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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 September 2021 (16.09.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/183781 A1

(51) International Patent Classification:
A61K 9/08 (2006.01) *A61K 47/34* (2017.01)
A61K 9/19 (2006.01) *A61K 31/436* (2006.01)

(21) International Application Number:
PCT/US2021/021939

(22) International Filing Date:
11 March 2021 (11.03.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/988,347 11 March 2020 (11.03.2020) US

(71) Applicant: **SELECTA BIOSCIENCES, INC.** [US/US]; 65 Grove Street, Watertown, MA 02472 (US).

(72) Inventor: **JOHNSTON, Lloyd**; 24 Oakley Road, Belmont, MA 02478 (US).

(74) Agent: **VON REYN, Jessica, A.** et al.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (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, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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

Published:
— *with international search report (Art. 21(3))*

(54) **Title:** METHODS AND COMPOSITIONS RELATED TO SYNTHETIC NANOCARRIERS

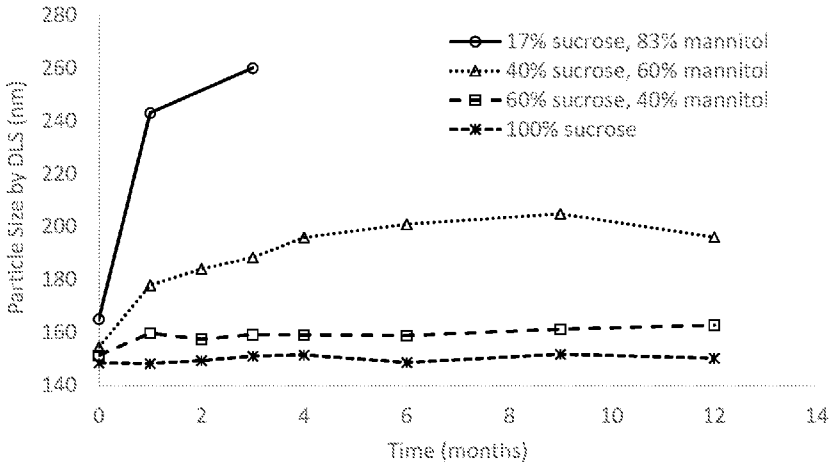


FIG. 1

(57) **Abstract:** This invention relates to synthetic nanocarriers, and related compositions and methods, including wherein the synthetic nanocarrier compositions can be lyophilized, are in a lyophilized form, or a reconstituted composition thereof.

WO 2021/183781 A1

METHODS AND COMPOSITIONS RELATED TO SYNTHETIC NANOCARRIERS

RELATED APPLICATION

This application claims the benefit of priority under 35 U.S.C. § 119 of U.S. Provisional Application No. 62/988,347, filed March 11, 2020, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

This invention relates to synthetic nanocarriers, and related compositions and methods, including wherein the synthetic nanocarrier compositions can be lyophilized, are in a lyophilized form, or a reconstituted composition thereof. In some embodiments, the synthetic nanocarriers comprise a hydrophobic carrier material, such as a hydrophobic polyester carrier material, and an immunosuppressant, such as a rapalog, such as rapamycin. The immunosuppressant, such as a rapalog, such as rapamycin, may be in a stable, super-saturated amount. In some embodiments, the synthetic nanocarriers are initially sterile filterable. In some embodiments, the synthetic nanocarriers also comprise a non-ionic surfactant with a hydrophilic-lipophilic balance (HLB) value less than or equal to 10.

SUMMARY OF THE INVENTION

Provided herein are compositions comprising synthetic nanocarriers that preferably can be lyophilized, are in a lyophilized form, or a reconstituted composition thereof. In some embodiments, upon reconstitution, the synthetic nanocarrier compositions can be used to inhibit or reduce immune responses, such as to an antigen and/or result in other beneficial *in vivo* effects.

In one aspect, provided herein are synthetic nanocarriers (which may be any one of the synthetic nanocarriers described herein) that can be lyophilized, are in a lyophilized form, or are in a reconstituted composition of a lyophilized form. It was found that different components can help facilitate lyophilization, reduce aggregation (e.g., following reconstitution), and/or allow for long-term storage at 2-8 °C (e.g., following lyophilization). In some embodiments of any one of the synthetic nanocarrier compositions or methods provided herein, the duration of long-term storage is 36 months or more. Thus, in some embodiments of any one of the synthetic nanocarrier compositions or methods provided herein, the synthetic nanocarrier compositions can further comprise one or more of such components.

In one embodiment of any one of the compositions provided herein, one or more of such components comprises a lyoprotectant. In one embodiment of any one of such compositions, the lyoprotectant comprises sucrose, trehalose, mannitol, or a sucrose/mannitol mixture. In one embodiment of any one of such compositions, the lyoprotectant comprises sucrose. In one embodiment of any one of such compositions, the sucrose is at a concentration ranging from 4 to 9.6 wt%.

Also found was that the use of surfactants may lead to solubilization of an immunosuppressant, such as rapamycin, and/or disruption of the synthetic nanocarriers. Thus, in one aspect, synthetic nanocarrier compositions (which may be any one of the synthetic nanocarriers described herein) that do not comprise such a surfactant are also provided. In one embodiment of any one of such synthetic nanocarrier compositions, the synthetic nanocarrier composition does not comprise a phosphate buffer or phosphate surfactant. In another embodiment of any one of such synthetic nanocarrier compositions, the synthetic nanocarrier composition comprises a non-phosphate buffer or non-phosphate surfactant.

Also found, is the benefit, in some embodiments, of buffer components that help maintain neutral or near-neutral pH. Thus, in one aspect synthetic nanocarrier compositions (which may be any one of the synthetic nanocarriers described herein) that comprise a buffer and/or that are at a neutral or near-neutral pH are also provided. In one embodiment of any one of such synthetic nanocarrier compositions, the buffer is a non-phosphate buffer. In one embodiment of any one of such synthetic nanocarrier compositions, the buffer is a Tris buffer. In one embodiment of any one of such synthetic nanocarrier compositions, the Tris buffer is at a concentration of 10 mM. In one embodiment of any one of such synthetic nanocarrier compositions, tromethamine (tris(hydroxymethyl)aminomethane) and Tris hydrochloride (Tris HCl) are components of the Tris buffer. In one embodiment of any one of such synthetic nanocarrier compositions, the Tris buffer comprises tromethamine at a concentration of 1.3 mM and Tris HCL at a concentration of 8.7 mM.

In one embodiment of any one of the synthetic nanocarrier compositions provided herein, the synthetic nanocarrier composition further comprises a lyoprotectant, such as sucrose (e.g., at a concentration of 4-9.6 wt%), and a buffer, such as a non-phosphate buffer or Tris buffer (e.g., 10 mM). In one embodiment of any one of such compositions, the Tris buffer comprises tromethamine (tris(hydroxymethyl)aminomethane) (e.g., 1.3 mM) and Tris

hydrochloride (Tris HCl) (e.g., 8.7 mM). The lyoprotectant and buffer may be any one of the lyoprotectants or buffers provided herein, respectively.

In one embodiment of any one of any one of the synthetic nanocarrier compositions or methods provided herein, the composition comprises 10-20 wt% synthetic nanocarrier, hydrophobic carrier material, and immunosuppressant; 80-90 wt% sucrose, 0.1-5 wt% tromethamine; and 0.1-5 wt% Tris HCL.

In one embodiment of any one of the synthetic nanocarrier compositions provided herein, the composition is at a pH of 7.3 (e.g., at 25˚C).

In one embodiment of any one of the synthetic nanocarrier compositions provided herein, the immunosuppressant, such as a rapalog, such as rapamycin is at a concentration of 2 mg/mL immunosuppressant.

In one embodiment of any one of the synthetic nanocarrier compositions provided herein, the composition is in a 20 mL vial.

In one embodiment of any one of the compositions and methods provided herein, the composition of synthetic nanocarriers is in a lyophilized form, such as a lyophilized powder form. In one embodiment of any one of the compositions and methods provided herein, the composition of synthetic nanocarriers is a composition to be lyophilized, such as to a lyophilized powder form. In one embodiment of any one of the compositions and methods provided herein, the composition of synthetic nanocarriers is a reconstituted composition of the lyophilized form. In one embodiment of any one of the compositions and methods provided herein, the composition of synthetic nanocarriers is stored in a glass vial. In one embodiment, of any one of the compositions or methods provided herein, the glass vial is a 20 mL glass vial. In one embodiment of any one of the compositions and methods provided herein, the composition of synthetic nanocarriers is stored at 2 to 8˚C.

In one embodiment of any one of the compositions and methods provided herein, the hydrophobic carrier material, such as hydrophobic polyester carrier material, comprises PLA, PLG, PLGA or polycaprolactone. In one embodiment of any one of the compositions and methods provided herein, the hydrophobic carrier material, such as hydrophobic polyester carrier material, further comprises PLA-PEG, PLGA-PEG or PCL-PEG.

In one embodiment of any one of the compositions and methods provided herein, the amount of the hydrophobic carrier material, such as hydrophobic polyester carrier material, in

the synthetic nanocarriers is 5-95 weight% hydrophobic carrier material/total solids. In one embodiment of any one of the compositions and methods provided herein, the amount of hydrophobic carrier material, such as hydrophobic polyester carrier material, in the synthetic nanocarriers is 60-95 weight% hydrophobic carrier material/total solids.

In one embodiment of any one of the compositions or methods provided herein, the rapalog, such as rapamycin, is in a stable, super-saturated amount that is less than 50 weight% based on the weight of rapalog, such as rapamycin, relative to the weight of hydrophobic carrier material, such as a hydrophobic polyester carrier material. In one embodiment of any one of the compositions and methods provided herein, the rapalog, such as rapamycin, is present in a stable, super-saturated amount that is less than 45 weight%, less than 40 weight%, less than 35 weight%, less than 30 weight%, less than 25 weight%, less than 20 weight%, less than 15 weight% or less than 10 weight%. In one embodiment of any one of the compositions and methods provided herein, the rapalog, such as rapamycin is present in a stable, super-saturated amount that is greater than 7 weight%.

In one embodiment of any one of the compositions or methods provided herein, the amount of rapalog is ≥ 6 but ≤ 50 weight% rapalog/hydrophobic carrier material. In one embodiment of any one of the compositions or methods provided herein, the amount of rapalog is ≥ 7 but ≤ 30 weight% rapalog/hydrophobic carrier material. In one embodiment of any one of the compositions or methods provided herein, the amount of rapalog is ≥ 8 but ≤ 24 weight% rapalog/hydrophobic carrier material.

In one embodiment of any one of the compositions or methods provided herein, the rapalog is encapsulated in the synthetic nanocarriers.

In one embodiment of any one of the compositions or methods provided herein, the rapalog is rapamycin.

In one embodiment of any one of the compositions and methods provided herein, the composition is initially sterile filterable through a 0.22 μm filter.

In one embodiment of any one of the compositions and methods provided herein, the synthetic nanocarriers further comprise a non-ionic surfactant with HLB value less than or equal to 10. In one embodiment of any one of the compositions and methods provided herein, the amount of non-ionic surfactant with HLB value less than or equal to 10 is ≥ 0.01 but ≤ 20 weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material.

In one embodiment of any one of the compositions and methods provided herein, the non-ionic surfactant with HLB value less than or equal to 10 is encapsulated in the synthetic nanocarriers, present on the surface of the synthetic nanocarriers, or both. In one embodiment of any one of the compositions and methods provided herein, the amount of non-ionic surfactant with HLB value less than or equal to 10 is $\geq$ 0.1 but $\leq$ 15 weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material. In one embodiment of any one of the compositions and methods provided herein, the amount of non-ionic surfactant with HLB value less than or equal to 10 is $\geq$ 1 but $\leq$ 13 weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material. In one embodiment of any one of the compositions and methods provided herein, the amount of non-ionic surfactant with HLB value less than or equal to 10 is $\geq$ 1 but $\leq$ 9 weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material.

In one embodiment of any one of the compositions and methods provided herein, the non-ionic surfactant with HLB value less than or equal to 10 comprises a sorbitan ester, fatty alcohol, fatty acid ester, ethoxylated fatty alcohol, poloxamer, fatty acid, cholesterol, cholesterol derivative, or bile acid or salt. In one embodiment of any one of the compositions and methods provided herein, the non-ionic surfactant with HLB value less than or equal to 10 comprises SPAN 40, SPAN 20, oleyl alcohol, stearyl alcohol, isopropyl palmitate, glycerol monostearate, BRIJ 52, BRIJ 93, Pluronic P-123, Pluronic L-31, palmitic acid, dodecanoic acid, glyceryl tripalmitate or glyceryl trilinoleate. In one embodiment of any one of the compositions and methods provided herein, the non-ionic surfactant with HLB value less than or equal to 10 is SPAN 40.

In one embodiment of any one of the compositions or methods provided herein, the weights are the recipe weights of the materials that are combined during the formulation of the synthetic nanocarriers. In one embodiment of any one of the compositions or methods provided herein, the weights are the weights of the materials in the resulting synthetic nanocarrier composition.

In one embodiment of any one of the compositions and methods provided herein, the mean of a particle size distribution obtained using dynamic light scattering of the synthetic nanocarriers is a diameter greater than 110nm. In one embodiment of any one of the compositions and methods provided herein, the diameter is greater than 120nm. In one embodiment of any one of the compositions and methods provided herein, the diameter is greater than 150nm. In one embodiment of any one of the compositions and methods provided

herein, the diameter is greater than 200nm. In one embodiment of any one of the compositions and methods provided herein, the diameter is greater than 250nm. In one embodiment of any one of the compositions and methods provided herein, the diameter is less than 300nm. In one embodiment of any one of the compositions and methods provided herein, the diameter is less than 250nm. In one embodiment of any one of the compositions and methods provided herein, the diameter is less than 200nm.

In another aspect, a kit comprising any one of the compositions provided herein is provided. In one embodiment of any one of the kits provided, the kit is for use in any one of the methods provided herein. In one embodiment of any one of the kits provided, the kit further comprises instructions for use. In one embodiment of any one of the kits provided, the instructions for use include a description of any one of the methods provided herein.

In another aspect, a method comprising administering any one of the compositions provided herein to a subject is provided. In one embodiment of any one of the methods provided herein, the method further comprises administering antigen to the subject. In one embodiment of any one of the methods provided herein, the administering is by intradermal, intramuscular, intravenous, intraperitoneal or subcutaneous administration.

In another aspect, a method of manufacturing any one of the compositions or kits provided herein is provided. In one embodiment of any one of these methods, the method of manufacturing comprises the steps of any one of the methods provided herein.

In another aspect, a use of any one of the compositions or kits provided herein for the manufacture of a medicament for promoting immune tolerance in a subject is provided. In another embodiment of any one of the uses provided herein, the use is for achieving any one of the methods provided herein.

In another aspect, any one of the compositions or kits provided herein may be for use in any one of the methods provided herein.

In another aspect, a method of manufacturing a medicament intended for promoting immune tolerance, is provided. In one embodiment, the medicament comprises any one of the compositions provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1 is a graph depicting the effects of particle size testing on stability of lyophilized formulations.

DETAILED DESCRIPTION OF THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified materials or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting of the use of alternative terminology to describe the present invention.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety for all purposes.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a polymer" includes a mixture of two or more such molecules or a mixture of differing molecular weights of a single polymer species, reference to "a synthetic nanocarrier" includes a mixture of two or more such synthetic nanocarriers or a plurality of such synthetic nanocarriers, and the like.

As used herein, the term “comprise” or variations thereof such as “comprises” or “comprising” are to be read to indicate the inclusion of any recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) but not the exclusion of any other integer or group of integers. Thus, as used herein, the term “comprising” is inclusive and does not exclude additional, unrecited integers or method/process steps.

In embodiments of any one of the compositions and methods provided herein, “comprising” may be replaced with “consisting essentially of” or “consisting of”. The phrase “consisting essentially of” is used herein to require the specified integer(s) or steps as well as those which do not materially affect the character or function of the claimed invention. As used herein, the term “consisting” is used to indicate the presence of the recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) alone.

A. INTRODUCTION

Surprisingly, certain components have been found that can ease lyophilization, maintain storage stability, reduce aggregation, etc. of compositions of synthetic nanocarriers, such as any one of the synthetic nanocarrier compositions described herein. Accordingly, provided herein are lyophilized forms of such synthetic nanocarrier compositions as well as reconstituted compositions thereof, and related methods.

The invention will now be described in more detail below.

B. DEFINITIONS

"Administering" or "administration" or "administer" means providing a material to a subject in a manner that is pharmacologically useful. The term is intended to include causing to be administered in some embodiments. "Causing to be administered" means causing, urging, encouraging, aiding, inducing or directing, directly or indirectly, another party to administer the material.

"Amount effective" in the context of a composition or dose for administration to a subject refers to an amount of the composition or dose that produces one or more desired responses in the subject, for example, the generation of a tolerogenic immune response. In some embodiments, the amount effective is a pharmacodynamically effective amount. Therefore, in some embodiments, an amount effective is any amount of a composition or dose provided herein that produces one or more of the desired therapeutic effects and/or immune responses as provided herein. This amount can be for *in vitro* or *in vivo* purposes. For *in vivo* purposes, the amount can be one that a clinician would believe may have a clinical benefit for a subject, such as one in need of antigen-specific immune tolerance. Any one of the compositions as provided herein can be in an amount effective.

