

HYALURONIDASE PARTICLES, COMPOSITIONS COMPRISING THE SAME AND METHODS OF MAKING AND USING THE SAME

Related Application Information

5 This application claims the benefit of U.S. Provisional Patent Application Serial No. 63/486,013, filed February 20, 2023, the disclosure of which is incorporated herein by reference in its entirety.

Statement Regarding Electronic Filing Of A Sequence Listing

10 A Sequence Listing in XML format, entitled 1458-5WO_ST26.xml, 1,557,299 bytes in size, generated on February 19, 2024, and filed herewith, is hereby incorporated by reference into the specification for its disclosures.

Field

15 The present invention relates to hyaluronidase particles, compositions comprising hyaluronidase particles, and to methods of making and using hyaluronidase particles.

Background

Subcutaneous injection offers a flexible and effective route of drug administration
20 wherein a medication is injected into the tissue layer between the skin and muscle. Subcutaneous injection is advantageous in that it is a less expensive and less painful parenteral administration method with a low risk of systemic infection and a slow adsorption rate, which allows for long-term effects of an injected drug. However, fluid injected subcutaneously largely remains at the injection site before slowly dissipating over the course of hours to days.
25 Injecting a large volume (e.g., more than about 2 mL) results in retention of the fluid and/or swelling at the injection area, which can lead to local adverse reactions causing pain, irritation, and/or skin damage. For this reason, subcutaneous injections are typically limited to a small dosage, such as a volume of less than about 2 mL.

Hyaluronidases are a family of enzymes, specifically endoglycosidases, which can
30 break down hyaluronic acid and may be able to degrade other glycosaminoglycans, such as chondroitin and chondroitin sulfates. Hyaluronidases are present both in organs, such as the testis, spleen, and liver, as well as in body fluids, such as tears and blood. Three distinct classes of hyaluronidases exist—mammalian or vertebrate, bacterial, and leech/hookworm. Within the mammalian class, the human genome contains six known genes which code for hyaluronidase-

like sequences. Purified human, such as testicular hyaluronidase PH-20, or ovine, such as ovine PH-20, hyaluronidases are commonly used for medical and experimental purposes.

Hyaluronidase in an aqueous solution has been used subcutaneously to modify tissue permeability by degrading the subcutaneous hyaluronic acid network. By breaking down the hyaluronic acid network in the subcutaneous space, subcutaneous administration of a hyaluronidase in an aqueous solution can allow for a subcutaneously injected fluid to dissipate more quickly than in the absence of the hyaluronidase, which can enable a larger volume of fluid to be administered. Hyaluronidase in an aqueous solution is approved by the U.S. Food and Drug Administration (FDA) for use in rapid fluid administration of volumes of 1,000 mL or more or as an adjuvant in the subcutaneous delivery of other drugs. Hyaluronidase is typically administered as an aqueous solution before injecting a large volume dose of greater than 2 mL or as a coformulation with a large-volume dose of greater than 2 mL.

New approaches are needed for administration of hyaluronidase and therapeutics, particularly for subcutaneous and intradermal administration.

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Summary

A first aspect of the present invention is directed to a particle comprising: hyaluronidase, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9.

20 A further aspect of the present invention is directed to a particle comprising: hyaluronidase and a therapeutic, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9.

Another aspect of the present invention is directed to a particle comprising: hyaluronidase and a stabilizer, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9.

25 A further aspect of the present invention is directed to a composition comprising a particle as described herein.

Another aspect of the present invention is directed to a composition comprising: a solvent and a particle comprising hyaluronidase, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9.

30 A further aspect of the present invention is directed to a method of increasing dissolution of a therapeutic in a liquid, the method comprising: combining the liquid, the therapeutic, and a particle comprising hyaluronidase, wherein the particle has a water content

of less than about 15% by weight of the particle and/or a water activity of less than about 0.9, thereby increasing dissolution of the therapeutic in the liquid.

Another aspect of the present invention is directed to a method of improving tolerance and/or increasing absorption of a therapeutic upon subcutaneous, intramuscular, and/or intradermal administration to a subject, the method comprising: subcutaneously, intramuscularly, and/or intradermally administering to the subject a particle comprising hyaluronidase, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9, thereby improving tolerance and/or increasing absorption of the therapeutic upon subcutaneous, intramuscular, or intradermal administration to the subject. In some embodiments, the therapeutic is subcutaneously, intramuscularly, and/or intradermally administered to the subject prior to, during, and/or after subcutaneously, intramuscularly, and/or intradermally administering the particle to the subject.

It is noted that aspects of the invention described herein with respect to one embodiment, may be incorporated in a different embodiment although not specifically described relative thereto. That is, all embodiments and/or features of any embodiment can be combined in any way and/or combination. Applicant reserves the right to change any originally filed claim and/or file any new claim accordingly, including the right to be able to amend any originally filed claim to depend from and/or incorporate any feature of any other claim or claims although not originally claimed in that manner. These and other objects and/or aspects of the present invention are explained in detail in the specification set forth below. Further features, advantages and details of the present invention will be appreciated by those of ordinary skill in the art from a reading of the figures and the detailed description of the preferred embodiments that follow, such description being merely illustrative of the present invention.

Brief Description of the Drawings

Fig. 1 is a graph of absorbance at 280 nm over time (hours) that shows the amount of bovine gamma globulin (BGG) released from a benzyl benzoate suspension into a media comprising phosphate buffered saline (PBS) and 0.25 mg/mL hyaluronidase (PBS-Hyal media) or a media comprising PBS, 0.5% hyaluronic acid, and 0.25 mg/mL hyaluronidase (PBS-HA-Hyal media).

Fig. 2 is a graph of absorbance at 280 nm over time (hours) that shows the amount of BGG released from a benzyl benzoate suspension into PBS for three, separate samples (i.e., PBS media-1, PBS media-2, and PBS media-3).

Fig. 3 is a graph of absorbance at 280 nm over time (hours) that shows the amount of BGG released from a benzyl benzoate suspension into a media comprising 0.5% hyaluronic acid (HA) and PBS for three, separate samples (i.e., PBS-HA media-1, PBS-HA media-2, and PBS HA media-3).

5 **Fig. 4** is a graph of absorbance at 280 nm over time (hours) that shows the amount of BGG released from a benzyl benzoate suspension into PBS for three, separate samples (i.e., PBS media-1, PBS media-2, and PBS media-3), the suspension comprising a plurality of particles that each comprise BGG and a hyaluronidase according to some embodiments of the present invention.

10 **Fig. 5** is a graph of absorbance at 280 nm over time (hours) that shows the amount of BGG released from a benzyl benzoate suspension into a media comprising 0.5% hyaluronic acid (HA) and PBS for three, separate samples (i.e., PBS-HA media-1, PBS-HA media-2, and PBS-HA media-3), the suspension comprising a plurality of particles that each comprise BGG and a hyaluronidase according to some embodiments of the present invention.

15 **Fig. 6** is a graph of the stability of microbatches of various hyaluronidase powder formulations stored at 25 °C, indicated by enzymatic activity compared to the level of activity of the stock solution pre-storage (i.e., at initial formation of the stock solution, $t=0$).

Fig. 7 is a graph of the stability of microbatches of various hyaluronidase powder formulations stored at 2-8 °C, indicated by enzymatic activity compared to the level of activity
20 of the stock solution pre-storage.

Fig. 8 is a graph of the activity of hyaluronidase over time when stored in a powder formulation containing the enzyme and bovine serum albumin (BSA) at the indicated temperatures.

Fig. 9 is a graph of the change in the monomer percentage of BSA over time in powders
25 containing only BSA or a combination of BSA and hyaluronidase.

Fig. 10 is a microscopy image showing the spherical morphology of particles of monoclonal antibody (mAb) and ~0.3% hyaluronidase suspended in octanol.

