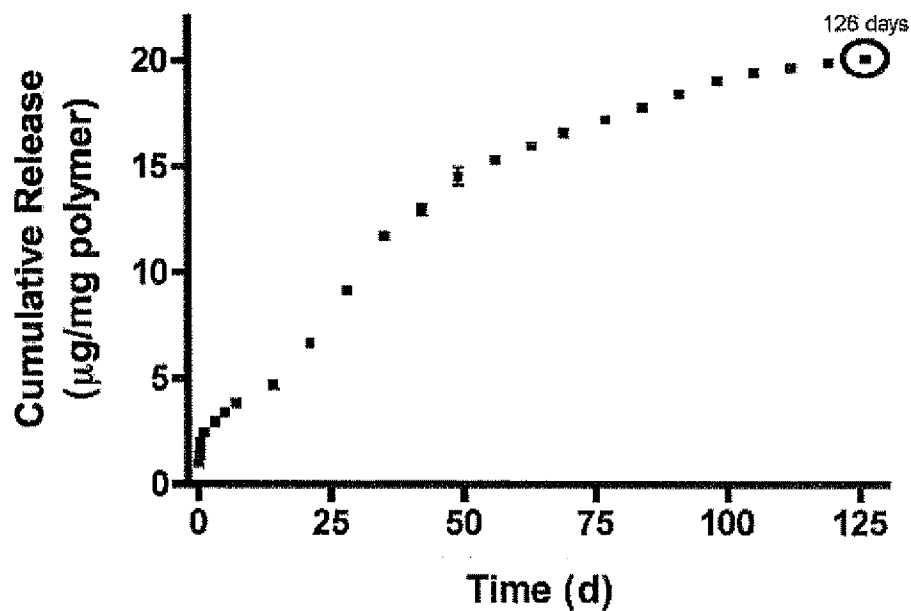
**AG1478 encapsulated in PLGA 503H**

Figure 7

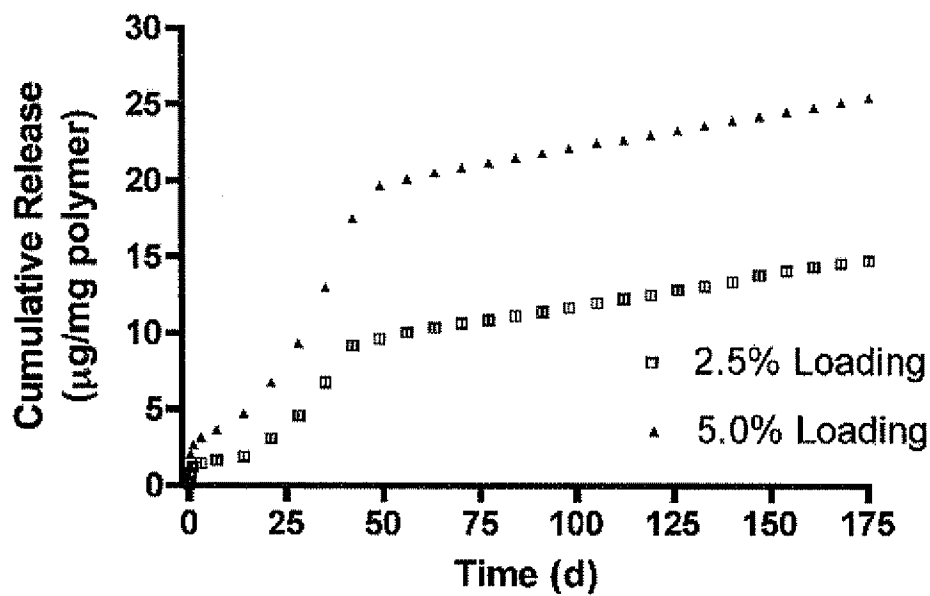
**AG1478 Release  
(o/w, 503H)**

Figure 8

5/6

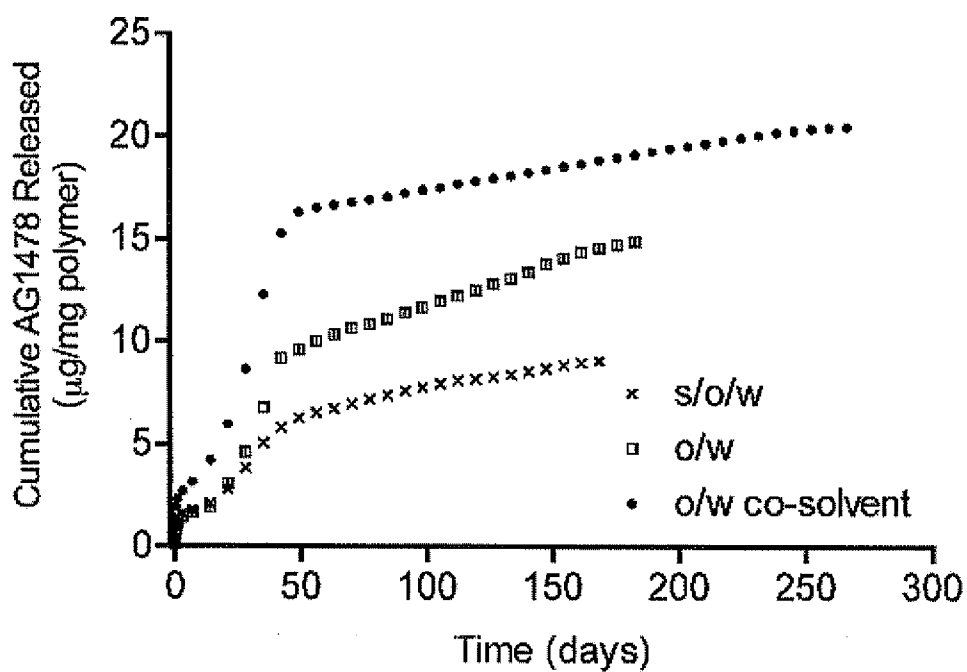


Figure 9

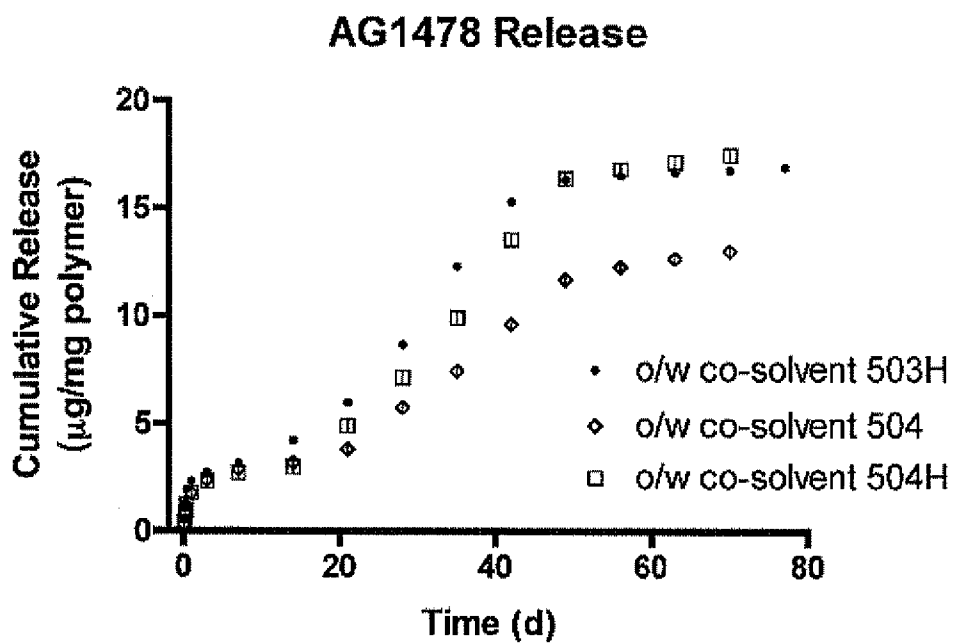


Figure 10

6/6

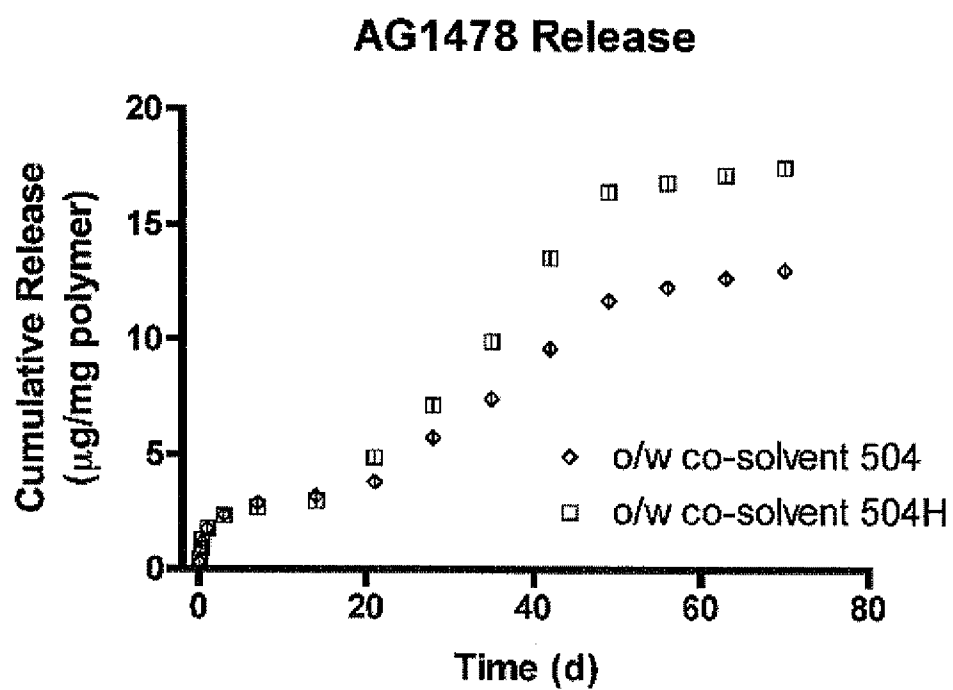


Figure 11

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2009/044732

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K9/00 A61K9/16 A61K31/58 A61K31/57 A61K31/519

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 practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                           | Relevant to claim No.  |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| X         | WO 2005/107707 A (ALLERGAN INC [US];<br>HUGHES PATRICK M [US]; OLEJNIK OREST [US];<br>HUANG GL) 17 November 2005 (2005-11-17)<br>figures 23-28<br>example 12 | 1-5,<br>7-20,<br>22-31 |
| X         | WO 2005/107706 A (ALLERGAN INC [US];<br>HUGHES PATRICK M [US]; MALONE THOMAS [US];<br>DEVRIES) 17 November 2005 (2005-11-17)<br>figures 3-21<br>example 3    | 1-5,<br>7-20,<br>22-31 |
| X         | WO 2008/019265 A (ALLERGAN INC [US];<br>TROGDEN JOHN T [US]; LYONS ROBERT T [US];<br>CHANG JAM) 14 February 2008 (2008-02-14)<br>example 2<br>example 6      | 1-5,<br>7-20,<br>22-31 |

☒ 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 document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*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.
- \*G\* document member of the same patent family