Amounts effective can involve reducing the level of an undesired immune response, although in some embodiments, it involves preventing an undesired immune response altogether. Amounts effective can also involve delaying the occurrence of an undesired immune response. An amount that is effective can also be an amount that produces a desired therapeutic endpoint or a desired therapeutic result. In other embodiments, the amounts effective can involve enhancing the level of a desired response, such as a therapeutic endpoint or result. Amounts effective, in some embodiments, result in a tolerogenic immune response in a subject to an antigen. The achievement of any of the foregoing can be monitored by routine methods.

Amounts effective will depend, of course, on the particular subject being treated; the severity of a condition, disease or disorder; the individual patient parameters including age, physical condition, size and weight; the duration of the treatment; the nature of concurrent therapy (if any); the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reason.

In general, doses of the components in the compositions of the invention refer to the amount of the components. Alternatively, the dose can be administered based on the number of synthetic nanocarriers that provide the desired amount.

"Antigen-specific" refers to any immune response that results from the presence of the antigen, or portion thereof, or that generates molecules that specifically recognize or bind the antigen. For example, where the immune response is antigen-specific antibody production, antibodies are produced that specifically bind the antigen. As another example, where the immune response is antigen-specific B cell or CD4+ T cell proliferation and/or activity, the proliferation and/or activity results from recognition of the antigen, or portion thereof, alone or in complex with MHC molecules, B cells, etc.

"Average", as used herein, refers to the arithmetic mean unless otherwise noted.

"Encapsulate" means to enclose at least a portion of a substance within a synthetic nanocarrier. In some embodiments, a substance is enclosed completely within a synthetic nanocarrier. In other embodiments, most or all of a substance that is encapsulated is not exposed to the local environment external to the synthetic nanocarrier. In other embodiments, no more than 50%, 40%, 30%, 20%, 10% or 5% (weight/weight) is exposed to the local environment. Encapsulation is distinct from absorption, which places most or all of a substance on a surface of a synthetic nanocarrier, and leaves the substance exposed to the local environment external to the synthetic nanocarrier.

"Hydrophobic carrier material" refers to any pharmaceutically acceptable carrier that can deliver one or more molecules that comprises one or more polymers or units thereof and that has hydrophobic characteristics. In some preferred embodiments, the hydrophobic carrier material is a "hydrophobic polyester carrier material" which refers to any pharmaceutically

acceptable carrier that can deliver one or more molecules that comprises one or more polyester polymers or units thereof and that has hydrophobic characteristics. Polyester polymers include, but are not limited to, PLA, PLGA, PLG and polycaprolactone. The hydrophobic carrier materials include materials that can form a synthetic nanocarrier or a portion thereof and that can include or be loaded with one or more molecules (e.g., an immunosuppressant, such as a rapalog, a non-ionic surfactant with a HLB value less than or equal to 10). Generally, carrier materials can allow for delivery of one or more molecules to a target site or target cell, controlled-release of the one or more molecules, and other desired activities. "Hydrophobic" refers to a material that does not substantially participate in hydrogen bonding to water. Such materials are generally non-polar, primarily non-polar, or neutral in charge. A carrier material suitable for the compositions described herein may be selected based on it exhibiting hydrophobicity at some level. Hydrophobic polyester carrier materials, therefore, are those that are hydrophobic overall and may be completely comprised of hydrophobic polyesters or units thereof. In some embodiments, however, the hydrophobic polyester carrier materials are hydrophobic overall and comprise hydrophobic polyesters or units thereof but are in combination with other polymers or units thereof. These other polymers or units thereof may by hydrophobic but are not necessarily so. Hydorphobic carrier materials may include one or more other polymers or units thereof provided that the matrix of polymers or units thereof is considered hydrophobic.

"Initially sterile filterable" refers to a composition of synthetic nanocarriers that has not previously been filtered but can be filtered through a filter, such as a 0.22 μm filter, with a throughput of at least 50 grams nanocarrier/m^2 of filter membrane surface area. In some embodiments of any one of the compositions or methods provided herein, the throughput is determined by taking a 9 mL volume of synthetic nanocarrier suspension and placing it in a 10 mL syringe with any one of the filters as provided herein. The synthetic nanocarrier suspension is then pushed through the filter until no further suspension materials pass through the filter. The throughput can then be calculated based on the material that was pushed through the filter and the remaining suspension material in the syringe. In some embodiments of any one of the compositions or methods provided herein, the initially sterile filterable composition is non-sterile and/or not suitable for *in vi*vo administration (i.e., not substantially pure and comprising soluble components that are less than desirable for administration *in vivo*). In other embodiments of any one of the compositions or methods provided herein, the initially sterile filterable composition comprises synthetic nanocarriers that have been produced but

have not been further processed to produce a clinical grade material. In some embodiments of any one of the compositions or methods provided herein, the initially sterile filterable composition has not previously been filtered but can be filtered through a filter, such as a 0.22 μm filter, with a throughput of at least 60, 70, 80, 90, 100, 120, 130, 140, 160, 200, 250, 300, 350, 500, 750, 1000 or 1500 grams nanocarrier/m2 of a filter membrane surface area. The 0.22 μm filter can be any filter with a 0.22 μm pore size. Such filters can be made of a variety of materials, such as polyethylene sulfone, polyvinylidene fluoride, mixed cellulose esters, solvent free cellulose acetate, regenerated cellulose, nylon, etc. Specific examples of filters include Millipore SLGPM33R, Millipore SLGVM33RS, Millipore SLGSM33SS, Sartorius 16534, Sartorius 17764, Sartorius 17845, etc.

"Lyophilized" as used herein, refers to a synthetic nanocarrier composition that has been dried by freezing the formulation and then subliming the ice from the frozen content using any freeze-drying methods known in the art (e.g., with a commercially available freeze drying device). In some embodiments, the resulting lyophilisate has a residual moisture level of 0.1%(w/w) to 5%(w/w) and is present as a stable powder. The lyophilisate may be reconstituted in a reconstitution medium. "Reconstituted synthetic nanocarriers" are those which have been prepared by dissolving a lyophilized composition comprising the synthetic nanocarriers in a diluent or reconstitution medium, such that the synthetic nanocarriers are dispersed throughout the diluent. In some embodiments, the diluent or reconstitution medium comprises sterile water for injection. In some embodiments, the reconstituted synthetic nanocarriers are suitable for administration to a subject. The lyophilized or to be lyophilized or reconstituted compositions, in some embodiments, comprise a buffer and/or a lyoprotectant as provided herein. In some embodiments, the buffer is a non-phosphate buffer. In some embodiments, the buffer is sodium phosphate, potassium phosphate, citrate, histidine, tromethamine (tris(hydroxymethyl)aminomethane), Tris hydrochloride (Tris HCl), or a combination thereof. In some embodiments, the lyoprotectant comprises sucrose, trehalose, maltose, lactose, sorbitol, dextran, or a combination thereof. In one embodiment, the lyoprotectant is a disaccharide (e.g., sucrose). In one embodiment, the compositions comprise a buffer and a disaccharide (e.g., sucrose). In one embodiment, the compositions comprise Tris buffer and sucrose. In one embodiment, the compositions comprise tromethamine, Tris HCl, and sucrose. The amounts of any one or all of these components can be at any one of the concentrations provided herein, respectively.

In some embodiments, the tromethamine is present in any one of the compositions provided herein at a concentration of 0.5 mM – 3 mM, 0.5 mM – 2.5 mM, 0.5 mM – 2.0 mM, 0.5 mM – 1.5 mM, 0.5 mM – 1 mM, 1 mM- 3 mM, 1 mM – 2.5 mM, 1 mM – 2 mM, 1 mM – 1.9 mM, 1 mM – 1.8 mM, 1 mM – 1.7 mM, 1 mM – 1.6 mM, 1 mM – 1.5 mM, 1 mM – 1.4 mM, 1 mM – 1.3 mM, 1 mM- 1.2 mM, 1 mM – 1.1 mM, 1.2 mM – 3 mM, 1.2 mM – 2.5 mM, 1.2 mM – 2 mM, 1.2 mM – 1.9 mM, 1.2 mM – 1.8 mM, 1.2 mM – 1.7 mM, 1.2 mM – 1.6 mM, 1.2 mM – 1.5 mM, 1.2 mM - 1.4 mM, 1.2 mM – 1.3 mM, 1.4 mM – 3 mM, 1.4 mM – 2.5 mM, 1,4 mM – 2 mM, 1.4 mM – 1.9 mM, 1.4 mM – 1.8 mM, 1.4 mM – 1.7 mM, 1.4 mM – 1.6 mM, 1.4 mM – 1. 5 mM, 1.5 mM – 3 mM, 1.5 mM -2.5 mM, 1.5 mM – 2 mM, 2 mM – 3 mM, or 2 mM – 2.5 mM. In some embodiments, the tromethamine is present in any one of the compositions provided herein at a concentration of 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM, 1.6 mM, 1.7 mM, 1.8 mM, 1.9 mM, 2 mM, or more.

In some embodiment, the Tris HCl is present in any one of the compositions provided herein at a concentration of 7.5 mM – 10 mM, 7.5 mM – 9.5 mM, 7.5 mM - 9 mM, 7.5 mM – 8.5 mM, 7.5 mM – 8 mM, 8 mM – 10 mM, 8 mM – 9.5 mM, 8 mM – 9 mM, 8 mM – 8.9 mM, 8 mM – 8.8 mM, 8 mM – 8.7 mM, 8 mM – 8.6 mM, 8 mM – 8.5 mM, 8 mM – 8.4 mM, 8 mM – 8.3 mM, 8 mM – 8.2 mM, 8 mM – 8.1 mM, 8.2 mM – 10 mM, 8.2 mM – 9.5 mM, 8.2 mM – 9 mM, 8.2 mM – 8.9 mM, 8.2 mM – 8.8 mM, 8.2 mM – 8.7 mM, 8.2 mM – 8.6 mM, 8.2 mM – 8.5 mM, 8.2 mM – 8.4 mM, 8.2 mM – 8.3 mM, 8.4 mM – 10 mM, 8.4 mM – 9.5 mM, 8.4 mM – 9 mM, 8.4 mM – 8.9 mM, 8.4 mM – 8.8 mM, 8.4 mM – 8.7 mM, 8.4 mM – 8.6 mM, 8.4 mM – 8.5 mM, 8.6 mM – 10 mM, 8.6 mM – 9.5 mM, 8.6 mM – 9 mM, 8.6 mM – 8.9 mM, 8.6 mM – 8.8 mM, 8.6 mM – 8.7 mM, 8.8 mM – 10 mM, 8.8 mM – 9.5 mM, 8.8 mM – 9 mM, 8.8 mM – 8.9 mM, 8.8 mM – 10 mM, 8.8 mM – 9.5 mM, 8.8 mM – 9 mM, or 8.8 mM – 8.9 mM. In some embodiments, the Tris HCl is present in any one of the compositions provided herein at a concentration of 7.5 mM, 7.6 mM, 7.7 mM, 7.8 mM, 7.9 mM, 8 mM, 8.1 mM, 8.2 mM, 8.3 mM, 8.4 mM, 8.5 mM, 8.6 mM, 8.7 mM, 8.8 mM, 8.9 mM, 9 mM, 9.1 mM, 9.2 mM, 9.3 mM, 9.4 mM, 9.5 mM, 9.6 mM, 9.7 mM, 9.8 mM, 9.9 mM, 10 mM, or more.

In some embodiments, the sucrose is present in any one of the compositions provided herein at 8.5 wt% - 10.5 wt%, 8.5 wt% – 10 wt%, 8.5 wt% – 9.5 wt%, 8.5 wt% – 9 wt%, 9 wt% – 10.5 wt%, 9 – 10 wt%, 9 wt% – 9.9 wt%, 9 wt%-9.8 wt%, 9 wt%-9.7 wt%, 9 wt%-9.6 wt%, 9 wt%-9.5 wt%, 9 wt%-9.4 wt%, 9 wt%-9.3 wt%, 9 wt%-9.2 wt%, 9 wt%-9.1 wt%, 9.2 wt% – 10.5 wt%, 9.2 – 10 wt%, 9.2 wt% – 9.9 wt%, 9.2 wt%-9.8 wt%, 9.2 wt%-9.7 wt%, 9.2

wt%-9.6 wt%, 9.2 wt%-9.5 wt%, 9.2 wt%-9.4 wt%, 9.2 wt%-9.3 wt%, 9.4 wt% – 10.5 wt%, 9.4 – 10 wt%, 9.4 wt% – 9.9 wt%, 9.4 wt%-9.8 wt%, 9.4 wt%-9.7 wt%, 9.4 wt%-9.6 wt%, 9.4 wt%-9.5 wt%, 9.6 wt% – 10.5 wt%, 9.6 – 10 wt%, 9.6 wt% – 9.9 wt%, 9.6 wt%-9.8 wt%, 9.6 wt%-9.7 wt%, 9.8 wt% – 10.5 wt%, 9.8 – 10 wt%, 9.8 wt% – 9.9 wt%, or 10 wt% – 10.5 wt%. In some embodiments, the sucrose is present in the in any one of the compositions provided herein at 8.5 wt%, 8.6 wt%, 8.7 wt%, 8.8 wt%, 8.9 wt%, 9 wt%, 9.1 wt%, 9.2 wt%, 9.3 wt%, 9.4 wt%, 9.5 wt%, 9.6 wt%, 9.7 wt%, 9.8 wt%, 9.9 wt%, 10 wt%, 10.1 wt%, 10.2 wt%, 10.3 wt%, 10.4 wt%, 10.5 wt%, or more,

In some embodiments, the compositions described herein comprise 10-20 wt% synthetic nanocarrier, hydrophobic carrier material, and immunosuppressant; 80-90 wt% sucrose, 0.1-5 wt% tromethamine; and 0.1-5 wt% Tris HCL. In some embodiments, the synthetic nanocarrier, hydrophobic carrier material, and immunosuppressant are present in any one of the compositions provided herein at 5-10 wt%, 5-15 wt%, 5-20 wt%, 5-25 wt%, 10-15 wt%, 10-20 wt%, 10-25 wt%, 15-20 wt%, 15-25 wt%, or 20-25 wt%. In some embodiments, the composition may comprise 5 wt%, 6 wt%, 7 wt%, 8 wt%, 9 wt%, 10 wt%, 11 wt%, 12 wt%, 13 wt%, 14 wt%, 15 wt%, 16 wt%, 17 wt%, 18 wt%, 19 wt%, 20 wt%, 21 wt%, 22 wt%, 23 wt%, 24 wt%, or 25 wt% synthetic nanocarrier, hydrophobic carrier material, and immunosuppressant. In some embodiments, the sucrose is present in any one of the compositions provided herein at 75-95 wt%, *e.g.*, 75-80 wt%, 75-85 wt%, 75-90 wt%, 80-85 wt%, 80-90 wt%, 80-95 wt%, 85-90 wt%, 85-95 wt%, or 90-95 wt%. In some embodiments, the composition may comprise 75 wt%, 76 wt%, 77 wt%, 78 wt%, 79 wt%, 80 wt%, 81 wt%, 82 wt%, 83 wt%, 84 wt%, 85 wt%, 86 wt%, 87 wt%, 88 wt%, 89 wt%, 90 wt%, 91 wt%, 92 wt%, 93 wt%, 94 wt%, or 95 wt% sucrose. In some embodiments, the tromethamine is present in any one of the compositions provided herein at 0.1-5 wt%, *e.g.*, 0.1-0.2 wt%, 0.1-0.3 wt%, 0.1-0.4 wt%, 0.1-0.5 wt%, 0.1-0.6 wt%, 0.1-0.7 wt%, 0.1-0.8 wt%, 0.1-0.9 wt%, 0.1-1 wt%, 0.1-1.5 wt%, 0.1-2 wt%, 0.1-2.5 wt%, 0.1-3 wt%, 0.1-3.5 wt%, 0.1-4 wt%, 0.1-4.5 wt%, 0.2-0.3 wt%, 0.2-0.4 wt%, 0.2-0.5 wt%, 0.2-0.6 wt%, 0.2-0.7 wt%, 0.2-0.8 wt%, 0.2-0.9 wt%, 0.3-0.4 wt%, 0.3-0.5 wt%, 0.3-0.6 wt%, 0.3-0.7 wt%, 0.3-0.8 wt%, 0.3-0.9 wt%, 0.4-0.5 wt%, 0.4-0.6 wt%, 0.4-0.7 wt%, 0.4-0.8 wt%, 0.4-0.9 wt%, 0.5-0.6 wt%, 0.5-0.7 wt%, 0.5-0.8 wt%, 0.5-0.9 wt%, 0.6-0.7 wt%, 0.6-0.8 wt%, 0.6-0.9 wt%, 0.7-0.8 wt%, 0.7-0.9 wt%, 0.8-0.9 wt%, 0.5-1.5 wt%, 0.5-2 wt%, 0.5-2.5 wt%, 0.5-3 wt%, 0.5-4 wt%, 0.5-5 wt%, 1-2 wt%, 1-3 wt%, 1-4 wt%, or 1-5 wt%. In some embodiments, the composition may comprise 0.1 wt%, 0.2 wt%, 0.3 wt%, 0.4 wt%, 0.5 wt%, 0.6 wt%, 0.7 wt%, 0.8 wt%, 0.9 wt%, 1 wt%, 1.2 wt%, 1.4 wt%,

1.5 wt%, 1.6 wt%, 1.8 wt%, 2 wt%, 2.5 wt%, 3 wt%, 3.5 wt%, 4 wt%, 4.5 wt%, or 5 wt% tromethamine. In some embodiments, the Tris HCL is present in any one of the compositions provided herein at 0.1-5 wt%, *e.g.*, 0.1-0.2 wt%, 0.1-0.3 wt%, 0.1-0.4 wt%, 0.1-0.5 wt%, 0.1-0.6 wt%, 0.1-0.7 wt%, 0.1-0.8 wt%, 0.1-0.9 wt%, 0.1-1 wt%, 0.1-1.5 wt%, 0.1-2 wt%, 0.1-2.5 wt%, 0.1-3 wt%, 0.1-3.5 wt%, 0.1-4 wt%, 0.1-4.5 wt%, 0.2-0.3 wt%, 0.2-0.4 wt%, 0.2-0.5 wt%, 0.2-0.6 wt%, 0.2-0.7 wt%, 0.2-0.8 wt%, 0.2-0.9 wt%, 0.3-0.4 wt%, 0.3-0.5 wt%, 0.3-0.6 wt%, 0.3-0.7 wt%, 0.3-0.8 wt%, 0.3-0.9 wt%, 0.4-0.5 wt%, 0.4-0.6 wt%, 0.4-0.7 wt%, 0.4-0.8 wt%, 0.4-0.9 wt%, 0.5-0.6 wt%, 0.5-0.7 wt%, 0.5-0.8 wt%, 0.5-0.9 wt%, 0.6-0.7 wt%, 0.6-0.8 wt%, 0.6-0.9 wt%, 0.7-0.8 wt%, 0.7-0.9 wt%, 0.8-0.9 wt%, 0.5-1.5 wt%, 0.5-2 wt%, 0.5-2.5 wt%, 0.5-3 wt%, 0.5-4 wt%, 0.5-5 wt%, 1-2 wt%, 1-3 wt%, 1-4 wt%, or 1-5 wt%. In some embodiments, the composition may comprise 0.1 wt%, 0.2 wt%, 0.3 wt%, 0.4 wt%, 0.5 wt%, 0.6 wt%, 0.7 wt%, 0.8 wt%, 0.9 wt%, 1 wt%, 1.2 wt%, 1.4 wt%, 1.5 wt%, 1.6 wt%, 1.8 wt%, 2 wt%, 2.5 wt%, 3 wt%, 3.5 wt%, 4 wt%, 4.5 wt%, or 5 wt% Tris HCL.