Fig. 11 is a graph of the mAb stability as indicated by the monomer percentage from particles containing only mAb or a combination of mAb and hyaluronidase when stored at 2-8
30 °C. **Fig. 12** is a graph of the mAb stability as indicated by the percentage of high-molecular weight species (HMWS) from particles containing only mAb or a combination of mAb and hyaluronidase when stored at 2-8 °C.

Fig. 13 is a graph of the mAb stability as indicated by the monomer percentage from particles containing only mAb or a combination of mAb and hyaluronidase when stored at 25 °C.

Fig. 14 is a graph of the mAb stability as indicated by the percentage of HMWS from
5 particles containing only mAb or a combination of mAb and hyaluronidase when stored at 25 °C.

Fig. 15 is a graph of the mAb stability as indicated by the monomer percentage from particles containing only mAb or a combination of mAb and hyaluronidase when stored at 40 °C.

Fig. 16 is a graph of the mAb stability as indicated by the percentage of HMWS from
10 particles containing only mAb or a combination of mAb and hyaluronidase when stored at 40 °C.

Fig. 17 is a graph of the activity of hyaluronidase over time in particles containing only mAb or a combination of mAb and hyaluronidase when stored at 2-8, 25, or 40 °C.

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Detailed Description of Example Embodiments

The present invention is now described more fully hereinafter with reference to the accompanying drawings, in which embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to
20 the embodiments set forth herein; rather these embodiments are provided so that this disclosure will be thorough and complete and will fully convey the scope of the invention to those skilled in the art.

The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As
25 used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

It will be understood that, although the terms "first," "second," etc. may be used herein to describe various elements, these elements should not be limited by these terms. These terms
30 are only used to distinguish one element from another. Thus, a "first" element discussed below could also be termed a "second" element without departing from the teachings of the present invention. The sequence of operations (or steps) is not limited to the order presented in the claims or figures unless specifically indicated otherwise.

Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with
5 their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. In case of a conflict in terminology, the present specification is controlling.

10 Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the
15 present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed.

As used herein, the transitional phrase "consisting essentially of" (and grammatical
20 variants) is to be interpreted as encompassing the recited materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. *See, In re Herz*, 537 F.2d 549, 551-52, 190 U.S.P.Q. 461, 463 (CCPA 1976) (emphasis in the original); see also MPEP § 2111.03. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising."

25 It will also be understood that, as used herein, the terms "example," "exemplary," and grammatical variations thereof are intended to refer to non-limiting examples and/or variant embodiments discussed herein, and are not intended to indicate preference for one or more embodiments discussed herein compared to one or more other embodiments.

The term "about," as used herein when referring to a measurable value such as an
30 amount or concentration and the like, is meant to encompass variations of $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified value as well as the specified value. For example, "about X" where X is the measurable value, is meant to include X as well as variations of $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of X. A range provided herein for a measurable value may include any other range and/or individual value therein.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if the range 10 to 15 is disclosed, then 11, 12, 13, and 14 are also disclosed.

A “portion” or “fragment” of a material or component as used herein refers to less than all (e.g., less than 100%) of the material or component or of a measurable value thereof. In some embodiments, a “portion” or “fragment” of a material or component refers to about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% of the material or component or of a measurable value of the material or component (e.g., a portion of the length, volume, weight, sequence, etc.). In some embodiments, a “portion” or “fragment” of a particle or a plurality of particles refers to about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% of the particle or of the plurality of particles, respectively. In some embodiments, the material or component may be a nucleotide sequence or polypeptide (optionally including a domain) and so a “portion” or “fragment” of a nucleotide sequence or polypeptide will be understood to mean a nucleotide sequence or polypeptide of reduced length (e.g., reduced by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more residue(s) (e.g., nucleotide(s) or peptide(s)) relative to a reference nucleotide sequence or polypeptide, respectively, and comprising, consisting essentially of and/or consisting of a nucleotide sequence or polypeptide of contiguous residues, respectively, identical or almost identical (e.g., 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical) to the reference nucleotide sequence or polypeptide. In some embodiments, a “portion” or “fragment” of a nucleotide sequence or polypeptide will have similar or the same biological activity (e.g., 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120%, 125%, or 130% similar biological activity) compared to the reference nucleotide sequence or polypeptide. In some embodiments, a “portion” or “fragment” of a nucleotide sequence or polypeptide will have reduced biological activity (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% reduced biological activity) or increased biological activity (e.g., 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, 200%, or more increased biological activity) compared to the reference nucleotide sequence or polypeptide.

The term “comprise,” “comprises” and “comprising” as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

5 As used herein, the terms “increase,” “increased,” “increasing,” “enhance,” “enhancing,” “improve” and “improving” (and grammatical variations thereof) describe an elevation of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500% or more such as compared to another measurable property or quantity (e.g., a control value).

10 As used herein, the terms “reduce,” “reduced,” “reducing,” “reduction,” “diminish,” and “decrease” (and grammatical variations thereof), describe, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% such as compared to another measurable property or quantity (e.g., a control value). In some embodiments, the reduction can result in
15 no or essentially no (*i.e.*, an insignificant amount, *e.g.*, less than about 10% or even 5%) detectable activity or amount.

Provided according to embodiments of the present invention are particles that include hyaluronidase. In some embodiments, a particle comprising hyaluronidase further comprises an excipient (e.g., a stabilizer) and/or a therapeutic. A particle of the present invention may be
20 a solid and may have a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9. In some embodiments, the particle has a water content of less than about 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.5% by weight of the particle. In some embodiments, a particle of the present invention has a water content in a range of about 0%, 0.5%, 1%, 2%, 3%, or 4% to about 5%, 6%, 7%, 8%, 9%, 10%,
25 11%, 12%, 13%, 14%, or 15% by weight of the particle. In some embodiments, a particle of the present invention has a water content of less than about 10% by weight of the particle or less than about 5% by weight of the particle. In some embodiments, a particle of the present invention has a water content of about 0%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% by weight of the particle. In some embodiments, a particle
30 of the present invention has a water activity of less than about 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1, 0.05, or 0.01. In some embodiments, a particle of the present invention has a water activity of less than about 0.5 or less than about 0.1. In some embodiments, the particle has a water activity in a range of about 0, 0.01, 0.05,

0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, or 0.45 to about 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, or 0.9.

Water content can be measured using methods known in the art such as by using Karl Fischer titration and/or thermogravimetric analysis. In some embodiments, water content of a particle of the present invention may be measured using a Mettler Toledo coulometric KF titrator. A sample comprising a particle of the present invention may be contacted with a solvent (e.g., methanol) to extract water from the sample and then the amount of water in the solvent is measured (e.g., using a Mettler Toledo coulometric KF titrator) and the amount of water present in the sample is calculated from the measured water content of the solvent after subtracting any water present in a solvent blank (the solvent before contact with the particle). For example, in some embodiments, about 15 mg to about 30 mg of a particle of the present invention (e.g., in the form of a powder) or a composition (e.g., a suspension) comprising a particle of the present invention is weighed into a glass vial in a dry box (relative humidity (RH) less than 20%) and about 1.5 mL of anhydrous methanol is added to the particle or composition to extract the water from the sample. The methanol may then be injected into a titration cell (e.g., of a Mettler Toledo coulometric KF titrator) and the amount of water present in the methanol may be measured. The amount of water present in the particle or composition may then be calculated from the measured water content of the methanol after subtracting any water present in a methanol blank. In some embodiments, water content of a suspension may be measured by directly injecting the suspension into a titration cell. Water content may also be measured by loss on drying (e.g., using thermogravimetric analysis (TGA)) such as when no volatile components are present in the sample or no components are present in the sample that will evaporate at a similar temperature to water.