Date of the actual completion of the international search

10 August 2009

Date of mailing of the international search report

19/08/2009

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Schifferer, Hermann

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2009/044732

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                               | Relevant to claim No.  |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| X         | US 2004/137059 A1 (NIVAGGIOLI THIERRY [US]<br>ET AL) 15 July 2004 (2004-07-15)<br><br>examples 4-7<br>paragraph [0030] - paragraph [0095]<br>-----                                                                                                                                                                                                                                                                                                                                               | 1-5,<br>7-20,<br>22-31 |
| X         | XU JIANFENG ET AL: "Inhibitory efficacy<br>of intravitreal dexamethasone<br>acetate-loaded PLGA nanoparticles on<br>choroidal neovascularization in a<br>laser-induced rat model"<br>JOURNAL OF OCULAR PHARMACOLOGY AND<br>THERAPEUTICS, MARY ANN LIEBERT, INC., NEW<br>YORK, NY, US,<br>vol. 23, no. 6,<br>1 December 2007 (2007-12-01), pages<br>527-539, XP009095714<br>ISSN: 1080-7683<br>page 529, left-hand column, line 3 - page<br>533, left-hand column, line 8<br>figures 4,5<br>----- | 1-5,<br>7-20,<br>22-31 |
| X,P       | EP 1 944 032 A (ALLERGAN INC [US])<br>16 July 2008 (2008-07-16)<br><br>examples 3,4<br>-----                                                                                                                                                                                                                                                                                                                                                                                                     | 1-5,<br>7-20,<br>22-31 |

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2009/044732

### Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 15-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable-claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/044732

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| WO 2005107707 A                           | 17-11-2005          | AR 056254 A1               | 03-10-2007          |
|                                           |                     | AU 2005240078 A1           | 17-11-2005          |
|                                           |                     | BR PI0509459 A             | 04-09-2007          |
|                                           |                     | CA 2565285 A1              | 17-11-2005          |
|                                           |                     | EP 1765284 A1              | 28-03-2007          |
|                                           |                     | JP 2007535563 T            | 06-12-2007          |
|                                           |                     | US 2005271705 A1           | 08-12-2005          |
|                                           |                     | US 2009163550 A1           | 25-06-2009          |
| WO 2005107706 A                           | 17-11-2005          | AU 2005240093 A1           | 17-11-2005          |
|                                           |                     | AU 2005244780 A1           | 01-12-2005          |
|                                           |                     | BR PI0509461 A             | 04-09-2007          |
|                                           |                     | CA 2565182 A1              | 01-12-2005          |
|                                           |                     | EP 1742610 A1              | 17-01-2007          |
|                                           |                     | EP 1750665 A1              | 14-02-2007          |
|                                           |                     | JP 2007535556 T            | 06-12-2007          |
|                                           |                     | JP 2007535567 T            | 06-12-2007          |
|                                           |                     | US 2008260803 A1           | 23-10-2008          |
|                                           |                     | US 2008254096 A1           | 16-10-2008          |
|                                           |                     | US 2005244477 A1           | 03-11-2005          |
|                                           |                     | US 2005244470 A1           | 03-11-2005          |
|                                           |                     | WO 2005112884 A1           | 01-12-2005          |
| WO 2008019265 A                           | 14-02-2008          | US 2008033351 A1           | 07-02-2008          |
|                                           |                     | US 2008097335 A1           | 24-04-2008          |
|                                           |                     | WO 2008019267 A2           | 14-02-2008          |
| US 2004137059 A1                          | 15-07-2004          | AU 2004204744 A1           | 29-07-2004          |
|                                           |                     | BR PI0406692 A             | 20-12-2005          |
|                                           |                     | CA 2512315 A1              | 29-07-2004          |
|                                           |                     | CN 1735405 A               | 15-02-2006          |
|                                           |                     | CN 101259280 A             | 10-09-2008          |
|                                           |                     | EP 1581193 A2              | 05-10-2005          |
|                                           |                     | JP 4223040 B2              | 12-02-2009          |
|                                           |                     | JP 2006525953 T            | 16-11-2006          |
|                                           |                     | KR 20050099008 A           | 12-10-2005          |
|                                           |                     | MX PA05007342 A            | 30-09-2005          |
|                                           |                     | NZ 540451 A                | 29-02-2008          |
|                                           |                     | WO 2004062649 A2           | 29-07-2004          |
| EP 1944032                                | A                   | 16-07-2008                 | NONE                |