In some embodiments, the lyophilized composition is stable (e.g., maintained immunosuppressant content, purity, in vitro release, particle size, appearance, and pH) for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 1.5 weeks, 2 weeks, 2.5 weeks, 3 weeks, 3.5 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months, 30 months, 31 months, 32 months, 33 months, 34 months, 35 months, 36 months or longer. In some embodiments, the lyophilized composition is stable for at least 1-2 weeks, 2-4 weeks, 1-2 months, 2-4 months, 3-6 months, 3-9 months, 3-12 months, 6-12 months, 6-18 months, 6-24 months, 6-30 months, 6-36 months, 1-2 years, 1-3 years, or 2-3 years.

In some embodiments, the lyophilized composition is stored at -20°C ± 5°C (e.g., -25°C, -24°C, -23°C, -22°C, -21°C, -20°C, -19°C, -18°C, -17°C, -16°C, or -15°C). In some embodiments, the lyophilized composition is stored at 5°C ± 3°C (e.g., 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, or 8°C) or at 25°C ± 5°C (e.g., 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, or 30°C).

"Maximum dimension of a synthetic nanocarrier" means the largest dimension of a nanocarrier measured along any axis of the synthetic nanocarrier. "Minimum dimension of a synthetic nanocarrier" means the smallest dimension of a synthetic nanocarrier measured along any axis of the synthetic nanocarrier. For example, for a spheroidal synthetic nanocarrier, the

maximum and minimum dimension of a synthetic nanocarrier would be substantially identical, and would be the size of its diameter. Similarly, for a cuboidal synthetic nanocarrier, the minimum dimension of a synthetic nanocarrier would be the smallest of its height, width or length, while the maximum dimension of a synthetic nanocarrier would be the largest of its height, width or length. In an embodiment, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100 nm. In an embodiment, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or less than 5 μm. Preferably, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is greater than 110 nm, more preferably greater than 120 nm, more preferably greater than 130 nm, and more preferably still greater than 150 nm. Aspects ratios of the maximum and minimum dimensions of synthetic nanocarriers may vary depending on the embodiment. For instance, aspect ratios of the maximum to minimum dimensions of the synthetic nanocarriers may vary from 1:1 to 1,000,000:1, preferably from 1:1 to 100,000:1, more preferably from 1:1 to 10,000:1, more preferably from 1:1 to 1000:1, still more preferably from 1:1 to 100:1, and yet more preferably from 1:1 to 10:1.

Preferably, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample is equal to or less than 3 μm, more preferably equal to or less than 2 μm, more preferably equal to or less than 1 μm, more preferably equal to or less than 800 nm, more preferably equal to or less than 600 nm, and more preferably still equal to or less than 500 nm. In preferred embodiments, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100 nm, more preferably equal to or greater than 120 nm, more preferably equal to or greater than 130 nm, more preferably equal to or greater than 140 nm, and more preferably still equal to or greater than 150 nm. Measurement of synthetic nanocarrier dimensions (e.g., effective diameter) may be obtained, in some embodiments, by suspending the synthetic nanocarriers in a liquid (usually aqueous) media and using dynamic light scattering (DLS) (e.g., using a Brookhaven ZetaPALS instrument). For example, a suspension of synthetic nanocarriers can

be diluted from an aqueous buffer into purified water to achieve a final synthetic nanocarrier suspension concentration of approximately 0.01 to 0.5 mg/mL. The diluted suspension may be prepared directly inside, or transferred to, a suitable cuvette for DLS analysis. The cuvette may then be placed in the DLS, allowed to equilibrate to the controlled temperature, and then scanned for sufficient time to acquire a stable and reproducible distribution based on appropriate inputs for viscosity of the medium and refractive indicies of the sample. The effective diameter, or mean of the distribution, is then reported. Determining the effective sizes of high aspect ratio, or non-spheroidal, synthetic nanocarriers may require augmentative techniques, such as electron microscopy, to obtain more accurate measurements. "Dimension" or "size" or "diameter" of synthetic nanocarriers means the mean of a particle size distribution, for example, obtained using dynamic light scattering.

"Non-ionic surfactant with a HLB value less than or equal to 10", or "low HLB surfactant", as used herein, refers to a non-ionic amphiphilic molecule that has a structure comprising at least one hydrophobic tail and a hydrophilic head or that has hydrophobic groups or regions and hydrophilic groups or regions. The tail portion of surfactants generally consists of a hydrocarbon chain. Surfactants can be classified based on the charge characteristics of the hydrophilic head portion or groups or regions. As used herein, "HLB" refers to the hydrophilic-lipophilic balance or hydrophile-lipophile balance of a surfactant and is a measure of the hydrophilic or lipophilic nature of a surfactant.

The HLB of any one of surfactants provided herein may be calculated using the Griffin's method or the Davie's method. For example, using the Griffin's method, the HLB of a surfactant is the product of 20 multiplied by the molecular mass of the hydrophilic portion of the surfactant divided by the molecular mass of the entire surfactant. The HLB value is on a scale from 0 to 20, with 0 corresponding to a completely hydrophobic (lipophilic) molecule, and 20 corresponding to a completely hydrophilic (lipophobic) molecule. In some embodiments, the HLB of the surfactant of any one of the compositions or methods provided herein is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (e.g., as determined by Griffin's or Davie's method). Examples of such surfactants for use in any one of the compositions and methods provided herein include, without limitation, sorbitan esters, such as SPAN 40, SPAN 20; fatty alcohols, such as oleyl alcohol, stearyl alcohol; fatty acid esters, such as isopropyl palmitate, glycerol monostearate; ethoxylated fatty alcohols, such as BRIJ 52, BRIJ 93; poloxamers, such as Pluronic P-123, Pluronic L-31; fatty acids, such as palmitic acid, dodecanoic acid; triglycerides, such as glyceryl tripalmitate, glyceryl trilinoleate; cholesterol; cholesterol

derivatives, such as sodium cholesteryl sulfate, cholesteryl dodecanoate; and bile salts or acids, such as lithocholic acid, sodium lithocholate. Further examples of such surfactants include sorbitan monostearate (SPAN 60), sorbitan tristearate (SPAN 65), sorbitan monooleate (SPAN 80), sorbitan sesquioleate (SPAN 83), sorbitan trioleate (SPAN 85), sorbitan sesquioleate (Arlacel 83), sorbitan dipalmitate, mono and diglycerides of fatty acids, polyoxyethylene sorbitan trioleate (Tween 85), polyoxyethylene sorbitan hexaoleate (G 1086), sorbitan monoisostearate (Montane 70), polyoxyethylene alcohols, polyoxyethylene glycol alkyl ethers, polyoxyethylene (2) oleyl ether (BRIJ 93), polyoxyethylene cetyl ether (BRIJ 52), polyethylene glycol dodecyl ether (BRIJ L4); 1-monotetradecanoyl-rac-glycerol; glyceryl monostearate; glycerol monopalmitate; ethylenediamine tetradkis tetrol (Tetronic 90R4, Tetronic 701), polyoxyethylene (5) nonylphenylether (IGEPAL CA-520), MERPOL A surfactant, MERPOL SE surfactant, and poly(ethylene glycol) sorbitol hexaoleate. Further examples would also be apparent to one of ordinary skill in the art.

"Pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" means a pharmacologically inactive material used together with a pharmacologically active material to formulate the compositions. Pharmaceutically acceptable excipients comprise a variety of materials known in the art, including but not limited to saccharides (such as glucose, lactose, and the like), preservatives such as antimicrobial agents, reconstitution aids, colorants, saline (such as phosphate buffered saline), and buffers.

"Providing" means an action or set of actions that an individual performs that supplies a needed item or set of items or methods for the practice of the present invention. The action or set of actions may be taken either directly oneself or indirectly.

"Rapalog" refers to rapamycin and molecules that are structurally related to (an analog) of rapamycin (sirolimus), and are preferably hydrophobic. Examples of rapalogs include, without limitation, temsirolimus (CCI-779), deforolimus, everolimus (RAD001), ridaforolimus (AP-23573), zotarolimus (ABT-578). Additional examples of rapalogs may be found, for example, in WO Publication WO 1998/002441 and U.S. Patent No. 8,455,510, the disclosure of such rapalogs are incorporated herein by reference in its entirety.

"Subject" means animals, including warm blooded mammals such as humans and primates; avians; domestic household or farm animals such as cats, dogs, sheep, goats, cattle, horses and pigs; laboratory animals such as mice, rats and guinea pigs; fish; reptiles; zoo and wild animals; and the like.

"Super-saturation" refers to a composition (e.g., a synthetic nanocarrier composition) containing more of a solute (e.g., immunosuppressant) than can be dissolved within it under equilibrium conditions. In other words, a composition with a super-saturation concentration has a concentration that is beyond the concentration of saturation. In some embodiments, the immunosuppressant can be above its saturation limit for a hydrophobic carrier material, such as hydrophobic polyester carrier material, (e.g., alone or in combination with a solvent in the aqueous phase of a formulation process). The amount of immunosuppressant in a composition may be determined to be super-saturated by any method known in the art, for example, by determining the concentration of the molecule in a composition and comparing that concentration to the predicted saturation concentration.

Other methods for determining whether or not an immunosuppressant is in a super-saturated amount include film casting, X-ray scattering and electron microscopy. Forms of electron microscopy include, but are not limited to, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and cryogenic transmission electron microscopy (cryo-TEM). A super-saturated amount of immunosuppressant is preferably "stable". A super-saturated amount of immunosuppresasnt is stable in synthetic nanocarriers if the synthetic nanocarriers retain such an amount when in suspension, in some embodiments. Preferably, synthetic nanocarriers with stable, super-saturated amounts of immunosuppressant are initially sterile filterable, and initial sterile filterability may serve as a test of the stability of a super-saturated amount of immunosuppressant in synthetic nanocarriers.

"Surfactant" refers to a compound that can lower the surface tension between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants and can be used in the formation of synthetic nanocarriers as provided herein. In some embodiments, the surfactants are non-ionic surfactants with a HLB value less than or equal to 10.

"Synthetic nanocarrier(s)" means a discrete object that is not found in nature, and that possesses at least one dimension that is less than or equal to 5 microns in size. As provided herein the synthetic nanocarriers comprise a hydrophobic carrier material, such as hydrophobic polyester carrier material. A synthetic nanocarrier can be, but is not limited to, synthetic nanocarriers comprising hydrophobic polyester nanoparticles. Synthetic nanocarriers may be a variety of different shapes, including but not limited to spheroidal, cuboidal, pyramidal, oblong, cylindrical, toroidal, and the like. Synthetic nanocarriers according to the invention

comprise one or more surfaces. In embodiments, synthetic nanocarriers may possess an aspect ratio greater than or equal to 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7, or greater than 1:10.

Synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface with hydroxyl groups that activate complement or alternatively comprise a surface that consists essentially of moieties that are not hydroxyl groups that activate complement. In a preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface that substantially activates complement or alternatively comprise a surface that consists essentially of moieties that do not substantially activate complement. In a more preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface that activates complement or alternatively comprise a surface that consists essentially of moieties that do not activate complement.

"Total solids" refers to the total weight of all components contained in a composition or suspension of synthetic nanocarriers. In some embodiments of any one of the compositions or methods provided herein, the amount of total solids is determined as the total dry-nanocarrier mass per mL of suspension. This can be determined by a gravimetric method.

"Weight%" refers to the ratio of one weight to another weight times 100. For example, the weight% can be the ratio of the weight of one component to another times 100 or the ratio of the weight of one component to a total weight of more than one component times 100. Generally, the weight% is measured as an average across a population of synthetic nanocarriers or an average across the synthetic nanocarriers in a composition or suspension.

C. COMPOSITIONS AND RELATED METHODS

Provided herein are compositions of synthetic nanocarriers that have improved lyophilization, storage, etc. properties. Provided herein as lyophilized forms of the synthetic nanocarrier compositions, reconstituted compositions thereof, as well as synthetic nanocarrier compositions that are to be lyophilized. In one embodiment of any one of the compositions provided herein, the composition of synthetic nanocarriers has a neutral or near-neutral pH (e.g., a pH of 7.3, such as at 25˚C). In one embodiment of any one of the compositions provided herein, the composition of synthetic nanocarriers is in a lyophilized form, such as a lyophilized powder form. In one embodiment of any one of the compositions provided herein,

the composition of synthetic nanocarriers is one to be lyophilized, such as to a lyophilized powder form. In one embodiment of any one of the compositions provided herein, the composition of synthetic nanocarriers is a reconstituted composition of the lyophilized form. In one embodiment of any one of the compositions provided herein, the composition of synthetic nanocarriers is stored in a glass vial. In one embodiment of any one of the compositions provided herein, the glass vial is a 20 mL glass vial, optionally comprising a 20 mm stopper. In one embodiment of any one of the compositions provided herein, the composition of synthetic nanocarriers is stored at 2 to 8°C.

The compositions provided herein can be administered to a subject in need thereof, such as to promote a tolerogenic immune response.

Preferably, in some embodiments of any one of the compositions provided herein, the amount of hydrophobic carrier material, such as hydrophobic polyester carrier material, in the synthetic nanocarrier composition is 5-95 weight% hydrophobic carrier material/total solids. In other embodiments of any one of the compositions provided herein, the amount of hydrophobic carrier material, such as hydrophobic polyester carrier material, in the synthetic nanocarriers is 10-95, 15-90, 20-90, 25-90, 30-80, 30-70, 30-60, 30-50, etc. weight% hydrophobic carrier material/total solids. In still other embodiments of any one of the compositions provided herein, the amount of hydrophobic carrier materials, such as hydrophobic polyester carrier materials, in the synthetic nanocarriers is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95 weight% hydrophobic carrier material/total solids.

In some embodiments of any one of the compositions provided herein, the synthetic nanocarriers that comprise a rapalog, such as rapamycin, in a stable, super-saturated amount comprise ≥ 6 but ≤ 50 weight% rapalog, such as rapamycin,/hydrophobic carrier material, such as hydrophobic polyester carrier material. In some embodiments of any one of the compositions provided herein, the synthetic nanocarriers comprise ≥ 6 but ≤ 45, ≥ 6 but ≤ 40, ≥ 6 but ≤ 35, ≥ 6 but ≤ 30, ≥ 6 but ≤ 25, ≥ 6 but ≤ 20, ≥ 6 but ≤ 15 weight% rapalog, such as rapamycin,/hydrophobic carrier material, such as hydrophobic polyester carrier material. In other embodiments of any one of the compositions provided herein, the synthetic nanocarriers comprise ≥ 7 but ≤ 45, ≥ 7 but ≤ 40, ≥ 7 but ≤ 35, ≥ 7 but ≤ 30, ≥ 7 but ≤ 25, ≥ 7 but ≤ 20, ≥ 7 but ≤ 15 weight% rapalog, such as rapamycin,/hydrophobic carrier material, such as hydrophobic polyester carrier material. In still other embodiments of any one of the compositions provided herein, the synthetic nanocarriers comprise ≥ 8 but ≤ 24 weight%

rapalog, such as rapamycin,/hydrophobic carrier material, such as hydrophobic polyester carrier material. In some embodiments of any one of the compositions provided herein, the synthetic nanocarriers comprise 6, 7, 8, 9, 10, 12, 15, 17, 20, 22, 25, 27, 30, 35, 45 or more weight% rapalog, such as rapamycin/hydrophobic carrier material, such as hydrophobic polyester carrier material.