Water activity is a measure of the chemical availability of water in a sample (e.g., in a particle of the present invention) and can quantify the amount of unbound water in a sample and/or how tightly water is bound to a component in the sample (e.g., how tightly water is bound to a biologic present in the particle). In some embodiments, water activity is measured by equilibrating a sample comprising a particle of the present invention in a closed chamber and measuring the relative humidity of the headspace in the chamber. Alternatively or in addition, water activity may be measured by correlating water content and water activity in a known system. To correlate water content and water activity, the water activity of a sample comprising a particle of the present invention is determined by equilibrating the sample through a vapor phase with a known water activity (e.g., a saturated salt solution or using a dynamic vapor sorption instrument) and measuring the change in water content by Karl Fischer titration

or change in mass to calculate the water activity of the sample. Thus, the water content and/or mass of the sample may be measured before and after contact with the vapor phase and the change used to measure the water activity of the sample. In some embodiments, water content and water activity for a single particle of the present invention or a droplet comprising a particle
5 of the present invention may be correlated by measuring volume changes of the single particle or droplet upon contact (e.g., exposure) to a solvent having a known water activity or a gas having a known water activity such as described in Rickard, et al., *Biophysical Journal*, 2010;98(6):1075-84. The volume of the single particle or droplet may be measured before and after contact with the solvent or gas and the change in volume used to measure the water
10 activity of the sample.

In some embodiments, a particle of the present invention has a water content (e.g., a total moisture content) of less than about 3% of the total particle mass. The particle may have a total moisture content of less than about 2.5%, 2%, 1.5%, 1%, or 0.5% of the total particle mass. In some embodiments, total moisture content of a particle is in a range of about 0%,
15 0.1%, or 0.5% to about 1%, 1.5%, 2%, 2.5%, or 3% of the total particle mass.

A particle of the present invention may have a residual content in an amount of less than about 3% by weight of the particle (e.g., total particle mass). The particle may have a residual content of less than about 2.5%, 2%, 1.5%, 1%, or 0.5% by weight of the particle. In some embodiments, a residual content of a particle of the present invention is in a range of
20 about 0%, 0.01%, 0.05%, 0.1%, or 0.5% to about 1%, 1.5%, 2%, 2.5%, or 3% by weight of the particle. In some embodiments, a particle of the present invention has a total residual content (e.g., the sum of all residuals) of less than 3% by weight of the particle such as in an amount less than 2.5%, 2%, 1.5%, 1%, or 0.5% by weight of the particle. In some embodiments, a single residual may be present in a particle of the present invention in an amount of less than
25 1% by weight of the particle such as in an amount of about 0.01%, 0.05%, 0.1%, or 0.2% to about 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, or 0.9% by weight of the particle. In some embodiments, a single residual may be present in a particle of the present invention in an amount of less than 0.5% by weight of the particle such as in an amount of about 0.01%, 0.05%, 0.1%, 0.2% to 0.3%, or 0.4 by weight of the particle. A residual may be one or more
30 component(s) present in a composition used to prepare (e.g., form, dehydrate, solidify, wash, and/or isolate) a particle of the present invention. Exemplary residuals include, but are not limited to, solvents (e.g., octanol and/or pentanol). In some embodiments, a residual may be a Class II or Class III residual solvent as classified by the U.S. Food & Drug Administration.

A particle of the present invention may be a microparticle or a nanoparticle. In some embodiments, the particle may have a size in at least one dimension and/or a diameter in a range of about 1, 5, 10, 20, 30, 40, or 50 nm to about 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or 500 nm. In some embodiments, a plurality of particles of the present invention have an average size of about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or 500 nm. In some embodiments, a particle of the present invention may have a size in at least one dimension and/or a diameter in a range of about 0.1, 0.5, 1, 5, 10, or 15 microns to about 20, 25, or 30 microns. In some embodiments, the particle may have a size in at least one dimension and/or a diameter of about 0.1, 0.5, 1, or 2 microns to about 3, 4, 5, 6, 7, 8, 9, or 10 microns. In some embodiments, the particle may have a size in at least one dimension and/or a diameter of about 5, 6, 7, 8, 9, or 10 microns to about 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 microns. In some embodiments, the particle may have a size in at least one dimension and/or a diameter of about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 microns to about 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 microns.

A particle of the present invention may be spherical in shape. In some embodiments, the particle may be amorphous. In some embodiments, at least a portion of the particle is amorphous, so the particle may be partially amorphous. For example, all or a portion of hyaluronidase may be amorphous in a particle of the present invention, but molecules (e.g., a salt) within a matrix of hyaluronidase may be crystalline in a particle of the present invention. A particle of the present invention may comprise one or more molecule(s) of hyaluronidase that may be the same as or different than each other and/or a matrix of one or more molecule(s) of hyaluronidase that may be the same as or different than each other, optionally wherein the hyaluronidase in the particle is amorphous.

In some embodiments, a particle of the present invention is in the form of a solid such as a solid particulate and/or a powder. In some embodiments, a particle of the present invention is in the form of a solid when it is in the form of a powder (e.g., a dry powder such as a powder in the absence of a liquid in which the powder is dispersed or suspended) and/or when the particle is present in a vehicle (e.g., a non-aqueous solvent and/or an aqueous solvent). In some embodiments, particles of the present invention are in the form of a powder. In some embodiments, a particle of the present invention may be a microglassified particle and/or hyaluronidase may be in the form of microglassified hyaluronidase that is a particle and/or that is comprised in a particle. "Microglassification" as used herein refers to a process of removing water from (e.g., dehydrating) a component (e.g., a hyaluronidase or a therapeutic) that is present in an aqueous composition (e.g., dissolved or suspended in an aqueous composition).

by contacting the aqueous composition and an organic phase (e.g., a non-aqueous organic phase) to provide a solid comprising the component, and the solidified component may be referred to herein as a "microglassified" component. A microglassification process may be performed in any manner that allows for an organic phase and an aqueous composition comprising a component to be in contact to thereby remove water from the component. For example, in some embodiments, a microglassification process may be performed using a homogenizer (e.g., an inline homogenizer) and/or a microfluidic device to bring an organic phase and an aqueous composition comprising a component into contact, and/or by spraying droplets of an aqueous composition comprising a component into an organic phase or vice versa to thereby bring the organic phase and aqueous composition into contact. In a microglassification process the organic phase is a liquid organic phase (e.g., a liquid dehydration composition) and the aqueous composition is a liquid. Thus, a microglassification process comprises a liquid-liquid mixture and/or system. A microglassification process may comprise contacting at least two different liquids. In some embodiments, a microglassification process is devoid of a spray-drying step or method and/or does not involve spraying droplets of an aqueous composition comprising a component into an organic phase or vice versa. In some embodiments, the microglassification process that is devoid of a spray-drying step or method, does not involve spraying droplets of an aqueous composition comprising a component into a gas (e.g., a heated gas phase) and/or the microglassification process is devoid of a liquid-gas mixture and/or system. A microglassified component is not a precipitate. In some embodiments, a particle of the present invention is not prepared by a method that uses a phase separating agent. A "phase separating agent" as used herein refers to an agent that causes a biologic to precipitate from a solution or that causes a liquid-liquid phase separation where one of the liquid phases includes the majority of a biologic. Exemplary phase separating agents include, but are not limited to, salts (such as those that can be used for "salting out protein") and polyethylene glycols (PEGs). Phase separation, which is a process of nucleation and growth, may occur during a microglassification method (e.g., liquid-liquid and/or liquid-solid), but a microglassified component is not formed by phase separation. Instead, microglassification is a continuous phase transition that does not require a nucleating event in order to form the solidified component. A microglassified component (e.g., microglassified hyaluronidase) may dissolve back into the initial composition in which it was present (e.g., an aqueous solution) and/or into an aqueous composition (e.g., an aqueous buffer).

In some embodiments, a microglassified component (e.g., microglassified hyaluronidase) may be more stable (e.g., have an increased shelf-life duration, increased range

of acceptable storage temperatures, and/or reduced degradation optionally following repeated freeze/thaw cycles) than a solution of said component (e.g., a hyaluronidase solution, wherein the hyaluronidase has not been microglassified). In some embodiments, microglassification of a component (e.g., hyaluronidase) increases the stability of said component optionally compared to the stability of the same component that has not been microglassified that is present in a solution. In some embodiments, a particle comprising hyaluronidase of the present invention has increased stability compared to hyaluronidase in a solution, wherein the hyaluronidase in the solution is not in the form of a particle and has not been microglassified.

A particle comprising hyaluronidase may be present in any form such as, for example, a microparticle, nanoparticle, microsphere, or nanosphere. Particles of the present invention may be amorphous and/or crystalline. In some embodiments, a plurality of particles of the present invention comprises amorphous particles. In some embodiments, a plurality of particles of the present invention comprises crystalline particles such as, e.g., when small molecules (e.g., salts) are present that can be crystalized. A plurality of particles of the present invention may be uniform in size or may be polydisperse. In some embodiments, at least a portion of the plurality of particles have a size that is within about $\pm 5\%$, 10% , 15% , 20% , 25% , 50% , 75% , 100% , 150% , 200% or more of the average particle size. In some embodiments, a composition of the present invention comprises discrete particles. A particle of the present invention may have a density of about 0.5 , 0.6 , 0.7 , 0.8 , 0.9 , or 1 g/cm^3 to about 1.1 , 1.2 , 1.3 , 1.4 , 1.5 , 1.6 , 1.7 , 1.8 , 1.9 , or 2 g/cm^3 . In some embodiments, a particle of the present invention may have a density of about 0.5 , 0.6 , 0.7 , 0.8 , 0.9 , 1 , 1.1 , 1.2 , 1.3 , 1.4 , 1.5 , 1.6 , 1.7 , 1.8 , 1.9 , or 2 g/cm^3 . In some embodiments, a particle of the present invention may have a density of about 1.1 g/cm^3 to about 1.5 g/cm^3 . Density can be measured and/or determined using methods known in the art such as, but not limited to, using a gas pycnometer and/or by sedimentation experiments.

“Hyaluronidase” as used herein refers to an enzyme or a portion thereof (e.g., a domain thereof) that cleaves a glycosidic linkage of a glycosaminoglycan such as, but not limited to, hyaluronic acid. As used herein, “hyaluronidase” refers to all forms of hyaluronidase from any organism (e.g., from a bacteria and/or mammal such as a human and/or bovine) along with naturally occurring (e.g., wild-type hyaluronidases) and recombinant hyaluronidases. In some embodiments, the hyaluronidase present in a particle of the present invention at least cleaves hyaluronic acid such as human hyaluronic acid that is optionally present in the skin and/or tissue of a subject. A hyaluronidase of the present invention may cleave a beta 1-4 linkage of a glycosaminoglycan (e.g., a beta 1-4 linkage of hyaluronic acid). Exemplary hyaluronidases

include, but are not limited to, a human hyaluronidase (e.g., human Hyal-1, human Hyal-2, human Hyal-3, human Hyal-4, and/or human PH-20/Spam1), a bovine testicular hyaluronidase, and/or an ovine hyaluronidase (e.g., ovine PH-20). For example, in some embodiments, the hyaluronidase may be ovine testicular Hyaluronidase V (for example, Millipore Sigma Catalog
5 Number: H6254), optionally with a specific activity of $\geq 1,500$ units/mg solid. Hyaluronidase present in a particle of the present invention may be synthetically obtained (e.g., through laboratory synthesis) and/or obtained and/or derived from nature (e.g., from a living or previously living organism). In some embodiments, hyaluronidase may be the same as a hyaluronidase found in nature (i.e., a native hyaluronidase) or may be modified from that found
10 in nature (e.g., a modified hyaluronidase and/or recombinant hyaluronidase). For example, a hyaluronidase of the present invention may be modified to have a different structure, sequence, charge, chemical modification, and/or length than that of a native hyaluronidase and/or a hyaluronidase may be a portion of a native hyaluronidase (e.g., an active domain of a native hyaluronidase). In some embodiments, a hyaluronidase of the present invention may be and/or
15 may have an activity comparable to a commercially available hyaluronidase (e.g., a hyaluronidase such as, but not limited to, TERGASE® (recombinant human hyaluronidase), ALT-BC4 (recombinant human hyaluronidase), ALT-B4 (recombinant human hyaluronidase), MK-5180 (recombinant human hyaluronidase), HYLENEX® (recombinant human hyaluronidase; **SEQ ID NO:1**), AMPHADASE® (bovine testicular hyaluronidase; **SEQ ID**
20 **NO:2**), HYDASE™ (bovine testicular hyaluronidase; **SEQ ID NO:2**), VITRASE® (ovine testicular hyaluronidase; optionally having a sequence of **SEQ ID NO:3** and/or **SEQ ID NO:4**), a *Vespula vulgaris* hyaluronidase such as having a sequence of one of **SEQ ID NOs: 21** or **22**, an *Apis mellifera* hyaluronidase such as having a sequence of **SEQ ID NO: 23**, a *Dolichovespula maculata* hyaluronidase such as having a sequence of **SEQ ID NO: 24**, a
25 *Polistes annularis* hyaluronidase such as having a sequence of **SEQ ID NO: 25**, a *Mus musculus* hyaluronidase such as having a sequence of any one of **SEQ ID NOs: 26-28** or **39**, a *Sus scrofa* hyaluronidase such as having a sequence of any one of **SEQ ID NOs: 29** or **30**, a *Rattus norvegicus* hyaluronidase such as having a sequence of any one of **SEQ ID NOs: 31-33** or **38**, an *Oryctolagus cuniculus* hyaluronidase such as having a sequence of **SEQ ID NO:**
30 **34**, a *Pongo pygmaeus* hyaluronidase such as having a sequence of **SEQ ID NO: 35**, a *Macaca fascicularis* hyaluronidase such as having a sequence of **SEQ ID NO: 36**, a *Cavia porcellus* hyaluronidase such as having a sequence of **SEQ ID NO: 37**, a *Staphylococcus aureus* hyaluronidase such as having a sequence of **SEQ ID NO: 40**, a *Staphylococcus pyogenes* hyaluronidase such as having a sequence of **SEQ ID NO: 41**, a *Clostridium perfringens*

hyaluronidase such as having a sequence of **SEQ ID NO: 42**, and/or a hyaluronidase according to any one of **SEQ ID NOs: 5-20 or 43-896**). In some embodiments, a hyaluronidase of the present invention is a human hyaluronidase, a bovine hyaluronidase (e.g., a bovine testicular hyaluronidase), an ovine hyaluronidase (e.g., an ovine testicular hyaluronidase), a *Vespula vulgaris* hyaluronidase, an *Apis mellifera* hyaluronidase, a *Dolichovespula maculata* hyaluronidase, a *Polistes annularis* hyaluronidase, a *Mus musculus* hyaluronidase, a *Sus scrofa* hyaluronidase, a *Rattus norvegicus* hyaluronidase, an *Oryctolagus cuniculus* hyaluronidase, a *Pongo pygmaeus* hyaluronidase, a *Macaca fascicularis* hyaluronidase, a *Cavia porcellus* hyaluronidase, a *Staphylococcus aureus* hyaluronidase, a *Staphylococcus pyogenes* hyaluronidase, a *Clostridium perfringens* hyaluronidase, and/or a recombinant hyaluronidase of any of the foregoing. In some embodiments, a hyaluronidase of the present invention has an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more of **SEQ ID NOs:1-896**. In some embodiments, a hyaluronidase of the present invention comprises an amino acid sequence of one of **SEQ ID NOs:1-896**. In some embodiments, a hyaluronidase may be as described in U.S. Patent No. 8,431,380; U.S. Patent No. 10,857,213; U.S. Patent No. 9,284,543; U.S. Patent No. 9,447,401; U.S. Patent Publication No. 2021/0155913; U.S. Patent Publication No. US 2023/0250408; and Stern, R. and Jedrzejewski, M.J., Hyaluronidases: Their Genomics, Structures, and Mechanisms of Action. *Chem Rev.* (2006), 818-839, the contents of each of which are incorporated herein by reference in their entirety.