In some embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is ≥ 0.01 but ≤ 20 weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. In some embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is ≥ 0.1 but ≤ 15, ≥ 0.5 but ≤ 13, ≥ 1 but ≤ 9 or 10 weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. In other embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is ≥ 0.01 but ≤ 17, ≥ 0.01 but ≤ 15, ≥ 0.01 but ≤ 13, ≥ 0.01 but ≤ 12, ≥ 0.01 but ≤ 11, ≥ 0.01 but ≤ 10, ≥ 0.01 but ≤ 9, ≥ 0.01 but ≤ 8, ≥ 0.01 but ≤ 7, ≥ 0.01 but ≤ 6, ≥ 0.01 but ≤ 5, etc. weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. In still other embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is ≥ 0.1 but ≤ 15, ≥ 0.1 but ≤ 14, ≥ 0.1 but ≤ 13, ≥ 0.1 but ≤ 12, ≥ 0.1 but ≤ 11, ≥ 0.1 but ≤ 10, ≥ 0.1 but ≤ 9, ≥ 0.1 but ≤ 8, ≥ 0.1 but ≤ 7, ≥ 0.1 but ≤ 6, ≥ 0.1 but ≤ 5, etc. weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. In still other embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is ≥ 0.5 but ≤ 15, ≥ 0.5 but ≤ 14, ≥ 0.5 but ≤ 13, ≥ 0.5 but ≤ 12, ≥ 0.5 but ≤ 11, ≥ 0.5 but ≤ 10, ≥ 0.5 but ≤ 9, ≥ 0.5 but ≤ 8, ≥ 0.5 but ≤ 7, ≥ 0.5 but ≤ 6, ≥ 0.5 but ≤ 5, etc. weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. In still other embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is ≥ 1 but ≤ 9, ≥ 1 but ≤ 8, ≥ 1 but ≤ 7, ≥ 1 but ≤ 6, ≥ 1 but ≤ 5, etc.

weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. In still other embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is ≥ 5 but ≤ 15, ≥ 5 but ≤ 14, ≥ 5 but ≤ 13, ≥ 5 but ≤ 12, ≥ 5 but ≤ 11, ≥ 5 but ≤ 10, ≥ 5 but ≤ 9, ≥ 5 but ≤ 8, ≥ 5 but ≤ 7, ≥ 5 but ≤ 6, etc. weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. In some embodiments of any one of the compositions or methods provided herein, the amount of the non-ionic surfactant with HLB value less than or equal to 10 in the synthetic nanocarriers is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 weight% non-ionic surfactant with a HLB value less than or equal to 10/hydrophobic carrier material, such as hydrophobic polyester carrier material. Any one of the HLB values provided herein may be determined using Griffin's or Davie's method.

The amounts of components or materials as recited herein for any one of the compositions provided herein can be determined using methods known to those of ordinary skill in the art or otherwise provided herein. For example, amounts of the non-ionic surfactant with a HLB value less than or equal to 10 can be measured by extraction followed by quantitation by an HPLC method. Amounts of hydrophobic carrier material, such as hydrophobic polyester carrier material, can be determined using HPLC. The determination of such an amount may, in some embodiments, follow the use of proton NMR or other orthogonal methods, such as MALDI-MS, etc. to determine the identity of a hydrophobic carrier material. Similar methods can be used to determine the amounts of immunosuppressant (e.g., rapalog, such as rapamycin) in any one of the compositions provided herein. In some embodiments, the amount of immunosuppressant (e.g., rapalog, such as rapamycin) is determined using HPLC. For any one of the compositions or methods provided herein the amounts of the components or materials can also be determined based on the recipe weights of a nanocarrier formulation. Accordingly, in some embodiments of any one of the compositions or methods provided herein, the amounts of any one of the components provided herein are those of the components in an aqueous phase during formulation of the synthetic nanocarriers. In some embodiments of any one of the compositions or methods provided herein, the amounts of any one of the components are those of the components in a synthetic nanocarrier composition that is produced and the result of a formulation process.

The synthetic nanocarriers as provided herein comprise hydrophobic carrier materials, such as hydrophobic polymers or lipids. Therefore, in some embodiments, the synthetic nanocarriers provided herein comprise one or more lipids. In some embodiments, a synthetic nanocarrier may comprise a lipid bilayer. In some embodiments, a synthetic nanocarrier may comprise a lipid monolayer. In some embodiments, a synthetic nanocarrier may comprise a core comprising a polymeric matrix surrounded by a lipid layer (e.g., lipid bilayer, lipid monolayer, etc.). Further hydrophobic carrier materials include lipids (synthetic and natural), lipid-polymer conjugates, lipid-protein conjugates, and crosslinkable-oils, waxes, fats, etc. Further examples of lipid materials for use as hydrophobic carrier materials as provided herein can be found, for example, in PCT Publication No. WO2000/006120 and WO2013/056132, the disclosures of such materials being incorporated herein by reference in their entirety.

Accordingly, in some embodiments the synthetic nanocarriers provided herein can be liposomes. Liposomes can be produced by standard methods such as those reported by Kim et al. (1983, Biochim. Biophys. Acta 728, 339-348); Liu et al. (1992, Biochim. Biophys. Acta 1104, 95-101); Lee et al. (1992, Biochim. Biophys. Acta. 1103, 185-197), Brey et al. (U.S. Pat. Appl. Pub. 20020041861), Hass et al. (U.S. Pat. Appl. Pub. 20050232984), Kisak et al. (U.S. Pat. Appl. Pub. 20050260260) and Smyth-Templeton et al. (U.S. Pat. Appl. Pub. 20060204566), the disclosure of such liposomes and methods for their production are incorporated herein by reference in their entirety.

The hydrophobic carrier material as provided herein comprises one or more hydrophobic polymers or units thereof. However, in some embodiments, while the hydrophobic carrier material is hydrophobic overall, the hydrophobic carrier material may also comprise polymers or units thereof that are not hydrophobic.

The hydrophobic carrier materials as provided herein may comprise polyesters, which can include copolymers comprising lactic acid and glycolic acid units, such as poly(lactic acid-co-glycolic acid) and poly(lactide-co-glycolide), collectively referred to herein as "PLGA"; and homopolymers comprising glycolic acid units, referred to herein as "PGA," and lactic acid units, such as poly-L-lactic acid, poly-D-lactic acid, poly-D,L-lactic acid, poly-L-lactide, poly-D-lactide, and poly-D,L-lactide, collectively referred to herein as "PLA." In some embodiments, exemplary polyesters include, for example, polyhydroxyacids; PEG copolymers and copolymers of lactide and glycolide (e.g., PLA-PEG copolymers, PGA-PEG copolymers, PLGA-PEG copolymers, and derivatives thereof. In some embodiments, polyesters include, for example, poly(caprolactone), poly(caprolactone)-PEG copolymers, poly(L-lactide-co-L-

lysine), poly(serine ester), poly(4-hydroxy-L-proline ester), poly[α-(4-aminobutyl)-L-glycolic acid], and derivatives thereof.

In some embodiments, the polyester may be PLGA. PLGA is a biocompatible and biodegradable co-polymer of lactic acid and glycolic acid, and various forms of PLGA are characterized by the ratio of lactic acid:glycolic acid. Lactic acid can be L-lactic acid, D-lactic acid, or D,L-lactic acid. The degradation rate of PLGA can be adjusted by altering the lactic acid:glycolic acid ratio. In some embodiments, PLGA to be used in accordance with the present invention is characterized by a lactic acid:glycolic acid ratio of approximately 85:15, approximately 75:25, approximately 60:40, approximately 50:50, approximately 40:60, approximately 25:75, or approximately 15:85.

The hydrophobic polyester carrier material as provided herein may comprise one or more non-polyester hydrophobic polymers or units thereof and/or polymers or units thereof that are not hydrophobic provided that overall the hydrophobic polyester carrier material is hydrophobic and contains one or more polyesters or units thereof.

Hydrophobic carrier materials as provided herein may comprise one or more polymers that are a non-methoxy-terminated, pluronic polymer, or a unit thereof. "Non-methoxy-terminated polymer" means a polymer that has at least one terminus that ends with a moiety other than methoxy. In some embodiments, the polymer has at least two termini that ends with a moiety other than methoxy. In other embodiments, the polymer has no termini that ends with methoxy. "Non-methoxy-terminated, pluronic polymer" means a polymer other than a linear pluronic polymer with methoxy at both termini.

Hydrophobic carrier materials may comprise, in some embodiments, polyhydroxyalkanoates, polyamides, polyethers, polyolefins, polyacrylates, polycarbonates, polystyrene, silicones, fluoropolymers, or a unit thereof. Further examples of polymers that may be comprised in the hydrophobic carrier materials provided herein include polycarbonate, polyamide, or polyether, or unit thereof. In other embodiments, the polymers of the hydrophobic carrier material may comprise poly(ethylene glycol) (PEG), polypropylene glycol, or unit thereof.

In some embodiments, it is preferred that the hydrophobic carrier material comprises polymer that is biodegradable. Therefore, in such embodiments, the polymers of the hydrophobic carrier materials may include a polyether, such as poly(ethylene glycol) or polypropylene glycol or unit thereof. Additionally, the polymer may comprise a block-co-polymer of a polyether and a biodegradable polymer such that the polymer is biodegradable. In other

embodiments, the polymer does not solely comprise a polyether or unit thereof, such as poly(ethylene glycol) or polypropylene glycol or unit thereof.

Other examples of polymers suitable for use in the present invention include, but are not limited to polyethylenes, polycarbonates (e.g. poly(1,3-dioxan-2one)), polyanhydrides (e.g. poly(sebacic anhydride)), polypropylfumerates, polyamides (e.g. polycaprolactam), polyacetals, polyethers, polyesters (e.g., polylactide, polyglycolide, polylactide-co-glycolide, polycaprolactone, polyhydroxyacid (e.g. poly(β-hydroxyalkanoate))), poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polyureas, polystyrenes, and polyamines, polylysine, polylysine-PEG copolymers, and poly(ethyleneimine), poly(ethylene imine)-PEG copolymers.

Still other examples of polymers that may be included in a hydrophobic carrier material include acrylic polymers, for example, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, aminoalkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), methacrylic acid alkylamide copolymer, poly(methyl methacrylate), poly(methacrylic acid anhydride), methyl methacrylate, polymethacrylate, poly(methyl methacrylate) copolymer, polyacrylamide, aminoalkyl methacrylate copolymer, glycidyl methacrylate copolymers, polycyanoacrylates, and combinations comprising one or more of the foregoing polymers.

In some embodiments, the polymers of the hydrophobic carrier material can associate to form a polymeric matrix. A wide variety of polymers and methods for forming polymeric matrices therefrom are known conventionally. In some embodiments, a synthetic nanocarrier comprising a hydrophobic polymeric matrix generates a hydrophobic environment within the synthetic nanocarrier.

In some embodiments, polymers may be modified with one or more moieties and/or functional groups. A variety of moieties or functional groups can be used in accordance with the present invention. In some embodiments, polymers may be modified with polyethylene glycol (PEG), with a carbohydrate, and/or with acyclic polyacetals derived from polysaccharides (Papisov, 2001, ACS Symposium Series, 786:301). Certain embodiments may be made using the general teachings of US Patent No. 5543158 to Gref et al., or WO publication WO2009/051837 by Von Andrian et al.

In some embodiments, polymers may be modified with a lipid or fatty acid group. In some embodiments, a fatty acid group may be one or more of butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, or lignoceric acid. In some embodiments,

a fatty acid group may be one or more of palmitoleic, oleic, vaccenic, linoleic, alpha-linoleic, gamma-linoleic, arachidonic, gadoleic, arachidonic, eicosapentaenoic, docosahexaenoic, or erucic acid.

In some embodiments, it is preferred that the polymer is biodegradable. In some embodiments, polymers in accordance with the present invention include polymers which have been approved for use in humans by the U.S. Food and Drug Administration (FDA) under 21 C.F.R. § 177.2600.

Polymers may be natural or unnatural (synthetic) polymers. Polymers may be homopolymers or copolymers comprising two or more monomers. In terms of sequence, copolymers may be random, block, or comprise a combination of random and block sequences. Typically, polymers in accordance with the present invention are organic polymers.

In some embodiments, polymers can be linear or branched polymers. In some embodiments, polymers can be dendrimers. In some embodiments, polymers can be substantially cross-linked to one another. In some embodiments, polymers can be substantially free of cross-links. In some embodiments, polymers can be used in accordance with the present invention without undergoing a cross-linking step. It is further to be understood that the synthetic nanocarriers may comprise block copolymers, graft copolymers, blends, mixtures, and/or adducts of any of the foregoing and other polymers. Those skilled in the art will recognize that the polymers listed herein represent an exemplary, not comprehensive, list of polymers that can be of use in accordance with the present invention provided they meet the desired criteria.

The properties of these and other polymers and methods for preparing them are well known in the art (see, for example, U.S. Patents 6,123,727; 5,804,178; 5,770,417; 5,736,372; 5,716,404; 6,095,148; 5,837,752; 5,902,599; 5,696,175; 5,514,378; 5,512,600; 5,399,665; 5,019,379; 5,010,167; 4,806,621; 4,638,045; and 4,946,929; Wang et al., 2001, J. Am. Chem. Soc., 123:9480; Lim et al., 2001, J. Am. Chem. Soc., 123:2460; Langer, 2000, Acc. Chem. Res., 33:94; Langer, 1999, J. Control. Release, 62:7; and Uhrich et al., 1999, Chem. Rev., 99:3181). More generally, a variety of methods for synthesizing certain suitable polymers are described in Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, Ed. by Goethals, Pergamon Press, 1980; Principles of Polymerization by Odian, John Wiley & Sons, Fourth Edition, 2004; Contemporary Polymer Chemistry by Allcock et al., Prentice-Hall, 1981; Deming et al., 1997, Nature, 390:386; and in U.S. Patents 6,506,577, 6,632,922, 6,686,446, and 6,818,732.

A wide variety of synthetic nanocarriers can be used according to the invention. In some embodiments, synthetic nanocarriers are spheres or spheroids. In some embodiments, synthetic nanocarriers are flat or plate-shaped. In some embodiments, synthetic nanocarriers are cubes or cubic. In some embodiments, synthetic nanocarriers are ovals or ellipses. In some embodiments, synthetic nanocarriers are cylinders, cones, or pyramids.

In some embodiments, it is desirable to use a population of synthetic nanocarriers that is relatively uniform in terms of size or shape so that each synthetic nanocarrier has similar properties. For example, at least 80%, at least 90%, or at least 95% of the synthetic nanocarriers, based on the total number of synthetic nanocarriers, may have a minimum dimension or maximum dimension that falls within 5%, 10%, or 20% of the average diameter or average dimension of the synthetic nanocarriers.

Compositions according to the invention can comprise elements in combination with pharmaceutically acceptable excipients, such as preservatives, buffers, saline, or phosphate buffered saline. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. In an embodiment, compositions, such as those comprising the synthetic nanocarriers are suspended in sterile saline solution for injection together with a preservative.

In some embodiments, any component of the synthetic nanocarriers as provided herein may be isolated. Isolated refers to the element being separated from its native environment and present in sufficient quantities to permit its identification or use. This means, for example, the element may be purified as by chromatography or electrophoresis. Isolated elements may be, but need not be, substantially pure. Because an isolated element may be admixed with a pharmaceutically acceptable excipient in a pharmaceutical preparation, the element may comprise only a small percentage by weight of the preparation. The element is nonetheless isolated in that it has been separated from the substances with which it may be associated in living systems, i.e., isolated from other lipids or proteins. Any of the elements provided herein may be isolated and included in the compositions or used in the methods in isolated form.

D. METHODS OF MAKING AND USING THE COMPOSITIONS AND RELATED METHODS

Synthetic nanocarriers may be prepared using a wide variety of methods known in the art. For example, synthetic nanocarriers can be formed by methods such as nanoprecipitation, flow focusing using fluidic channels, spray drying, single and double emulsion solvent

evaporation, solvent extraction, phase separation, milling (including cryomilling), supercritical fluid (such as supercritical carbon dioxide) processing, microemulsion procedures, microfabrication, nanofabrication, sacrificial layers, simple and complex coacervation, and other methods well known to those of ordinary skill in the art. Alternatively or additionally, aqueous and organic solvent syntheses for monodisperse semiconductor, conductive, magnetic, organic, and other nanomaterials have been described (Pellegrino et al., 2005, Small, 1:48; Murray et al., 2000, Ann. Rev. Mat. Sci., 30:545; and Trindade et al., 2001, Chem. Mat., 13:3843). Additional methods have been described in the literature (see, e.g., Doubrow, Ed., "Microcapsules and Nanoparticles in Medicine and Pharmacy," CRC Press, Boca Raton, 1992; Mathiowitz et al., 1987, J. Control. Release, 5:13; Mathiowitz et al., 1987, Reactive Polymers, 6:275; and Mathiowitz et al., 1988, J. Appl. Polymer Sci., 35:755; US Patents 5578325 and 6007845; P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010)).

Various materials may be encapsulated into synthetic nanocarriers as desirable using a variety of methods including but not limited to C. Astete et al., "Synthesis and characterization of PLGA nanoparticles" J. Biomater. Sci. Polymer Edn, Vol. 17, No. 3, pp. 247–289 (2006); K. Avgoustakis "Pegylated Poly(Lactide) and Poly(Lactide-Co-Glycolide) Nanoparticles: Preparation, Properties and Possible Applications in Drug Delivery" Current Drug Delivery 1:321-333 (2004); C. Reis et al., "Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles" Nanomedicine 2:8– 21 (2006); P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010). Other methods suitable for encapsulating materials into synthetic nanocarriers may be used, including without limitation methods disclosed in United States Patent 6,632,671 to Unger issued October 14, 2003.