Hyaluronidase may be present in a particle of the present invention in an amount of about 0.1%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% by weight of the particle. In some embodiments, hyaluronidase is present in a particle of the present invention in an amount of about 0.1%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70% to about 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% by weight of the particle. In some embodiments, hyaluronidase is present in a particle of the present invention in an amount of about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, or 1% to about 2%, 3%, 4%, or 5% by weight of the particle. In some embodiments, hyaluronidase is present in a particle of the present invention in an amount of about 50%, 55%, 60%, 65%, or 70% to about 75%, 80%, 85%, 90%, 95%, 99%, or 100% by weight of the particle.

A particle of the present invention and/or a hyaluronidase and/or a therapeutic present in the particle may dissolve in an aqueous composition upon and/or following contact with the aqueous composition. In some embodiments, contacting the particle of the present invention,

and the aqueous composition may be carried out and/or performed *ex vivo* and/or *in vitro*. In some embodiments, contacting the particle of the present invention and the aqueous composition may be carried out and/or performed *in vivo* such as during and/or following administration (e.g., subcutaneous administration) to a subject by contacting the particle and a
5 bodily fluid. All or a portion of the particle may dissolve in the aqueous composition and/or all or a portion of the hyaluronidase and/or another component (e.g., a therapeutic) present in the particle may dissolve in the aqueous composition. In some embodiments, a particle of the present invention and/or a component thereof (e.g. a hyaluronidase and/or a therapeutic) may dissolve over a period of time that starts at the time of contact with an aqueous composition to
10 about 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 hours or more following contact with the aqueous composition, optionally at room temperature (e.g., about 20°C to about 22°C) and/or body temperature (e.g., about 33°C, 34°C, 35°C, or 36°C to about 38°C). In some embodiments, a particle of the present invention and/or a component thereof (e.g. a hyaluronidase and/or a therapeutic) dissolves in in an aqueous composition in about 15, 30, or
15 45 seconds or about 1, 5, 15, 30, or 45 minutes or about 1, 1.25, 1.5, 1.75, or 2 hours following contact of the particle and aqueous composition, optionally at a temperature in a range of about 20°C to about 40°C. Exemplary aqueous compositions include, but are not limited to, water, saline, a buffer (e.g., phosphate buffered saline), and/or a bodily fluid (e.g., blood and/or interstitial fluid).

20 A particle of the present invention may maintain and/or preserve one or more properties and/or functions of a hyaluronidase and/or therapeutic present in the particle compared to the same one or more properties and/or functions of the hyaluronidase and/or therapeutic prior to formation of the particle and/or compared to the same one or more properties and/or functions of a control hyaluronidase (e.g., free hyaluronidase) and/or a control therapeutic that was not
25 provided in the form of a particle. In some embodiments, a particle of the present invention may increase the stability of a hyaluronidase and/or therapeutic present in the particle compared to the stability of the hyaluronidase and/or therapeutic prior to formation of the particle. A control hyaluronidase may be the same hyaluronidase used in a particle of the present invention and/or is comparable thereto, and a control therapeutic may the same therapeutic used in a
30 particle of the present invention and/or is comparable thereto. In some embodiments, a control hyaluronidase is a commercially available hyaluronidase that has not been provided in the form of a particle of the present invention, and the control hyaluronidase may be the same type of hyaluronidase used in a particle of the present invention.

In some embodiments, following dissolution (e.g., all or a portion) of a particle of the present invention and/or a component thereof (e.g. a hyaluronidase and/or a therapeutic) in an aqueous composition, an activity of the component (e.g., the hyaluronidase and/or therapeutic) is within about $\pm$ 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the activity of a control component and/or of the activity of the component prior to formation in a particle of the present invention. The activity of the component (e.g. a hyaluronidase and/or a therapeutic) may be measured in the aqueous composition in which the particle is dissolved. Activity of a component (e.g., enzymatic activity of hyaluronidase) may be measured by a method known in the art. For example, activity of a hyaluronidase may be measured using the USP XXII assay for hyaluronidase, EC 3.2.1.35, HAse, and/or USP29-NF24, page 1057. In some embodiments, the activity of hyaluronidase and/or changes in activity of hyaluronidase may be determined and/or measured by measuring viscosity of a composition comprising hyaluronidase and/or by comparing viscosity of a composition comprising hyaluronidase after contact with hyaluronic acid for a certain amount of time. For example, in some embodiments, viscosity of a composition comprising hyaluronidase may be measured and the viscosity may be compared to a standard curve that was prepared using the viscosity of a composition including a specific amount of hyaluronidase after contact with hyaluronic acid for two or more different periods of time. In some embodiments, the control component is a control hyaluronidase and/or a control therapeutic. In some embodiments, the activity of a hyaluronidase may be measured using a turbidimetric assay such as that described in Dorfman, A. (1955) Methods in Enzymology, Volume I, 166-173 (One unit is equivalent to one NF unit as determined by the method described in USP XXII-NF XVII combined edition, p. 644 (1990)). For example, in some embodiments, the turbidity (e.g., optical density) of a composition comprising a hyaluronidase may be measured as the percent transmittance of 600 nm light through 1 cm of a hyaluronic acid composition (e.g., a composition (e.g., solution) comprising about 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, or about 0.5 mg/ml hyaluronic acid present (e.g., dissolved in phosphate buffered saline). In some embodiments, a reaction mixture comprising or consisting of 160 mM sodium phosphate, 39 mM sodium chloride, 0.005% (w/v) bovine serum albumin, 0.015% (w/v) hyaluronic acid, and 1.5-5 units hyaluronidase is incubated over 45 minutes at 37 °C, and 0.5 mL of the reaction mixture is then added to 2.5 mL of acid albumin solution (0.1% (w/v) bovine serum albumin pH 3.75 containing 1:1 HCl:H₂O) at 37°C and incubated for 10 min at room temperature. The percent transmittance at 600 nm is measured and compared to a standard curve. In some embodiments, one unit of hyaluronidase activity is equivalent to a change in A₆₀₀ of 0.330 per minute at pH

5.35 at 37 °C in a 2.0 ml reaction mixture (e.g., a reaction mixture comprising or consisting of 160 mM sodium phosphate, 39 mM sodium chloride, 0.005% (w/v) bovine serum albumin, 0.015% (w/v) hyaluronic acid, and 1.5-5 units hyaluronidase). In some embodiments, the activity of hyaluronidase and/or a therapeutic following dissolution is compared to the activity of the hyaluronidase and/or therapeutic prior to formation in a particle of the present invention. In some embodiments, following dissolution of a particle of the present invention in an aqueous composition, an enzymatic activity of the hyaluronidase that was present in the particle is within about $\pm$ 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the enzymatic activity of a control hyaluronidase (e.g., a free hyaluronidase) and/or of the enzymatic activity of the same hyaluronidase prior to formation in a particle of the present invention. For example, hyaluronidase having a specific activity of 1,500 units/mg solid (wherein the solid is the form of the hyaluronidase (e.g., a lyophilized powder) prior to formation in a particle) may be in a particle of the present invention and/or may be used to form a particle of the present invention (e.g., the particle comprises 1,500 hyaluronidase activity units per mg of solid wherein the solid is hyaluronidase lyophilized powder prior to formation of the particle), and, upon dissolution (e.g., all or a portion) of the particle comprising the hyaluronidase in an aqueous composition, the dissolved hyaluronidase may have a specific activity of at least about 750 units/mg solid. "Free hyaluronidase" as used herein refers to hyaluronidase that has not been in a particle of the present invention.

20 In some embodiments, following storage of a particle of the present invention and then dissolution (e.g., all or a portion) of the particle in an aqueous composition, an activity of a component (e.g., hyaluronidase and/or therapeutic) that was within the particle may be within about $\pm$ 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the activity of a control component (e.g., a control hyaluronidase and/or control therapeutic, respectively) and/or of the activity of the component prior to formation in the particle. In some embodiments, following storage of a particle of the present invention and then dissolution (e.g., all or a portion) of the particle in an aqueous composition, the enzymatic activity of hyaluronidase which was present in the particle may be within about $\pm$ 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the enzymatic activity of a control hyaluronidase and/or the enzymatic activity of the hyaluronidase prior to formation in the particle.

In some embodiments, an activity of a component (e.g., hyaluronidase and/or therapeutic) present in a particle following storage of the particle at about 4°C to about 40°C for at least about 3 months is within about $\pm$ 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the activity of a control component and/or of the activity of the component prior to

formation in the particle of the present invention, optionally following dissolution of the particle and/or component in an aqueous composition. In some embodiments, the enzymatic activity of hyaluronidase following storage of a particle comprising the hyaluronidase at about 4°C to about 40°C for at least about 3 months is within about $\pm$ 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the enzymatic activity of a control hyaluronidase and/or the enzymatic activity of hyaluronidase prior to formation in the particle.

A particle of the present invention may be stored at a temperature of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20°C to about 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40°C for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more weeks, months, and/or years. In some embodiments, a particle of the present invention is stored at a temperature of about 2°C to about 8 or 10°C for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 months. In some embodiments, a particle of the present invention is stored at a temperature of about 25°C or about 20°C to about 22°C for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 months. In some embodiments, a particle of the present invention is stored at a temperature of about 40°C for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 months.

A therapeutic may be present in a particle of the present invention and/or in a composition of the present invention. In some embodiments, the therapeutic may be an agent that can be dehydrated to provide a particle of the present invention and that can provide a therapeutic benefit to a subject. In some embodiments, the therapeutic may be an agent that can be microglassified and that can provide a therapeutic benefit to a subject. In some embodiments, a therapeutic is hydrophilic. In some embodiments, a therapeutic is hydrophobic. In some embodiments, the therapeutic is hydrophobic and is dissolved in a vehicle (e.g., a non-aqueous solvent and/or an aqueous solvent). In some embodiments, a therapeutic is a biologic such as, but not limited to, an amino acid, peptide, protein, enzyme or fragment thereof, antibody or fragment thereof (e.g., heavy chain, light chain, fusion protein, Fv, and/or Fc), nucleotide, and/or a polynucleotide such as, e.g., an oligonucleotide, DNA, and/or RNA. Exemplary antibody therapeutics include, but are not limited to, immunoglobulin G (IgG; human IgG), [fam]-trastuzumab deruxtecan, abagovomab, abciximab, adalimumab, ado-trastuzumab emtansine, aducanumab, alemtuzumab, alirocumab, amivantamab, odesivimab-ebgn, anifrolumab, ansuvimab, atezolizumab, atoltivimab, avelumab, axatilimab, bamlanivimab, basiliximab, bebtelovimab, bedinvetmab, belantamab mafodotin, belimumab, benralizumab, bevacizumab, bezlotoxumab, bimekizumab, blinatumomab, brentuximab

vedotin, brodalumab, brolocizumab, burosumab, camrelizumab, canakinumab, caplacizumab, casirivimab, catumaxomab, cemiplimab, certolizumab pegol, cetuximab, cilgavimab, concizumab, cosibelimab, crizanlizumab, crovalimab, daclizumab, daratumumab, denosumab, dinutuximab, dinutuximab beta, donanemab, dostarlimab, dupilumab, durvalumab, 5 eculizumab, edrecolomab, efalizumab, elotuzumab, elranatamab, emapalumab, emicizumab, enfortumab vedotin, epcoritamab, eptinezumab, erenumab, ertumaxomab, etesevimab, evinacumab, evolocumab, faricimab, fremanezumab, frunevetmab, galcanezumab, garadacimab, gemtuzumab, gemtuzumab ozogamicin, glofitamab, golimumab, guselkumab, ibalizumab, ibritumomab tiuxetan, idarucizumab, imdevimab, inebilizumab, infliximab, 10 inotuzumab, inotuzumab ozogamicin, ipilimumab, isatuximab, itolizumab, ixekizumab, lanadelumab, lebrikizumab, lecanemab, lokivetmab, loncastuximab tesirine, maftivimab, margetuximab, marstacimab, mepolizumab, mirikizumab, mirvetuximab soravtansine, mogamulizumab, mosunetuzumab, moxetumomab pasudotox, muromonab-cd3, narsoplimab, natalizumab, naxitamab, nebacumab, necitumumab, nimotuzumab, nirsevimab, nivolumab, 15 obiltoxaximab, obinutuzumab, ocrelizumab, odronextamab, ofatumumab, olaratumab, omalizumab, palivizumab, panitumumab, patritumab deruxtecan, pembrolizumab, pertuzumab, polatuzumab vedotin, pozelimab, racotumomab, ramucirumab, ranibizumab, ravulizumab, raxibacumab, regdanvimab, relatlimab, reslizumab, retifanlimab, risankizumab, rituximab, romosozumab, rozanolixizumab, ruplizumab, sacituzumab govitecan, sarilumab, 20 satralizumab, secukinumab, serplulimab, siltuximab, sintilimab, sotrovimab, spesolimab, sugemalimab, sutimlimab, tafasitamab, talquetamab, tarlatamab, tebentafusp, teclistamab, teplizumab, teprotumumab, tezepelumab, tildrakizumab, tislelizumab, tisotumab vedotin, tixagevimab, tocilizumab, toripalimab, tositumomab, tralokinumab, trastuzumab, tremelimumab, ublituximab, ustekinumab, vedolizumab, veltuzumab, and zolbetuximab and a 25 fragment of any of the foregoing.

In some embodiments, the therapeutic is IgG (human IgG), abagovomab, adalimumab, alirocumab, bedinvetmab, belimumab, benralizumab, bimekizumab, brodalumab, burosumab, canakinumab, casirivimab, certolizumab pegol, concizumab, crovalimab, daclizumab, denosumab, dupilumab, efalizumab, elranatamab, emicizumab, epcoritamab, erenumab, 30 evolocumab, fremanezumab, frunevetmab, galcanezumab, garadacimab, guselkumab, ixekizumab, lanadelumab, lebrikizumab, lokivetmab, marstacimab, mepolizumab, mirikizumab, ofatumumab, omalizumab, pozelimab, risankizumab, romosozumab, rozanolixizumab, sarilumab, satralizumab, secukinumab, talquetamab, teclistamab, tezepelumab, tildrakizumab, tocilizumab, tralokinumab, ustekinumab, veltuzumab, a fragment

of any of the foregoing, or a combination of any thereof. In some embodiments, the antibody therapeutic is IgG (human IgG), abagovomab, alemtuzumab, daratumumab, denosumab, pertuzumab, rituximab, trastuzumab, veltuzumab, a fragment of any of the foregoing, or a combination of any thereof.

5 In some embodiments, the therapeutic is an antibody conjugated (e.g., covalently bound) to another moiety (e.g., a second therapeutic), such as, but not limited to, an antibody drug conjugate (ADC) and/or an antibody-oligo conjugate (AOC). In some embodiments, a therapeutic comprises an antibody that is conjugated to a tubulin inhibitor (e.g., auristatin, a maytansinoid, and/or tubulysin), a DNA damaging agent (e.g., calicheamicin, duocarmycin, exatecan, deruxitecan, govitecan, and/or pyrrolobenzodiazepine), and/or an immunomodulator (e.g., a toll like receptor agonist and/or a stimulator of interferon genes agonist).