In certain embodiments, synthetic nanocarriers are prepared by a nanoprecipitation process or spray drying. Conditions used in preparing synthetic nanocarriers may be altered to yield particles of a desired size or property (e.g., hydrophobicity, hydrophilicity, external morphology, "stickiness," shape, etc.). The method of preparing the synthetic nanocarriers and the conditions (*e.g.*, solvent, temperature, concentration, air flow rate, etc.) used may depend on the materials to be included in the synthetic nanocarriers and/or the composition of the carrier matrix.

If synthetic nanocarriers prepared by any of the above methods have a size range outside of the desired range, such synthetic nanocarriers can be sized, for example, using a sieve.

In embodiments, the synthetic nanocarriers can be combined with an antigen or other composition by admixing in the same vehicle or delivery system.

Compositions provided herein may comprise inorganic or organic buffers (*e.g.*, sodium or potassium salts of phosphate, carbonate, acetate, or citrate) and pH adjustment agents (*e.g.*, hydrochloric acid, sodium or potassium hydroxide, salts of citrate or acetate, amino acids and their salts) antioxidants (*e.g.*, ascorbic acid, alpha-tocopherol), surfactants (*e.g.*, polysorbate 20, polysorbate 80, polyoxyethylene9-10 nonyl phenol, sodium desoxycholate), solution and/or cryo/lyo stabilizers (*e.g.*, sucrose, lactose, mannitol, trehalose), osmotic adjustment agents (*e.g.*, salts or sugars), antibacterial agents (*e.g.*, benzoic acid, phenol, gentamicin), antifoaming agents (*e.g.*, polydimethylsilozone), preservatives (*e.g.*, thimerosal, 2-phenoxyethanol, EDTA), polymeric stabilizers and viscosity-adjustment agents (*e.g.*, polyvinylpyrrolidone, poloxamer 488, carboxymethylcellulose) and co-solvents (*e.g.*, glycerol, polyethylene glycol, ethanol).

Compositions according to the invention may comprise pharmaceutically acceptable excipients. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. Techniques suitable for use in practicing the present invention may be found in Handbook of Industrial Mixing: Science and Practice, Edited by Edward L. Paul, Victor A. Atiemo-Obeng, and Suzanne M. Kresta, 2004 John Wiley & Sons, Inc.; and Pharmaceutics: The Science of Dosage Form Design, 2nd Ed. Edited by M. E. Auten, 2001, Churchill Livingstone. In an embodiment, compositions are suspended in a sterile saline solution for injection together with a preservative.

It is to be understood that the compositions of the invention can be made in any suitable manner, and the invention is in no way limited to compositions that can be produced using the methods described herein. Selection of an appropriate method of manufacture may require attention to the properties of the particular elements being associated.

In some embodiments, compositions are manufactured under sterile conditions or are initially or terminally sterilized. This can ensure that resulting compositions are sterile and non-infectious, thus improving safety when compared to non-sterile compositions. This provides a valuable safety measure, especially when subjects receiving the compositions have immune defects, are suffering from infection, and/or are susceptible to infection. In some

embodiments, the compositions may be lyophilized and stored in suspension or as lyophilized powder depending on the formulation strategy for extended periods without losing activity.

Administration according to the present invention may be by a variety of routes, including but not limited to intradermal, intramuscular, subcutaneous, intravenous, and intraperitoneal routes. The compositions referred to herein may be manufactured and prepared for administration using conventional methods.

The compositions of the invention can be administered in effective amounts, such as the effective amounts described elsewhere herein. Doses of dosage forms may contain varying amounts of elements according to the invention. The amount of elements present in the inventive dosage forms can be varied according to their nature, the therapeutic benefit to be accomplished, and other such parameters. In embodiments, dose ranging studies can be conducted to establish optimal therapeutic amounts to be present in the dosage form. In embodiments, the elements are present in the dosage form in an amount effective to generate a desired effect and/or a reduced immune response upon administration to a subject. It may be possible to determine amounts to achieve a desired result using conventional dose ranging studies and techniques in subjects. Inventive dosage forms may be administered at a variety of frequencies. In an embodiment, at least one administration of the compositions provided herein is sufficient to generate a pharmacologically relevant response.

Another aspect of the disclosure relates to kits. In some embodiments of any one of the kits provided, the kit comprises any one of the synthetic nanocarrier compositions provided herein. In some embodiments of any one of the kits provided, the kit further comprises an antigen. In some embodiments of any one of the kits provided, the container comprising any one of the synthetic nanocarrier compositions provided herein is a vial or an ampoule. In some embodiments of any one of the kits provided, the compositions are in lyophilized form and may be reconstituted at a subsequent time. In some embodiments of any one of the kits provided, the kit further comprises instructions for reconstitution, mixing, administration, etc. In some embodiments of any one of the kits provided, the instructions include a description of the methods described herein. Instructions can be in any suitable form, *e.g.*, as a printed insert or a label. In some embodiments of any one of the kits provided herein, the kit further comprises one or more syringes or other device(s) that can deliver synthetic nanocarriers *in vivo* to a subject.

EXAMPLES

Example 1 – Lyophilized Synthetic Nanocarriers

It was found that different components of a lyophilization composition can help facilitate lyophilization, reduce aggregation (e.g., following reconstitution), and/or allow for long-term storage at 2-8 °C (e.g., following lyophilization). Also found was that the use of surfactants may lead to solubilization of an immunosuppressant, such as rapamycin, and/or disruption of the synthetic nanocarriers. Also found, is the benefit, in some embodiments, of buffer components that help maintain neutral pH.

As an example, it was found that Tris buffer can help avoid a drop in pH that can occur with phosphate buffers upon freezing. To make a Tris buffer, tromethamine (tris(hydroxymethyl)aminomethane) and Tris hydrochloride (Tris HCl) were mixed and, prefereably in some embodiments, maintain a pH near neutral. The Tris buffer, in some embodiments, was at a concentration of 10mM and/or at a pH 7.3 (at 25 °C). The Tris buffer, in some embodiments, comprised tromethamine at a concentration of 1.3 mM and Tris HCL at a concentration of 8.7 mM.

Experimental formulations were also evaluated based on their ability to prevent nanoparticle aggregation following lyophilization and during storage. Various formulations were tested that included sucrose, trehalose, mannitol, and sucrose/mannitol mixtures. Formulations, such as those containing sucrose, consistently yielded suitable product, rapid reconstitution, no visible aggregates upon reconstitution, and little to no particle size increase following lyophilization. The formulations with sucrose also continued to display these properties through 12 months of stability testing. A number of sucrose concentrations, such as those ranging from 4 to 9.6 wt%, were found to display similar protection against aggregation (**FIG. 1**).

Based on these studies, an exemplary formulation selected for lyophilization was found to be one that contains synthetic nanocarriers as provided herein at a concentration of 2 mg/mL rapamycin, sucrose at a concentration of 9.6 wt%, and 10 mM pH 7.3 Tris buffer. The vial size was 20 mL to help with the drying rate during lyophilization.

Example 2 – Synthetic Nanocarriers with Super-Saturated Amounts of Rapamycin

Nanocarrier compositions containing the polymers PLGA (3:1 lactide:glycolide, inherent viscosity 0.39 dL/g) and PLA-PEG (5 kDa PEG block, inherent viscosity 0.36 dL/g) as well as the agent rapamycin (RAPA) were synthesized using an oil-in-water emulsion evaporation method. The organic phase was formed by dissolving the polymers and RAPA in dichloromethane. The emulsion was formed by homogenizing the organic phase in an aqueous phase containing the surfactant polyvinylalcohol (PVA). The emulsion was then combined with a larger amount of aqueous buffer and mixed to allow evaporation of the solvent. The RAPA content in the different compositions was varied such that the compositions crossed the RAPA saturation limit of the system as the RAPA content was increased. The RAPA content at the saturation limit for the composition was calculated using the solubility of the RAPA in the aqueous phase and in the dispersed nanocarrier phase. For compositions containing PVA as the primary solute in the aqueous phase, it was found that the RAPA solubility in the aqueous phase is proportional to the PVA concentration such that the RAPA is soluble at a mass ratio of 1:125 to dissolved PVA. For compositions containing the described PLGA and PLA-PEG as the nanocarrier polymers, it was found that the RAPA solubility in the dispersed nanocarrier phase was 7.2% wt/wt. The following formula may be used to calculate the RAPA content at the saturation limit for the composition:

$$RAPA\ content = V(0.008c_{PVA} + 0.072c_{pol})$$

where c_{PVA} is the mass concentration of PVA, c_{pol} is the combined mass concentration of the polymers, and V is the volume of the nanocarrier suspension at the end of evaporation.

Sample ID	Calc. Over Saturation (%)	RAPA Load (%)	Diameter (nm)
1	-50	2.5	143
2	-25	3.8	146
3	1	4.9	147
4	23	4.9	130
5	48	8.1	160
6	73	9.8	189
7	98	12.4	203

For 1, 2 and 3, a consistent 60% of the RAPA is not recovered, indicating a sub-saturation equilibrium regime between the aqueous and organic phases. For the remaining nanocarriers containing higher amounts of RAPA, a consistent 6.8 mg of RAPA is not recovered. This consistent absolute mass loss indicates that the system is in an oversaturated regime (i.e., is super-saturated in one or more phases).

Example 3 - Synthetic Nanocarriers with Super-Saturated Amounts of Rapamycin

Nanocarrier compositions containing the polymers PLA (inherent viscosity 0.41 dL/g) and PLA-PEG (5 kDa PEG block, inherent viscosity 0.50 dL/g) as well as the agent RAPA were synthesized using the oil-in-water emulsion evaporation method described in **Example 2**. The RAPA content in the different compositions was varied such that the compositions crossed the RAPA saturation limit of the system as the RAPA content was increased. The RAPA content at the saturation limit for the composition was calculated using the method described in **Example 2**. For compositions containing the described PLA and PLA-PEG as the nanocarrier polymers, it was found that the RAPA solubility in the dispersed nanocarrier phase was 8.4% wt/wt. The following formula may be used to calculate the RAPA content at the saturation limit for the composition:

$$RAPA\ content = V(0.008c_{PVA} + 0.084c_{pol})$$

where c_{PVA} is the mass concentration of PVA, c_{pol} is the combined mass concentration of the polymers, and V is the volume of the nanocarrier suspension at the end of evaporation. All nanocarrier lots were filtered through 0.22 μm filters at the end of formation.

Sample ID	Calc. Over Saturation (%)	RAPA Load (%)	Unwashed Diameter (nm)	Final Diameter (nm)	Filter Throughput (g/m^2)
10	-10	5.4	145	149	>171
11	0	6.2	150	155	>180
12	10	6.1	151	154	>170
13	20	6.1	148	148	80
14	30	6.2	171	151	28
15	40	5.8	202	154	16

Despite adding increasing amount of RAPA to nanocarriers 12-15, the final RAPA content in the nanocarriers does not increase while filter throughput decreased. This indicates that the compositions were oversaturated with RAPA, and the excess RAPA was removed during washing and/or filtration.

Example 4 – More Rapid Solvent Evaporation and Low HLB Surfactant Results in Synthetic Nanocarriers with Super-Saturated Amounts of Rapamycin that are also Initially Sterile Filterable

Materials and Methods

PLA with an inherent viscosity of 0.41 dL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 100 DL 4A. PLA-PEG-OMe block co-polymer with a methyl ether terminated PEG block of approximately 5,000 Da and an overall inherent viscosity of 0.50 DL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 100 DL mPEG 5000 5CE. Rapamycin was purchased from Concord Biotech Limited, 1482-1486 Trasad Road, Dholka 382225, Ahmedabad India. Product code SIROLIMUS. EMPROVE® Polyvinyl Alcohol 4-88 (PVA), USP (85-89% hydrolyzed, viscosity of 3.4-4.6 mPa·s) was purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027), product code 1.41350. Cellgro PBS 1X (PBS), was purchased from Corning Incorporated, (One Riverfront Plaza Corning, NY 14831 USA), part number 21-040-CV. Dulbecco's phosphate buffered saline 1X (DPBS) was purchased from Lonza (Muenchensteinerstrasse 38, CH-4002 Basel, Switzerland), product code 17-512Q. Sorbitan monopalmitate was purchased from Croda International (300-A Columbus Circle, Edison, NJ 08837), product code SPAN 40.

For sample 1, solutions were prepared as follows:

Solution 1: A polymer and rapamycin mixture was prepared by dissolving PLA at 18.75 mg per mL, PLA-PEG-Ome at 6.25 mg per mL, and rapamycin at 4.7 mg per mL of dichloromethane. **Solution 2:** PVA was prepared at 50 mg/mL in 100 mM pH 8 phosphate buffer.

An O/W emulsion was prepared by combining Solution 1 (1.0 mL) and Solution 2 (3.0 mL) in a small glass pressure tube, vortex mixed for 10 seconds, and was then emulsified by sonication at 30% amplitude for 1 minute with the pressure tube immersed in an ice water bath using a Branson Digital Sonifier 250. The emulsion was then added to an open 500 mL beaker

containing DPBS (30 mL). A second O/W emulsion was prepared using the same materials and method as above and then added to the same container containing the first emulsion and DPBS. This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate and for the nanocarriers to form. A portion of the nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g and 4 °C for 50 minutes, removing the supernatant, and re-suspended the pellet in DPBS containing 0.25% w/v PVA. The wash procedure was repeated and then the pellet was re-suspended in DPBS containing 0.25% w/v PVA to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. An identical formulation was prepared in a separate 500 mL beaker, processed the same, and pooled together with the first formulation just prior to sterile filtration. The nanocarrier suspension was then filtered using a 33mm diameter 0.22 μm PES membrane syringe filter (Millipore part number SLGP033RB). The filtered nanocarrier suspension was then stored at -20°C.

For sample 2, solutions were prepared as follows:

Solution 1: A polymer and rapamycin mixture was prepared by dissolving PLA at 75 mg per mL, PLA-PEG-Ome at 25 mg per mL, and rapamycin at 16 mg per mL in dichloromethane. **Solution 2:** A sorbitan monopalmitate mixture was prepared by dissolving Span 40 at 20 mg/mL in dichloromethane. **Solution 3:** Polyvinyl alcohol was prepared at 50 mg per mL in 100 mM pH 8 phosphate buffer. **Solution 4:** Dichloromethane was filtered using a 0.20μm PTFE membrane syringe filter (VWR part number 28145-491).

An O/W emulsion was prepared by combining Solution 1 (0.5 mL), Solution 2 (0.125 mL), and Solution 4 (0.375 mL), and Solution 3 (3.0 mL) in a small glass pressure tube, vortex mixed for 10 seconds, and was then emulsified by sonication at 30% amplitude for 1 minute with the pressure tube immersed in an ice water bath using a Branson Digital Sonifier 250. The emulsion was then added to a 50 mL beaker containing DPBS (30 mL). A second O/W emulsion was prepared using the same materials and method as above and then added to the same beaker containing the first emulsion and DPBS. The nanocarrier suspension was then processed in the same way as sample 1.

For sample 3, solutions were prepared as follows:

Solution 1: A polymer and rapamycin mixture was prepared by dissolving PLA at 37.5 mg per mL, PLA-PEG-Ome at 12.5 mg per mL, and rapamycin at 8 mg per mL in dichloromethane. **Solution 2:** Polyvinyl alcohol was prepared at 75 mg per mL in 100 mM pH 8 phosphate buffer.

An O/W emulsion was prepared by combining Solution 1 (1 mL) and Solution 2 (3.0 mL) in a small glass pressure tube, vortex mixed for 10 seconds, and was then emulsified by sonication at 30% amplitude for 1 minute with the pressure tube immersed in an ice water bath using a Branson Digital Sonifier 250. An O/W emulsion was formed using the same method as described above for sample 1. After emulsification by sonication, the emulsion was added to a 50 mL beaker containing DPBS (30 mL). A second O/W emulsion was prepared using the same materials and method as above and then added to the same solvent evaporation container. The emulsion was allowed to stir for 2 hours to allow for the organic solvent to evaporate and for the nanocarriers to form. A portion of the nanocarriers was then washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g for 50 minutes, removing the supernatant, and re-suspended the pellet in PBS. The wash procedure was repeated and then the pellet was re-suspended in PBS to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The nanocarrier suspension was then filtered using a 33mm diameter 0.22 μm PES membrane syringe filter (Millipore part number SLGP033RB). The filtered nanocarrier suspension was then stored at -20°C.

Nanocarrier size was determined by dynamic light scattering. The amount of rapamycin in the nanocarrier was determined by HPLC analysis. The total dry-nanocarrier mass per mL of suspension was determined by a gravimetric method.

Lot number	SE container	Low HLB surfactant	Surface area of container (cm^2)	Calculated filter throughput (g NP/m^2)	Rapamycin load (%)	Size (nm)	Yield (%)
1	500 mL beaker	None	64	>133	9.84	148	81
2	50 mL beaker	SPAN 40	14	>178	9.32	165	93
3	50 mL beaker	None	14	47	7.38	119	73

Group	**Lot ID**	**SE Container**	**Low HLB Surfactant**	**DLS Diameter (nm)**	**RAPA Load (%)**	**Filterability (g NP/m^2)**
1	PBS	N/A	N/A	N/A	N/A	N/A

6	Sample 1	500 mL beaker	None	148	9.8	>133
12	Sample 2	50 mL beaker	SPAN 40	165	9.3	>178
All	KLH, Sigma #H7017					

Example 5 – Method for Determining Super-Saturation

Materials and Methods

PLA with an inherent viscosity of 0.41 dL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 100 DL 4A. Rapamycin was purchased from Concord Biotech Limited, 1482-1486 Trasad Road, Dholka 382225, Ahmedabad India. Product code SIROLIMUS.