 In some embodiments, the therapeutic is a small molecule having a molecular weight of less than 500 daltons, optionally a small organic molecule (i.e., an organic compound having a molecular weight of less than 500 daltons). Exemplary therapeutics include, but are not limited to, antimicrobial agents, anti-inflammatory agents, analgesic agents, anesthetic agents, antihistamine agents, antiseptic agents, immunosuppressants, antihemorrhagic agents, vasodilators, wound healing agents, antineoplastics, antacids, antianxiety drugs, antiarrhythmics, antibacterials, antibiotics, anticoagulants, thrombolytics, anticonvulsants, antidepressants, antidiarrheals, antiemetics, antifungals, antihypertensives, antipsychotics, antipyretics, antivirals, barbiturates, beta-blockers, bronchodilators, cold relief agents, corticosteroids, cough suppressants, cytotoxics, decongestants, diuretics, expectorants, hormones, hypoglycemics, laxatives, muscle relaxants, sedatives, sex hormones (female and/or male), sleeping drugs, tranquilizers, vitamins, and/or anti-biofilm agents. In some embodiments, the therapeutic is an anti-inflammatory agent such as a corticosteroid. In some
25 embodiments, the therapeutic is an anesthetic agent such as bupivacaine. In some embodiments, the therapeutic is a hormone, such as testosterone (e.g., testosterone enanthate), dihydrotestosterone, androstenedione, estrone, estradiol, estriol, estetrol, estrogen, and/or progesterone. In some embodiments, a hormone therapeutic is dissolved into a non-aqueous vehicle (e.g., an oil, e.g., sesame oil) that optionally includes a particle of the present invention.
30 In some embodiments, the therapeutic is XYOSTED® (e.g., testosterone enanthate). In some embodiments, a composition of the present invention comprises a non-aqueous vehicle (e.g., an oil, e.g., sesame oil), XYOSTED® that is optionally dissolved in the non-aqueous vehicle, and optionally a particle of the present invention. One or more (e.g., 1, 2, 3, 4, 5, or more) different therapeutic(s) may be present in a particle and/or composition of the present

invention. In some embodiments, two or more different therapeutics are present in the same particle or are present in different, separate particles.

A therapeutic may be present in a particle of the present invention in an amount of about 50%, 55%, 60%, 65%, or 70% to about 75%, 80%, 85%, 90%, 95%, or 99% by weight of the particle. In some embodiments, the particle comprises the therapeutic in an amount of about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% by weight of the particle. In some embodiments, the therapeutic is present in the particle in an amount of about 75% or 80% to about 85%, 90%, or 95% by weight of the particle such as in an amount of about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95% by weight of the particle. In a particle of the present invention, hyaluronidase and a therapeutic may not be covalently bound and/or an excipient (e.g., a stabilizer) may not be covalently bound to a therapeutic.

In some embodiments, a particle of the present invention may be devoid of a therapeutic.

A stabilizer may be present in a particle of the present invention and/or in a composition of the present invention. Exemplary stabilizers include, but are not limited to, sugars, amino acids, polymers (e.g., hyaluronic acid, gelatin, and/or gelatin), antioxidants, surfactants, and/or a protein such as a globular protein (e.g., an albumin) and/or a disordered protein (e.g., an intrinsically disordered protein). In some embodiments, the stabilizer is an albumin (e.g., a serum albumin and/or recombinant albumin such as a recombinant albumin commercially available from Alumedix Ltd. of Nottingham, United Kingdom). In some embodiments, the stabilizer is a hyaluronic acid. In some embodiments, a particle of the present invention may comprise a sugar such as, but not limited to, sucrose, glucose, trehalose, mannitol, sorbitol, dextrose, maltose, and/or lactose. In some embodiments, a particle of the present invention may comprise an amino acid such as, but not limited to, histidine, methionine, arginine, lysine, aspartic acid, glutamic acid, proline, glycine, and/or leucine. In some embodiments, a particle of the present invention may comprise an albumin such as, but not limited to, a serum albumin. One or more (e.g., 1, 2, 3, 4, or 5) different stabilizer(s) may be present in a particle and/or composition of the present invention. In a particle of the present invention, hyaluronidase and a stabilizer may not be covalently bound and/or a stabilizer may not be covalently bound to a therapeutic.

A stabilizer may be present in a particle of the present invention in an amount of about 0.1%, 0.5%, 1%, 5%, 10%, 15%, or 20% to about 25%, 30%, 35%, 40%, 45%, or 50% by weight of the particle. In some embodiments, a stabilizer may be present in a particle of the

present invention in an amount of about 0.1%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by weight of the particle. In some embodiments, a stabilizer (e.g., an amino acid) may be present in a particle of the present invention in an amount of about 0.1%, 0.5%, 1%, 5%, or 10% to about 15%, 20%, 25%, or 30% by weight of the particle.

5 According to some embodiments of the present invention, a composition comprising a particle of the present invention is provided. The composition may comprise a plurality of particles of the present invention. In some embodiments, the composition is a suspension and one or more particles, that may be the same as and/or different from one another, are suspended in a solvent (e.g., a vehicle) present in the composition. In some embodiments, the composition
10 is in the form of a powder and/or solid.

 In some embodiments, a composition (e.g., a suspension) of the present invention comprises a plurality of particles and each particle of the plurality of particles comprises a hyaluronidase and a therapeutic. In some embodiments, a composition of the present invention comprises a non-aqueous solvent (e.g., a non-aqueous vehicle) and a plurality of particles,
15 wherein each particle of the plurality of particles comprises a hyaluronidase and a therapeutic. In some embodiments, a composition of the present invention comprises an aqueous solvent (e.g., an aqueous vehicle) and a plurality of particles, wherein each particle of the plurality of particles comprises a hyaluronidase and a therapeutic. In some embodiments, a composition of the present invention comprises a plurality of particles, wherein each particle of the plurality
20 of particles comprises a hyaluronidase and a therapeutic, and wherein the composition is in the form of a powder.

 In some embodiments, a composition (e.g., a suspension) of the present invention comprises a plurality of particles and a first portion of the plurality of particles comprises particles that each comprise a hyaluronidase and are devoid of a different therapeutic, and a
25 second portion of the plurality of particles comprises particles that each comprise a therapeutic that is different than hyaluronidase and are devoid of hyaluronidase. In some embodiments, a composition of the present invention comprises a non-aqueous solvent (e.g., a non-aqueous vehicle), a first plurality of particles, wherein each particle of the first plurality of particles comprises a hyaluronidase and is devoid of a different therapeutic, and a second plurality of
30 particles, wherein each particle of the second plurality of particles comprises a therapeutic that is different than hyaluronidase and is devoid of hyaluronidase. In some embodiments, a composition of the present invention comprises an aqueous solvent (e.g., an aqueous vehicle), a first plurality of particles, wherein each particle of the first plurality of particles comprises a hyaluronidase and is devoid of a different therapeutic, and a second plurality of particles,

wherein each particle of the second plurality of particles comprises a therapeutic that is different than hyaluronidase and is devoid of hyaluronidase. In some embodiments, a composition of the present invention comprises a first plurality of particles, wherein each particle of the first plurality of particles comprises a hyaluronidase and is devoid of a different
5 therapeutic, and a second plurality of particles, wherein each particle of the second plurality of particles comprises a therapeutic that is different than hyaluronidase and is devoid of hyaluronidase, wherein the composition is in the form of a powder.

In some embodiments, a composition of the present invention comprises a plurality of particles that are suspended in the composition (i.e., are not dissolved in the composition) and
10 one or more component(s) (e.g., a therapeutic) that are dissolved in a vehicle (e.g., a solvent) present in the composition. In some embodiments, the one or more component(s) (e.g., a therapeutic) are dissolved in a non-aqueous vehicle present in the composition. In some embodiments, the one or more component(s) (e.g., a therapeutic) are dissolved in an aqueous vehicle present in the composition. The one or more component(s) may be different than
15 hyaluronidase. In some embodiments, hyaluronidase is not dissolved in a composition of the present invention and/or a composition of the present invention is devoid of dissolved hyaluronidase. In some embodiments, a composition of the present invention comprises a non-aqueous solvent; a plurality of particles of the present invention, wherein each particle comprises hyaluronidase and is suspended in the non-aqueous solvent; and a therapeutic that
20 is not a hyaluronidase and that is dissolved in the non-aqueous solvent.