Solutions were prepared as follows:

Solution 1: A polymer solution was prepared by dissolving PLA at 100 mg per mL of dichloromethane. **Solution 2:** A rapamycin solution was prepared by dissolving rapamycin at 100 mg per mL of dichloromethane.

Glass microscope slides were cleaned with 70% isopropanol and allowed to dry on a clean, flat surface in a chemical fume hood. Mixture 1 was prepared by mixing 100 µL of Solution 1 with 100 µL of dichloromethane in a glass vial with a solvent resistant screw cap and mixed by vortex mixing. Mixture 2 was prepared using the same method as Mixture 1, with 100 µL of Solution 1, 33.3 µL of Solution 2, and 66.7 µL of dichloromethane. Mixture 3 was prepared using the same method as Mixture 1, using 100 µL of Solution 1 with 66.7 µL of Solution 2, and 33.3 µL of dichloromethane. Next, 50 µL of each mixture was applied to separate locations on the clean glass slides and allowed to dry overnight in the fume hood at room temperature. A digital image was taken of each dry film and analyzed using image analysis software. Normalized mean intensity increases can show the film becoming opaque above the saturation point.

	Film						Bgrd				
Mix.	*Area*	*Mean Intensity*	*Standard Deviation*	*Min*	*Max*	*Area*	*Mean Intensity*	*Standard Deviation*	*Min*	*Max*	*Normalized Mean Intensity*
1	45153	39.9	7.3	18	174	45588	38.6	6	18	123	1.3

2	43444	47.6	7.7	16	148	49698	40.5	5.7	19	95	7.1
3	63995	57.1	35.9	12	232	64441	23.4	4.4	8	85	33.7

Example 6 - Low HLB Surfactant, SM, Increases RAPA Loading and Synthetic Nanocarrier Filterability

Nanocarrier compositions containing the polymers PLA (inherent viscosity 0.41 dL/g) and PLA-PEG (5 kDa PEG block, inherent viscosity 0.50 dL/g) as well as the hydrophobic drug rapamycin (RAPA) were synthesized, with or without the addition of the low HLB surfactant sorbitan monopalmitate (SM), using the oil-in-water emulsion evaporation method. The organic phase was formed by dissolving the polymers and RAPA in dichloromethane. The emulsion was formed by homogenizing the organic phase in an aqueous phase containing the surfactant PVA using a probe-tip sonicator. The emulsion was then combined with a larger amount of aqueous buffer and mixed to allow dissolution and evaporation of the solvent. The resulting nanocarriers were washed and filtered through a 0.22 μm filter. All compositions contained 100 mg of polymer. The RAPA content in the different compositions was varied.

Sample ID	RAPA Added to Composition (mg)	SM Added to Composition (mg)	Unwashed Diameter (nm)	Final Diameter (nm)	RAPA Load (%)	Filter Throughput (g/m^2)
1	12.2	0	148	148	6.1	80
2	13.3	0	171	151	6.2	28
3	14.3	0	202	154	5.8	16
4	13.6	5	156	161	9.2	>174
5	17	5	168	170	11.8	>184
6	20.4	5	181	179	14.9	77

For the compositions not containing the surfactant SM (samples 1, 2, and 3), several indications of a limiting ability to fully incorporate RAPA in the nanocarrier composition were observed as increasing amounts of RAPA were added. The increasing difference between the pre- and post-filtration nanocarrier sizes at the higher RAPA formulation levels in the absence of SM were indicative of the presence of larger particulates (individual particles or aggregates) being removed during the washing and/or filtration processes. This was also indicated by the decreased filter throughput before clogging. Finally, adding increasing amounts of RAPA to nanocarrier compositions without SM did not result in increased RAPA loading (for example,

sample 1 compared to sample 3), indicating that the additional RAPA was separable from the bulk of the nanocarriers and was removed during the washing and/or filtration steps.

By contrast, the compositions containing the surfactant SM readily incorporated increased amounts of RAPA. The nanocarrier size was not affected by filtration, and increasing the amount of RAPA added to the composition resulted in increased RAPA loading of the nanocarriers. Some filter throughput reduction was observed at the highest loading level (sample 6), but this may be due to the inherently larger nanocarrier size. In sum, the incorporation of SM helped to increase RAPA loading and filterability of the synthetic nanocarrier compositions.

Example 7 - SM and Cholesterol Increased RAPA Loading and Filterability

Nanocarrier compositions were produced using the materials and methods as described in **Example 6**. Nanocarriers containing polymer and RAPA were produced with varying RAPA load levels. In addition, nanocarriers highly loaded with RAPA were also produced using an excipient, the surfactant SM or cholesterol, in an excipient:RAPA mass ratio of 3.2:1.

Sample ID	Excipient	Diameter (nm)	RAPA Load (%)	Filter Throughput (g/m^2)
7	-	131	5.6	>148
8	-	138	7.9	37
9	SM	165	9.3	>178
10	cholesterol	166	14.3	>180

The samples of nanocarriers produced in the absence of excipients (samples 7 and 8) demonstrated that the increase in RAPA loading beyond a point of apparent nanocarrier saturation tends to lead to a reduction in filter throughput. The addition of either SM or cholesterol resulted in greater RAPA loading while maintaining stability (samples 9 and 10).

Example 8– Effects of Low HLB Surfactant on RAPA Load and Filterability

Materials and Methods

PLA with an inherent viscosity of 0.41 dL/g was purchased from Lakeshore Biomaterials (756 Tom Martin Drive, Birmingham, AL 35211), product code 100 DL 4A.

PLA-PEG-OMe block co-polymer with a methyl ether terminated PEG block of approximately 5,000 Da and an overall inherent viscosity of 0.50 DL/g was purchased from Lakeshore Biomaterials (756 Tom Martin Drive, Birmingham, AL 35211), product code 100 DL mPEG 5000 5CE. Rapamycin was purchased from Concord Biotech Limited (1482-1486 Trasad Road, Dholka 382225, Ahmedabad India), product code SIROLIMUS. EMPROVE® Polyvinyl Alcohol 4-88, USP (85-89% hydrolyzed, viscosity of 3.4-4.6 mPa·s) was purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027), product code 1.41350. Dulbecco's phosphate buffered saline 1X (DPBS) was purchased from Lonza (Muenchensteinerstrasse 38, CH-4002 Basel, Switzerland), product code 17-512Q. Sorbitan monopalmitate was purchased from Croda International (300-A Columbus Circle, Edison, NJ 08837), product code SPAN 40. Polysorbate 80 was purchased from NOF America Corporation (One North Broadway, Suite 912
White Plains, NY 10601), product code Polysorbate80 (HX2). Sorbitan monolaurate (SPAN 20) was purchased from Alfa Aesar (26 Parkridge Rd Ward Hill, MA 01835), product code L12099. Sorbitan stearate (SPAN 60) was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code S7010. Sorbitan monooleate (SPAN 80) was purchased from Tokyo Chemical Industry Co., Ltd. (9211 North Harborgate Street Portland, OR 97203), product code S0060. Octyl β-D-glucopyranoside was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code O8001. Oleyl alcohol was purchased from Alfa Aesar (26 Parkridge Rd Ward Hill, MA 01835), product code A18018. Isopropyl palmitate was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code W515604. Polyethylene glycol hexadecyl ether (BRIJ 52) was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code 388831. Polyethylene glycol oleyl ether (BRIJ 93) was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code 388866. Poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (Pluronic L-31) was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code 435406. Poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (Pluronic P-123) was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code 435465. Palmitic Acid was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code P0500. DL-α-palmitin was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code M1640. Glyceryl Tripalmitate was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code T5888.

For Sample 11, solutions were prepared as follows:

Solution 1: A polymer and rapamycin mixture was prepared by dissolving PLA at 75 mg/mL, PLA-PEG-Ome at 25 mg/mL, and rapamycin at 16 mg/mL in dichloromethane. **Solution 2**: A Polysorbate80 mixture was prepared by dissolving Polysorbate80 at 80 mg/mL in dichloromethane. **Solution 3:** Polyvinyl alcohol was prepared at 50 mg/mL in 100 mM pH 8 phosphate buffer.

An O/W emulsion was prepared by combining Solution 1 (0.5 mL), Solution 2 (0.1 mL), dichloromethane (0.4 mL) and Solution 3 (3.0 mL) in a small glass pressure tube, vortex mixed for 10 seconds, and was then sonicated at 30% amplitude for 1 minute with the pressure tube immersed in an ice water bath, using a Branson Digital Sonifier 250. The emulsion was then added to a 50 mL beaker containing DPBS (30 mL). A second O/W emulsion was prepared using the same materials and method as above and then added to the same container containing the first emulsion and DPBS. This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate and for the nanocarriers to form. A portion of the nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g and 4 °C for 50 minutes, removing the supernatant, and re-suspended the pellet in DPBS containing 0.25% w/v PVA. The wash procedure was repeated and then the pellet was re-suspended in DPBS containing 0.25% w/v PVA to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The nanocarrier suspension was then filtered using a 0.22 μm PES membrane syringe filter (Millipore part number SLGP033RB). The filtered nanocarrier suspension was then stored at -20°C.

For samples 12-25, solutions were prepared as follows:

Solution 1: A polymer and rapamycin mixture was prepared by dissolving PLA at 75 mg/mL, PLA-PEG-Ome at 25 mg/mL, and rapamycin at 16 mg/mL in dichloromethane. **Solution 2**: The HLB mixture was prepared by dissolving the HLB surfactant at 5.0 mg/mL in dichloromethane. HLB surfactants include SPAN 20, SPAN 40, SPAN 60, SPAN 80, octyl β-D-glucopyranoside, oleyl acid, isopropyl palmitate, BRIJ 52, BRIJ 93, Pluronic L-31, Pluronic P-123, palmitic acid, DL-α-palmitin, and glyceryl tripalmitate. **Solution 3:** Polyvinyl alcohol was prepared at 62.5 mg/mL in 100 mM pH 8 phosphate buffer.

An O/W emulsion was prepared by combining Solution 1 (0.5 mL), Solution 2 (0.5 mL), and Solution 3 (3.0 mL) in a small glass pressure tube, vortex mixed for 10 seconds, and was then sonicated at 30% amplitude for 1 minute with the pressure tube immersed in an ice water bath using a Branson Digital Sonifier 250. The emulsion was then added to a 50 mL

beaker containing DPBS (30 mL). A second O/W emulsion was prepared using the same materials and method as above and then added to the same beaker containing the first emulsion and DPBS. This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate and for the nanocarriers to form. A portion of the nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g and 4 °C for 50 minutes, removing the supernatant, and re-suspended the pellet in DPBS containing 0.25% w/v PVA. The wash procedure was repeated and then the pellet was re-suspended in DPBS containing 0.25% w/v PVA to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The nanocarrier suspension was then filtered using a 0.22 µm PES membrane syringe filter (Millipore part number SLGP033RB). The filtered nanocarrier suspension was then stored at -20°C.

Sample	Organic Phase Surfactant	HLB of Surfactant	Size (nm)	Filtration	Number of Filters	Calculated g NP/m^2	Yield (%)	Rapamycin Load (%)
11	Polysorbate 80	15	184	Millex 0.22 µm	>1	22	91	9.7
12	SPAN 20	8.6	148	Millex 0.22 µm	1	>144	71	11.2
13	SPAN 40	6.7	149	Millex 0.22 µm	1	>154	77	11.2
14	SPAN 60	4.7	151	Millex 0.22 µm	1	>154	77	11.0
15	SPAN 80	4.3	144	Millex 0.22 µm	1	>169	85	11.1
16	octyl β-D-glucopyranoside	12	127	Millex 0.22 µm	3	47	64	6.7
17	oleyl alcohol	1.3	165	Millex 0.22 µm	1	>157	78	12.5
18	isopropyl palmitate	2.9	171	Millex 0.22 µm	1	>144	71	10.9
19	Brij 52	5	182	Millex 0.22 µm	1	>138	77	11.2
20	Brij 93	4	174	Millex 0.22 µm	1	>158	79	11.9
21	Pluronic L-31	1-7	169	Millex 0.22 µm	4	31	70	8.5
22	Pluronic P-123	7-9	162	Millex 0.22 µm	1	>145	72	10.7
23	Palmitic Acid	3.2	132	Millex 0.22 µm	1	>141	71	1.0
24	DL-α-palmitin	7.2	153	Millex 0.22 µm	3	51	68	7.4

25	Glyceryl Tripalmitate	4.3	168	Millex 0.22 µm	1	>146	73	10.0

The HLB for most of the low HLB surfactants was determined using publicly available information. For DL-α-Palmitin, the HLB was calculated using the following formula: Mw = 330.5 g/mol, hydrophilic portion = 119.0 g/mol; HLB = 119.0 / 330.5 * 100 / 5 = 7.2. For Glyceryl Palmitate, the HLB was calculated using the following formula: Mw = 807.3 g/mol, hydrophilic portion = 173.0 g/mol; HLB = 173.0 / 807.3 * 100 / 5 = 4.3. For Isopropyl Palmitate, the HLB was calculated using the following formula: Mw = 298.5 g/mol, hydrophilic portion = 44.0 g/mol; HLB = 44.0 / 298.5 * 100 / 5 = 2.9. For Oleyl Alcohol, the HLB was calculated using the following formula: Mw = 268.5 g/mol, hydrophilic portion =17.0 g/mol; HLB = 17.0 / 268.5 * 100 / 5 = 1.3. In addition, the load of low HLB surfactant was measured by extraction followed by quantitation by an HPLC method.

Example 9 – Effect of Low HLB Surfactant on Synthetic Nanocarrier Filterability

Materials and Methods

PLA-PEG-OMe block co-polymer with a methyl ether terminated PEG block of approximately 5,000 Da and an overall inherent viscosity of 0.50 DL/g was purchased from Evonik Industries (Rellinghauser Straße 1—11 45128 Essen, Germany), product code 100 DL mPEG 5000 5CE. PLA with an inherent viscosity of 0.41 dL/g was purchased from Evonik Industries (Rellinghauser Straße 1—11 45128 Essen Germany), product code 100 DL 4A. Rapamycin was purchased from Concord Biotech Limited, 1482-1486 Trasad Road, Dholka 382225, Ahmedabad India. Product code SIROLIMUS. Sorbitan monopalmitate was purchased from Croda (315 Cherry Lane New Castle Delaware 19720), product code SPAN 40. Dichloromethane was purchased from Spectrum (14422 S San Pedro Gardena CA, 90248-2027). Part number M1266. EMPROVE® Polyvinyl Alcohol 4-88, USP (85-89% hydrolyzed, viscosity of 3.4-4.6 mPa·s) was purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027), product code 1.41350. Dulbecco's Phosphate Buffered Saline, 1X, 0.0095 M (PO4), without calcium and magnesium, was purchased from BioWhittaker (8316 West Route 24 Mapleton, IL 61547), part number #12001, product code Lonza DPBS. Emulsification was carried out using a Branson Digital Sonifier 250 with a 1/8" tapered tip titanium probe.

Solutions were prepared as follows:

Solution 1: A polymer mixture was prepared by dissolving PLA-PEG-OMe (100 DL mPEG 5000 5CE) at 50 mg per 1 mL and PLA (100 DL 4A) at 150 mg per mL in dichloromethane. **Solution 2:** Rapamycin was dissolved at 160 mg per 1 mL in dichloromethane. **Solution 5:** Sorbitan monopalmitate (SPAN 40) was dissolved at 50 mg per 1 mL in dichloromethane. **Solution 6:** Dichloromethane was sterile filtered using a 0.2 μm PTFE membrane syringe filter (VWR part number 28145-491). **Solution 7:** A polyvinyl alcohol solution was prepared by dissolving polyvinyl alcohol (EMPROVE® Polyvinyl Alcohol 4-88) at 75 mg per 1 mL in 100 mM pH 8 phosphate buffer. **Solution 8:** A polyvinyl alcohol and Dulbecco's phosphate buffered saline, 1X, 0.0095 M (PO4) mixture was prepared by dissolving polyvinyl alcohol (EMPROVE® Polyvinyl Alcohol 4-88) at 2.5 mg per 1 mL in Dulbecco's phosphate buffered saline, 1X, 0.0095 M (PO4) (Lonza DPBS).

For sample 26, an O/W emulsion was prepared by combining Solution 1 (0.5 mL), Solution 2 (0.1 mL), Solution 5 (0.1 mL), and Solution 6 (0.30 mL) in a small glass pressure tube. The solution was mixed by repeat pipetting. Next, Solution 7 (3.0 mL) was added, and the formulation was vortex mixed for ten seconds. The formulation was then sonicated with the pressure tube immersed in an ice bath for 1 minute at 30% amplitude. The emulsion was then added to an open 50 mL beaker containing Lonza DPBS (30 mL). This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate and for the nanocarriers to form. A portion of the nanocarriers were washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g and 4 °C for 50 minutes, removing the supernatant, and re-suspending the pellet in Solution 8. The wash procedure was repeated and then the pellet was re-suspended in Solution 8 to achieve a nanocarrier suspension having a nominal concentration of 10 mg per mL on a polymer basis. The nanocarrier formulation was filtered using a 0.22 μm PES membrane syringe filter (Millex part number SLGP033RS). The mass of the nanocarrier solution filter throughput was measured. The filtered nanocarrier solution was then stored at -20°C.

For sample 27, an O/W emulsion was prepared by combining Solution 1 (0.5 mL), Solution 2 (0.1 mL), and Solution 6 (0.40 mL) in a small glass pressure tube. The solution was mixed by repeat pipetting. Next, Solution 7 (3.0 mL) was added, and the formulation was vortex mixed for ten seconds. The formulation was then sonicated with the pressure tube immersed in an ice bath for 1 minute at 30% amplitude. The emulsion was then added to a 50 mL open beaker containing Lonza DPBS (30 mL). This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate and for the nanocarriers to form. A

portion of the nanocarriers were washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g and 4 °C for 50 minutes, removing the supernatant, and re-suspending the pellet in Solution 8. The wash procedure was repeated and then the pellet was re-suspended in Solution 8 to achieve a nanocarrier suspension having a nominal concentration of 10 mg per mL on a polymer basis. The nanocarrier formulation was filtered using a 0.22 μm PES membrane syringe filter (Millex part number SLGP033RS). The mass of the nanocarrier solution filter throughput was measured. The filtered nanocarrier solution was then stored at -20°C.

Nanocarrier size was determined by dynamic light scattering. The amount of rapamycin in the nanocarrier was determined by HPLC analysis. The total dry-nanocarrier mass per mL of suspension was determined by a gravimetric method. The filterability was evaluated by the amount of filtrate that passed through the first filter.

The data show that for rapamycin, the incorporation of SPAN 40 in the synthetic nanocarriers resulted in an increase in filterability of the synthetic nanocarrier compositions.

Nanocarrier ID	Rapamycin	Low HLB Surfactant	Effective Diameter (nm)	Rapamycin Content (% w/w)	Nanocarrier Yield (%)	0.22μm Filter Throughput (g/m^2)
26	Rapamycin	SPAN 40	179	17.19	80	98
27	Rapamycin	None	226	17.56	75	10

Example 10 - SPAN 40 Greatly Increases Filterability of Synthetic Nanocarriers Comprising Polyester Polymers

Materials and Methods

PLA (100 DL 4A), with an inherent viscosity of 0.41 dL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 100 DL 4A. PLA-PEG-OMe block co-polymer with a methyl ether terminated PEG block of approximately 5,000 Da and an overall inherent viscosity of 0.50 DL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 100 DL mPEG 5000 5CE. Rapamycin was purchased from Concord Biotech Limited (1482-1486 Trasad Road, Dholka 382225, Ahmedabad India), product code SIROLIMUS. EMPROVE® Polyvinyl Alcohol 4-88 (PVA), USP (85-89% hydrolyzed, viscosity of 3.4-4.6 mPa·s) was purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027), product code 1.41350.

Dulbecco's phosphate buffered saline 1X (DPBS) was purchased from Lonza (Muenchensteinerstrasse 38, CH-4002 Basel, Switzerland), product code 17-512Q. Sorbitan monopalmitate (SPAN 40), was purchased from Croda International (300-A Columbus Circle, Edison, NJ 08837), product code Span 40. PLGA (5050 DLG 2.5A), with approximately 54% by weight lactide and 46% by weight glycolide, and an inherent viscosity of 0.24 dL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 5050 DLG 2.5A. PLGA (7525 DLG 4A), with approximately 73% by weight lactide and 27% by weight glycolide, and an inherent viscosity of 0.39 dL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 7525 DLG 4A. Polycaprolactone (PCL), average Mw 14,000 Da and Mn of 10,000 Da, was purchased from Sigma-Aldrich (3050 Spruce St. St. Louis, MO 63103), product code 440752.

For samples 1, 3, 5 and 7, solutions were prepared as follows:

Solution 1: PLA-PEG-Ome at 50 mg per mL, Span 40 at 10 mg per mL and rapamycin at 32 mg per mL were dissolved in dichloromethane. Solution 2: 100 DL 4A was dissolved in dichloromethane at 150 mg per mL. Solution 3: 5050 DLG 2.5A was dissolved in dichloromethane at 150 mg per mL. Solution 4: 7525 DLG 4A was dissolved in dichloromethane at 150 mg per mL. Solution 5: PCL was dissolved in dichloromethane at 150 mg per mL. Solution 6: PVA was prepared at 75 mg per mL in 100 mM pH 8 phosphate buffer.

An O/W emulsion was prepared by transferring Solution 1 (0.5 mL), to a thick walled glass pressure tube. To this, lot 1 added Solution 2 (0.5 mL), lot 3 added Solution 3 (0.5 mL), lot 5 added 4 (0.5 mL), and lot 7 added Solution 5 (0.5 mL). The two solutions were then mixed by repeat pipetting. Next, Solution 6 (3.0 mL) was added, the tube was vortex mixed for 10 seconds, and was then emulsified by sonication at 30% amplitude for 1 minute with the pressure tube immersed in an ice water bath using a Branson Digital Sonifier 250. The emulsion was then added to a 50 mL beaker containing DPBS (30 mL). This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate and for the nanocarriers to form. A portion of the nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g for 50 minutes, removing the supernatant, and re-suspended the pellet in DPBS. The wash procedure was repeated and then the pellet was re-suspended in DPBS to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The nanocarrier suspension was then filtered using a 0.22 μm PES membrane syringe filter (Millipore part number SLGP033RB), and if

necessary: 0.45 µm PES membrane syringe filter (PALL part number 4614), and/or a 1.2 µm PES membrane syringe filter (PALL part number 4656). The filtered nanocarrier suspension was then stored at -20°C.

Nanocarrier size was determined by dynamic light scattering. The amount of rapamycin in the nanocarrier was determined by HPLC analysis. Filterability was determined by comparing the weight of flow through of the first sterile 0.22 µm filter to the yield to determine the actual mass of nanocarriers that passed through prior to blocking the filter, or the total through the first and only filter. The total dry-nanocarrier mass per mL of suspension was determined by a gravimetric method.

For samples 2, 4, 6 and 8, solutions were prepared as follows:

Solution 1: A polymer and rapamycin mixture was prepared by dissolving PLA-PEG-Ome at 50 mg per mL, and rapamycin at 32 mg per mL in dichloromethane. Solution 2: 100 DL 4A was dissolved in dichloromethane at 150 mg per mL. Solution 3: 5050 DLG 2.5A was dissolved in dichloromethane at 150 mg per mL. Solution 4: 7525 DLG 4A was dissolved in dichloromethane at 150 mg per mL. Solution 5: PCL was dissolved in dichloromethane at 150 mg per mL. Solution 6: Polyvinyl alcohol was prepared at 75 mg per mL in 100 mM pH 8 phosphate buffer.

An O/W emulsion was prepared by transferring Solution 1 (0.5 mL), to a thick walled glass pressure tube. To this, lot 2 added Solution 2 (0.5 mL), lot 4 added Solution 3 (0.5 mL), lot 6 added 4 (0.5 mL), and lot 8 added Solution 5 (0.5 mL). The two solutions were then mixed by repeat pipetting. The addition of PVA solution, wash, filtration and storage are the same as above.

Nanocarrier size was evaluated the same as above.

The results show a significant increase in filterability of synthetic nanocarriers comprising polyester polymers with the inclusion of SPAN 40 in the synthetic nanocarriers.

Lot number	Core polymer	Excipient	Size (nm)	Filter throughput (g NP/m2)	Rapa load (%)	NP yield (%)
1	100 DL 4A	SPAN 40	160	>148	12.65	75
2	100 DL 4A	None	197	17	10.88	71
3	5050 DLG 2.5A	SPAN 40	153	>139	13.09	70
4	5050 DLG 2.5A	None	188	59	13.40	64
5	7525 DLG 4A	SPAN 40	164	>158	11.81	78
6	7525 DLG 4A	None	196	28	11.64	73

7	Polycaprolactone	SPAN 40	164	112	10.62	75
8	Polycaprolactone	None	173	52	10.29	78

Example 11– SPAN 40 Increases Filterability of Rapamycin

Materials and Methods

PLA with an inherent viscosity of 0.41 dL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 100 DL 4A. PLA-PEG-OMe block co-polymer with a methyl ether terminated PEG block of approximately 5,000 Da and an overall inherent viscosity of 0.50 DL/g was purchased from Evonik Industries AG (Rellinghauser Straße 1-11, Essen Germany), product code 100 DL mPEG 5000 5CE. Rapamycin was purchased from Concord Biotech Limited (1482-1486 Trasad Road, Dholka 382225, Ahmedabad India), product code SIROLIMUS. EMPROVE® Polyvinyl Alcohol 4-88, USP (85-89% hydrolyzed, viscosity of 3.4-4.6 mPa·s) was purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027), product code 1.41350. Dulbecco's phosphate buffered saline 1X (DPBS) was purchased from Lonza (Muenchensteinerstrasse 38, CH-4002 Basel, Switzerland), product code 17-512Q. Sorbitan monopalmitate was purchased from Croda International (300-A Columbus Circle, Edison, NJ 08837), product code SPAN 40.

Solutions were prepared as follows. **Solution 1:** A polymer and rapamycin mixture was prepared by dissolving PLA at 150 mg/mL and PLA-PEG-Ome at 50 mg/mL. **Solution 2**: A rapamycin solution was prepared at 100 mg/mL in dichloromethane. **Solution 6**: A sorbitan monopalmitate solution was prepared by dissolving SPAN 40 at 50 mg/mL in dichloromethane. **Solution 7:** Polyvinyl alcohol was prepared at 75 mg/mL in 100 mM pH 8 phosphate buffer.

O/W emulsions were prepared by adding Solution 1 (0.5mL), to a thick walled pressure tube. For lot 1, this was combined with Solution 6 (0.1 mL), and dichloromethane (0.28 mL). Lot 1 was then combined these with Solution 2 (0.12 mL). In a similar manner, lot 2 was combined with dichloromethane (0.38 mL), and then lot 2 was combined with Solution 2 (0.12 mL). For each individual lot the total volume of the organic phase was therefore 1 mL. The combined organic phase solutions were mixed by repeat pipetting. Next, Solution 7 (3.0 mL) was added, the pressure tube was vortex mixed for 10 seconds, and was then sonicated at 30% amplitude for 1 minute with the pressure tube immersed in an ice water bath using a Branson Digital Sonifier 250. The emulsion was then added to a 50 mL beaker containing DPBS (30

mL). This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate rapidly for the nanocarriers to form. A portion of the nanocarriers was washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g and 4 °C for 50 minutes, removing the supernatant, and re-suspended the pellet in DPBS containing 0.25% w/v PVA. The wash procedure was repeated and then the pellet was re-suspended in DPBS containing 0.25% w/v PVA to achieve a nanocarrier suspension having a nominal concentration of 10 mg/mL on a polymer basis. The nanocarrier suspension was then filtered using a 0.22 µm PES membrane syringe filter (Millipore part number SLGP033RB). The filtered nanocarrier suspension was then stored at -20°C.

The results show that the incorporation of SPAN 40 in synthetic nanocarriers increased the filterability of rapamycin.

Lot number	Rapamycin	Low HLB Surfactant	Calculated Filter Throughput (g NP/m2)	Size (nm)	Yield (%)	Rapamycin load (%)
1	Rapamycin	SPAN 40	>117	163	60	10.41
2	Rapamycin	None	21	189	58	11.38

Example 12– Shows the Effects of the Amounts of the Components on Rapamycin Load and Synthetic Nanocarrier Filterability

Materials and Methods

PLA-PEG-OMe block co-polymer with a methyl ether terminated PEG block of approximately 5,000 Da and an overall inherent viscosity of 0.50 DL/g was purchased from Evonik Industries (Rellinghauser Straße 1—11 45128 Essen, Germany), product code 100 DL mPEG 5000 5CE. PLA with an inherent viscosity of 0.41 dL/g was purchased from Evonik Industries (Rellinghauser Straße 1—11 45128 Essen Germany), product code 100 DL 4A. Rapamycin was purchased from Concord Biotech Limited, 1482-1486 Trasad Road, Dholka 382225, Ahmedabad India. Product code SIROLIMUS. Sorbitan monopalmitate was purchased from Croda (315 Cherry Lane New Castle Delaware 19720), product code SPAN 40. Dichloromethane was purchased from Spectrum (14422 S San Pedro Gardena CA, 90248-2027). Part number M1266. EMPROVE® Polyvinyl Alcohol 4-88, (PVA), USP (85-89% hydrolyzed, viscosity of 3.4-4.6 mPa·s) was purchased from EMD Chemicals Inc. (480 South Democrat Road Gibbstown, NJ 08027), product code 1.41350. Dulbecco's Phosphate

Buffered Saline (DPBS), 1X, 0.0095 M (PO4), without calcium and magnesium, was purchased from BioWhittaker (8316 West Route 24 Mapleton, IL 61547), part number #12001, product code Lonza DPBS. Emulsification was carried out using a Branson Digital Sonifier 250 with a 1/8" tapered tip titanium probe.

Solutions were prepared as follows:

Polymer Solution: A polymer mixture was prepared by dissolving PLA-PEG-OMe (100 DL mPEG 5000 5CE) and PLA (100 DL 4A) at the indicated mg per mL in dichloromethane at a 1:3 ratio of PLA-PEG to PLA. **Rapamycin Solution:** Rapamycin was dissolved at the indicated mg per 1 mL in dichloromethane. **SPAN 40 Solution:** Sorbitan monopalmitate (SPAN 40) was dissolved at the indicated mg per mL in dichloromethane. **CH2Cl2 Solution:** Dichloromethane (CH2Cl2), was sterile filtered using a 0.2µm PTFE membrane syringe filter (VWR part number 28145-491). **PVA Solution:** A polyvinyl alcohol solution was prepared by dissolving polyvinyl alcohol (EMPROVE® Polyvinyl Alcohol 4-88) at the indicated mg per 1 mL in 100 mM pH 8 phosphate buffer. **DPBS PVA Solution:** A polyvinyl alcohol and Dulbecco's phosphate buffered saline, 1X, 0.0095 M (PO4) mixture was prepared by dissolving polyvinyl alcohol (EMPROVE® Polyvinyl Alcohol 4-88) at 2.5 mg per 1 mL in Dulbecco's phosphate buffered saline, 1X, 0.0095 M (PO4) (Lonza DPBS).

An O/W emulsion was prepared by combining the Polymer Solution, Rapamycin Solution, SPAN 40 Solution and/or CH2Cl2 Solution (Total volume 1-2 mL) in a thick walled glass pressure tube. The solution was mixed by repeat pipetting. Next, PVA Solution (3 to 6 mL) was added (ether as a single emulsion with 1 mL organic phase and 3 mL aqueous PVA Solution, or as two single emulsions prepared one after the other). The formulation was vortex mixed for ten seconds, and then sonicated with the pressure tube immersed in an ice bath for 1 minute at 30% amplitude. The emulsion was then added to an open 50 mL beaker containing Lonza DPBS (30 mL). This was then stirred at room temperature for 2 hours to allow the dichloromethane to evaporate and for the nanocarriers to form. A portion of the nanocarriers were washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600×g and 4 °C for 50 minutes, removing the supernatant, and re-suspending the pellet in DPBS PVA Solution. The wash procedure was repeated and then the pellet was re-suspended in DPBS PVA Solution to achieve a nanocarrier suspension having a nominal concentration of 10 mg per mL on a polymer basis. The nanocarrier formulation was filtered using a 0.22µm PES membrane syringe filter (Millex part number SLGP033RS). The mass of the nanocarrier

solution filter throughput was measured. The filtered nanocarrier solution was then stored at -20°C.

Filterability is given as g/m^2 of filter membrane surface area, of measured nanocarrier passing through one 33 mm PES membrane 0.22 µm syringe filter from Millipore, part number SLGP033RB.

The results show the amount of various components in a number of synthetic nanocarriers that can result in initial sterile filterable synthetic nanocarriers with an amount of rapamycin that is expected to be efficacious *in vivo*.

Lot#	Polymer (mg per mL)	SPAN 40 (mg per mL)	Rapamycin (mg per mL)	PVA (mg per mL)	Size (nm)	Filterability (g NP/m^2)	Yield	wt% HLB/Rapa	wt% HLB/Polymer
1[a]	50	0	8	62.5	135	52	70.7	0.00	0.00
2[a]	50	0.1	8	62.5	135	26	68.6	1.23	0.20
3[a]	50	0.25	8	62.5	148	27	70.9	3.03	0.50
4[a]	50	0.5	8	62.5	166	146	73.2	5.88	0.99
5[a]	50	1	8	62.5	147	151	75.7	11.11	1.96
6[a]	50	1.5	8	62.5	161	146	72.2	15.79	2.91
7[a]	50	2.5	8	62.5	149	176	85.0	23.81	4.76
8[a]	50	2.5	8	50	182	209	103.5	23.81	4.76
9[a]	50	2.5	8	75	132	155	76.7	23.81	4.76
10[a]	50	3	8	62.5	143	140	69.4	27.27	5.66
11[a]	62.5	3	8	62.5	151	205	80.9	27.27	4.58
12[a]	37.5	3	8	62.5	139	203	60.9	27.27	7.41
13[a]	50	4.5	8	62.5	149	149	73.6	36.00	8.26
14[a]	50	5	6.66	50	148	193	94.4	42.88	9.09
15[a]	50	5	8.33	50	176	176	86.2	37.48	9.09
16[a]	50	10	8	50	173	38	66.1	55.56	16.67
17	100	10	11.32	75	153	178	88.2	46.90	9.09
18	100	10	14.16	75	160	200	98.9	41.39	9.09
19	100	10	17	75	177	182	101.0	37.04	9.09
20	100	7.5	24	75	188	125	70.4	23.81	6.98
21	75	11.25	30	75	197	17	82.5	27.27	13.04
22	100	15	32	75	201	17	108.1	31.91	13.04
23	100	15	40	75	217	9	82.6	27.27	13.04
24	100	15	40	75	193	14	116.5	27.27	13.04

[a]These formulations were prepared with 2 mL organic phase, 6 mL PVA Solution.

What is claimed is:

CLAIMS

1. A composition comprising synthetic nanocarriers comprising:

a hydrophobic carrier material and an immunosuppressant;

wherein the composition can be lyophilized, is in a lyophilized form, such as a lyophilized powdered form, or is a reconstituted version thereof.

2. The composition of claim 1, wherein the synthetic nanocarriers are any one of the synthetic nanocarriers described herein.

3. The composition of claim 1 or 2, wherein the composition has no visible aggregates, such as visible by the eye, upon reconstitution; has a stable average particle diameter, such as within 10% for at least 12 months, upon reconstitution; can be stored at between 2-8 °C for at least 12-36 months following lyophilization; can be stored at between 20-30 °C for at least 12 months following lyophilization; and/or has a neutral or near-neutral pH (e.g., a pH of 7.3 at 25°C) when in solution.

4. The composition of any one of the preceding claims, wherein the composition does not comprise a phosphate buffer or phosphate surfactant.

5. The composition of any one of the preceding claims, wherein the composition comprises a buffer, such as a non-phosphate buffer.

6. The composition of any one of the preceding claims, wherein the composition comprises a lyoprotectant.

7. The composition of any one of the preceding claims, wherein the composition comprises a buffer, such as a non-phosphate buffer, and a lyoprotectant.

8. The composition of any one of claims 5-7, wherein the buffer is a Tris buffer.

9. The composition of claim 8, wherein the Tris buffer is at a concentration of 10 mM.

10. The composition of claim 8 or 9, wherein the Tris buffer comprises Tris HCl and tromethamine.

11. The composition of any one of the preceding claims, wherein the lyoprotectant comprises sucrose or a sucrose/mannitol mixture.

12. The composition of claim 11, wherein the lyoprotectant comprises sucrose.

13. The composition of any one of the preceding claims, wherein the composition comprises at least one of tromethamine, Tris HCl, and sucrose.

14. The composition of any one of the preceding claims, wherein the composition comprises tromethamine, Tris HCl, and sucrose.

15. The composition of any one of the preceding claims, wherein the tromethamine is at a concentration of 1.3 mM.

16. The composition of any one of the preceding claims, wherein the Tris HCL is at a concentration of 8.7 mM.

17. The composition of any one of the preceding claims, wherein the sucrose is at 4-9.6 wt%.

18. The composition of claim 17, wherein the sucrose is at 9.6 wt%.

19. The composition of any one of the preceding claims, wherein the composition is in a solution at a pH of 7.3 at 25˚C.

20. The composition of any one of the preceding claims, wherein the composition is stored in a glass vial (e.g., a 20 mL glass vial, optionally further comprising a 20 mm stopper).

21. The composition of any one of the preceding claims, wherein the composition is stored at 2 to 8°C (e.g., in a lyophilized form).

22. The composition of any one of the preceding claims, wherein the composition is about to be lyophilized.

23. The composition of any one of the preceding claims, wherein the composition is reconstituted.

24. The composition of any one of the preceding claims, wherein the composition is lyophilized.

25. The composition of claim 24, wherein the composition is lyophilized as a lyophilized powder.

26. The composition of any one of the preceding claims, wherein the immunosuppressant is a rapalog.

27. The composition of claim 26, wherein the rapalog is rapamycin.

28. The composition of claim 26 or 27, wherein the rapalog or rapamycin is at a concentration of 2 mg/mL.

29. The composition of any one of the preceding claims, wherein the immunosuppressant is encapsulated in the synthetic nanocarriers.

30. The composition of any one of the preceding claims, wherein the immunosuppressant is present in the synthetic nanocarriers in a stable, super-saturated amount that is less than 50 weight% rapamycin/hydrophobic carrier material.

31. The composition of any one of the preceding claims, wherein the immunosuppressant is present in a stable, super-saturated amount that is greater than 7 weight%.

32. The composition of any one of the preceding claims, wherein the hydrophobic carrier material comprises one or more hydrophobic polymers or lipids.

33. The composition of claim 32, wherein the hydrophobic carrier material comprises one or more hydrophobic polymers, and wherein the one or more hydrophobic polymers comprise a polyester.

34. The composition of claim 33, wherein the polyester comprises PLA, PLG, PLGA or polycaprolactone.

35. The composition of claim 33 or 34, wherein the hydrophobic carrier material comprises or further comprises PLA-PEG, PLGA-PEG or PCL-PEG.

36. The composition of any one of the preceding claims, wherein the amount of hydrophobic carrier material in the synthetic nanocarriers is 5-95 weight% hydrophobic carrier material/total solids.

37. The composition of claim 36, wherein the amount of hydrophobic carrier material in the synthetic nanocarriers is 60-95 weight% hydrophobic carrier material/total solids.

38. The composition of any one of the preceding claims, wherein the amount of immunosuppressant is ≥ 6 but ≤ 50 weight% immunosuppressant/weight hydrophobic carrier material.

39. The composition of claim 38, wherein the amount of immunosuppressant is ≥ 7 but $\leq$ 30 weight% immunosuppressant /weight hydrophobic carrier material.

40. The composition of claim 39, wherein the amount of immunosuppressant is ≥ 8 but $\leq$ 24 weight% immunosuppressant /weight hydrophobic carrier material.

41. The composition of any one of the preceding claims, wherein the synthetic nanocarriers further comprise a non-ionic surfactant with HLB value less than or equal to 10.

42. The composition of claim 41, wherein the non-ionic surfactant with HLB value less than or equal to 10 comprises a sorbitan ester, fatty alcohol, fatty acid ester, ethoxylated fatty alcohol, poloxamer or a fatty acid.

43. The composition of claim 41 or 42, wherein the non-ionic surfactant with HLB value less than or equal to 10 comprises SPAN 40, SPAN 20, oleyl alcohol, stearyl alcohol, isopropyl palmitate, glycerol monostearate, BRIJ 52, BRIJ 93, Pluronic P-123, Pluronic L-31, palmitic acid, dodecanoic acid, glyceryl tripalmitate or glyceryl trilinoleate.

44. The composition of claim 43, wherein the non-ionic surfactant with HLB value less than or equal to 10 is SPAN 40.

45. The composition of any one of claims 41-44, wherein the non-ionic surfactant with HLB value less than or equal to 10 is encapsulated in the synthetic nanocarriers, present on the surface of the synthetic nanocarriers, or both.

46. The composition of any one of claims 41-45, wherein the amount of non-ionic surfactant with HLB value less than or equal to 10 is ≥ 0.01 but ≤ 20 weight% non-ionic surfactant with a HLB value less than or equal to 10/weight hydrophobic carrier material.

47. The composition of claim 46, wherein the amount of non-ionic surfactant with HLB value less than or equal to 10 is ≥ 0.1 but ≤ 15 weight% non-ionic surfactant with a HLB value less than or equal to 10/weight hydrophobic carrier material.

48. The composition of any one of the preceding claims, wherein the composition is initially sterile filterable through a 0.22 μm filter when in a solution, such as prior to lyophilization.

49. The composition of any one of the preceding claims, wherein the mean of a particle size distribution obtained using dynamic light scattering of the synthetic nanocarriers is a diameter greater than 110nm.

50. The composition of claim 49, wheren the diameter is greater than 120nm.

51. The composition of claim 50, wherein the diameter is greater than 150nm.

52. The composition of claim 51, wherein the diameter is greater than 200nm.

53. The composition of claim 52, wherein the diameter is greater than 250nm.

54. The composition of any one of claims 49-53, wherein the diameter is less than 300nm.

55. The composition of claim 54, wherein the diameter is less than 250nm.

56. The composition of claim 55, wherein the diameter is less than 200nm.

57. The composition of any one of the preceding claims, wherein the composition comprises 10-20% synthetic nanocarrier, hydrophobic carrier material, and immunosuppressant; 80-90% sucrose, 0.1-5% tromethamine; and 0.1-5% Tris HCL.

58. The composition of any one of the preceding claims, wherein the composition further comprises an antigen.

59. A kit comprising:
the composition of any one of the preceding claims.

60. The kit of claim 59, wherein the kit is for use in any one of the methods provided herein.

61. The kit of claim 60, further comprising instructions for use.

62. The kit of claim 61, wherein the instructions for use include a description of any one of the methods provided herein.

63. The kit of any one of claims 59-62, wherein the kit further comprises an antigen.

64. A method comprising administering the composition of any one of the compositions of the preceding claims to a subject in need thereof.

65. The method of claim 64, further comprising administering antigen to the subject.

66. A method of producing a composition comprising synthetic nanocarriers, comprising:

producing or obtaining, in a solution, synthetic nanocarriers as described in any one of the preceding claims, and

adding to the solution at least one of a buffer and a lyoprotectant.

67. The method of claim 66, wherein the buffer is a non-phosphate buffer.

68. The method of claim 67, wherein the buffer is a Tris buffer (e.g., to a concentration of 10mM).

69. The method of claim 68, wherein the Tris buffer comprises Tris HCl and tromethamine.

70. The method of any one of claims 66-69, wherein the lyoprotectant comprises sucrose or a mannitol/sucrose mixture.

71. The method of claim 70, wherein the lyoprotectant comprises sucrose.

72. The method of any one of claims 66-71, wherein a buffer and a lyoprotectant are added.

73. The method of any one of claims 66-72, wherein at least one of tromethamine, Tris HCl, and sucrose is added to the solution.

74. The method of claim 73, wherein tromethamine, Tris HCl, and sucrose are added.

75. The method of any one of claims 66-74, wherein the tromethamine is added to a concentration of 1.3 mM in the solution.

76. The method of any one of claims 66-75, wherein the Tris HCL is added to a concentration of 8.7 mM in the solution.

77. The method of any one of claims 66-76, wherein the sucrose is added to a wt% of 4-9.6.

78. The method of any one of claims 66-77, wherein the solution is at a pH of 7.3 at 25˚C or adjusted to such a pH.

79. The method of any one of claims 66-78, further comprising lyophilizing the synthetic nanocarriers in the solution.

80. The method of claim 79, wherein the lyophilizing is to a powdered form.

81. The method of any one of claims 66-80, further comprising reconstituting the lyophilized synthetic nanocarriers.

82. The method of any one of claims 66-81, wherein the synthetic nanocarriers are stored in a glass vial (e.g., a 20 mL glass vial).

83. The method of any one of claims 66-82, wherein the synthetic nanocarriers are stored at 2 to 8˚C.

84. A composition produced by the method of any one of claims 66-83.

85. A method comprising administering the composition of claim 84 to a subject in need thereof.

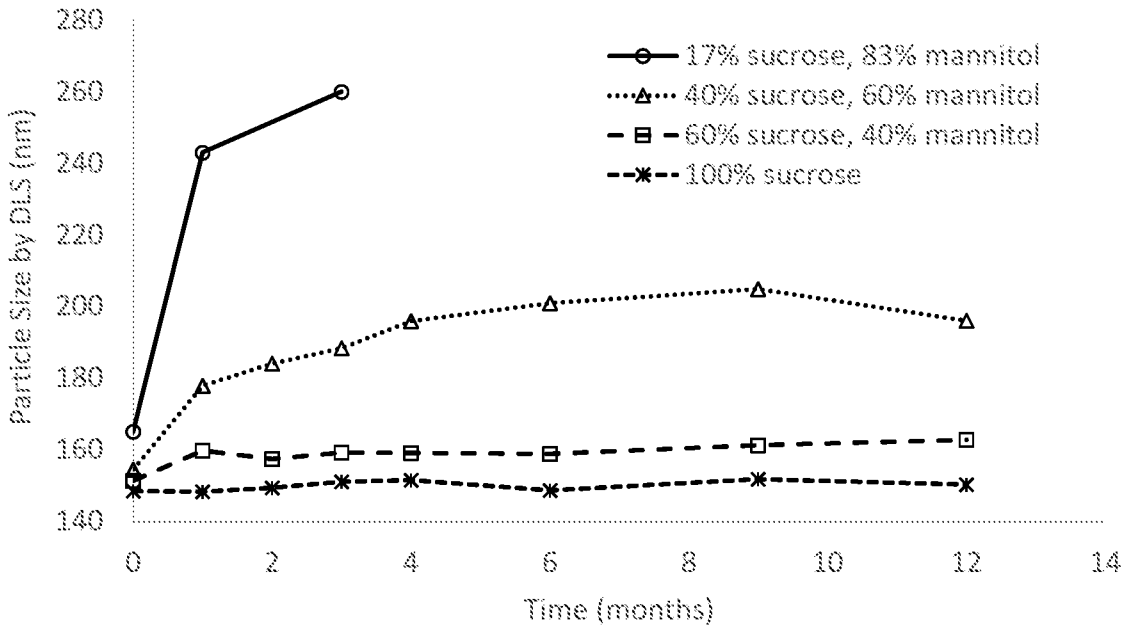

280
260
240
220
200
180
160
140
Particle Size by DLS (nm)
0
2
4
6
8
10
12
14
Time (months)
17% sucrose, 83% mannitol
40% sucrose, 60% mannitol
60% sucrose, 40% mannitol
100% sucrose

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/021939

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/08 A61K9/19 A61K47/34 A61K31/436
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/073798 A1 (SELECTA BIOSCIENCES INC [US]) 12 May 2016 (2016-05-12) the whole document claims 1,9,16-18,37,43,75 -----	1-85
X	US 2015/359865 A1 (KISHIMOTO TAKASHI KEI [US]) 17 December 2015 (2015-12-17) paragraph [0003]; examples 1,4 -----	1-85
X	WO 2019/162951 A1 (YISSUM RES DEV CO OF HEBREW UNIV JERUSALEM LTD [IL]) 29 August 2019 (2019-08-29) the whole document claims 1,7,19,44,49,51 -----	1-85
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2021	30/06/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Giese, Hans-Hermann

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKASHI K. KISHIMOTO ET AL: "Improving the efficacy and safety of biologic drugs with tolerogenic nanoparticles", NATURE NANOTECHNOLOGY, vol. 11, no. 10, 1 August 2016 (2016-08-01), pages 890-899, XP055371105, London ISSN: 1748-3387, DOI: 10.1038/nnano.2016.135 abstract -----	1-85
X	EP 2 522 338 A2 (SAMYANG BIOPHARMACEUTICALS [KR]) 14 November 2012 (2012-11-14) claims -----	1-85
A	US 2017/224620 A1 (ZALE STEPHEN E [US] ET AL) 10 August 2017 (2017-08-10) claims -----	1-85

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/021939

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016073798 A1	12-05-2016	AU 2015342968 A1	27-04-2017
		AU 2015342969 A1	27-04-2017
		BR 112017008499 A2	26-12-2017
		BR 112017008720 A2	19-12-2017
		CA 2966850 A1	12-05-2016
		CA 2966852 A1	12-05-2016
		CN 107072964 A	18-08-2017
		CN 107072965 A	18-08-2017
		DK 3215133 T3	01-02-2021
		DK 3215192 T3	03-05-2021
		EA 201790977 A1	31-10-2017
		EA 201790978 A1	29-09-2017
		EP 3215133 A1	13-09-2017
		EP 3215192 A1	13-09-2017
		EP 3834823 A1	16-06-2021
		HR P20210098 T1	05-03-2021
		IL 251620 A	28-02-2021
		JP 2017533243 A	09-11-2017
		JP 2017537081 A	14-12-2017
		KR 20170081659 A	12-07-2017
		KR 20170081660 A	12-07-2017
		LT 3215133 T	25-02-2021
		PT 3215133 T	29-01-2021
		US 2016128986 A1	12-05-2016
		US 2016128987 A1	12-05-2016
		WO 2016073798 A1	12-05-2016
		WO 2016073799 A1	12-05-2016
US 2015359865 A1	17-12-2015	NONE	
WO 2019162951 A1	29-08-2019	AU 2019226051 A1	10-09-2020
		CA 3092016 A1	21-08-2020
		CN 112004524 A	27-11-2020
		EP 3758677 A1	06-01-2021
		JP 2021514948 A	17-06-2021
		KR 20200130704 A	19-11-2020
		US 2021128534 A1	06-05-2021
		WO 2019162951 A1	29-08-2019
EP 2522338 A2	14-11-2012	CN 102740834 A	17-10-2012
		EP 2522338 A2	14-11-2012
		JP 2013516407 A	13-05-2013
		JP 2015078247 A	23-04-2015
		KR 20110079518 A	07-07-2011
		US 2012276169 A1	01-11-2012
		US 2014212462 A1	31-07-2014
		WO 2011081430 A2	07-07-2011
US 2017224620 A1	10-08-2017	AU 2016392719 A1	02-08-2018
		CA 3013896 A1	17-08-2017
		CN 108697815 A	23-10-2018
		EP 3413917 A1	19-12-2018
		JP 2019512006 A	09-05-2019
		US 2017224620 A1	10-08-2017
		US 2019029960 A1	31-01-2019
		WO 2017138983 A1	17-08-2017