A particle of the present invention may be present as a solid in a composition of the present invention. In some embodiments, the composition may comprise a particle of the present invention in an amount of about 1, 5, 10, 25, 50, 75, 100, 150, or 200 mg of particles (e.g., solids) per mL of the composition to about 300, 350, 400, 450, 500, 550, 600, 650, or 700
25 mg of particles (e.g., solids) per mL of the composition. In some embodiments, the composition is non-aqueous and/or the composition comprises a non-aqueous vehicle. "Non-aqueous" as used herein in reference to a composition or vehicle refers to a composition or vehicle, respectively, that is made from, with, or by means of a liquid other than water and which has properties and/or characteristics of a liquid other than water. In some embodiments, a non-
30 aqueous composition or vehicle comprises one or more (e.g., 1, 2, 3, 4, 5, or more) organic liquid(s) that do not dissolve in water and/or that are immiscible or miscible with water. In some embodiments, a composition of the present invention comprises a non-aqueous organic solvent. In some embodiments, a composition and/or vehicle of the present invention comprises water. In some embodiments, a composition and/or vehicle of the present invention

is anhydrous. "Anhydrous," as used herein, means that there is no direct addition of water to the composition or vehicle when it is being prepared. However, those skilled in the art will recognize that water may be physically and/or chemically absorbed by one or more components in the composition or vehicle at any time during the preparation, storage, and/or use of the composition or vehicle (*i.e.*, indirect addition of water to the composition). In some
5 embodiments, the term "anhydrous" means that the composition has a water content of less than 5% by weight of the composition or any range and/or individual value therein. A composition of the present invention may have a water content of less than 5%, 4.5%, 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, 1%, or 0.5%, or any range therein, by weight of the composition.
10 Water content may be measured by methods known to those of skill in the art, such as, but not limited to, Karl Fischer titration.

A composition of the present invention may comprise a solvent. One or more (e.g., 1, 2, 3, 4, 5, or more) different solvent(s) may be present in a composition of the present invention. A particle of the present invention may not dissolve in a solvent present in a composition of
15 the present invention, optionally wherein the particle does not dissolve in the solvent after storage in the composition at a temperature of about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20°C to about 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40°C for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 months or more. In some embodiments, a particle of the present invention
20 may not dissolve in a composition of the present invention, optionally wherein the particle does not dissolve in the composition after storage at a temperature of about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20°C to about 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40°C for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 months or more. In some embodiments, hyaluronidase
25 and/or a therapeutic is not soluble in a solvent and/or composition of the present invention. In some embodiments, hyaluronidase is not soluble in a solvent and/or composition of the present invention and a therapeutic is soluble in the solvent and/or composition of the present invention. Thus, a particle of the present invention and/or hyaluronidase may remain a solid in a composition of the present invention. Exemplary solvents that may be present in a
30 composition of the present invention include, but are not limited to, alcohols such as an alcohol having 1-20 carbons (*i.e.*, a C1-C20 alcohol) or a C4-C20 alcohol, alkanes such as an alkane having 1-20 carbon atoms (*i.e.*, a C1-C20 alkane) or a C4-C20 alkane (e.g., propane, pentane, hexane, etc.); alcohols (e.g., a C5-C14 alcohol); acetates; esters (e.g., benzyl benzoate, fatty acid esters such as ethyl oleate, triglyceride esters such as MIGLYOL® 812 (a triglyceride

ester of saturated coconut/palmkernel oil derived caprylic and capric fatty acids and plant derived glycerol) and/or MIGLYOL® 840 (an ester of saturated plant derived caprylic and capric fatty acids with propylene glycol that can be referred to as propylene glycol dicaprylocaprate)); ethers; carboxylic acids; (poly)heteroatomic cyclic, acyclic, linear or branched molecules; an oil (e.g., sesame oil, castor oil, soybean oil, and/or cotton seed oil); lactates such as butyl lactate and/or ethyl lactate; and/or fluorinated compounds such as perfluorinated carbons (e.g., perfluorodecaline, perfluorooctane, and/or perfluooctylbromide) and/or semifluorinated alkanes (e.g., perfluorobutylbutane, perfluorobutylpentane, perfluorobutylhexane, perfluorobutyloctane, perfluorohexylhexane, perfluorohexyloctane, and/or perfluorohexyldodecane). In some embodiments, the solvent may include a carbon, nitrogen, and/or sulfur atom. A composition of the present invention may comprise a solvent that is an ester such as an alkyl ester or aryl ester (e.g., benzyl benzoate). In some embodiments, a composition of the present invention comprises a C1-C20 alcohol, an ester containing compound (e.g., a C1-C20 ester containing compound such as, e.g., ethyl acetate, butyl acetate, triacetin, isobutyl acetate, isopropyl acetate, isopropyl myristate, methyl acetate, propyl acetate, and/or butyl lactate), acetic acid, acetone, anisole, tert butyl methyl ether, cumene, dimethyl sulfoxide, diethyl ether, ethyl formate, formic acid, alkanes (e.g., heptane, pentane, etc.), and/or methylethylketone. In some embodiments, a composition of the present invention comprises benzyl benzoate, ethyl oleate, a triglyceride ester, ethyl lactate, and/or sesame oil. In some embodiments, the solvent is an aqueous solvent (e.g., water, saline, etc.)

A composition of the present invention may have a viscosity of greater than 1 cP when measured at 25 degrees Celsius. In some embodiments, a composition of the present invention may be a suspension that comprises a plurality of particles suspended in one or more (e.g., 1, 2, 3, 4, 5, or more) non-aqueous phase(s). In some embodiments, a composition of the present invention may have a viscosity of about 20, 30, 40, 50, 60, or 70 centipoise (cP) to about 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 cP, when measured at a temperature of about 20 degrees Celsius and about 25 degrees Celsius and a shear rate of between about 1,000 s⁻¹ to about 3,000 s⁻¹.

In some embodiments, one or more (e.g., 1, 2, 3, 4, 5, or more) different therapeutic(s) may be present in a composition of the present invention. In some embodiments, a composition of the present invention comprises a first particle and a second particle and the first particle comprises a first therapeutic and a second particle comprises a second therapeutic that is different than the first therapeutic, optionally wherein a hyaluronidase may be present in the first particle and/or second particle. In some embodiments, a composition of the present

invention comprises a particle comprising a first therapeutic and/or a hyaluronidase that is suspended in the composition and a second therapeutic that is dissolved in a solvent present in the composition, wherein the first therapeutic, if present, is different than the second therapeutic.

5 One or more (e.g., 1, 2, 3, 4, 5, or more) different excipient(s) may be present in a particle and/or composition of the present invention. Exemplary excipients include, but are not limited to, stabilizers, surfactants (e.g., detergents), tonicity agents, sugars, salts, antimicrobial agents, antioxidants, reducing agents, etc. In some embodiments, an excipient is present in a composition of the present invention at a concentration of about 0.01%, 0.05%, 0.1%, or 0.5%
10 to about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% by weight of the composition. Exemplary surfactants include, but are not limited to, polysorbates (e.g., polysorbate-20 and/or polysorbate-80), sorbitan esters (spans; e.g., sorbitan monostearate, sorbitan tristearate, sorbitan monolaurate, sorbitan trioleate, sorbitan monopalmitate, sorbitan
15 monooleate, and/or sorbitan sesquioleate), pegylated fatty esters and ethers (e.g., Laureth-4), sucrose esters, block copolymers (e.g., poloxamers such as Poloxamer 188), and/or ethoxylated triglycerides (e.g., ethoxylated castor oil).

Exemplary antimicrobial agents include, but are not limited to, benzoic acid and/or benzyl alcohol. Exemplary antioxidants include, but are not limited to, butylated hydroxy
20 anisole, butylated hydroxy toluene, propyl gallate, tert-butyl hydroquinone, and/or Vitamin E (e.g., alpha-tocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol, alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol, and/or delta-tocotrienol). Exemplary reducing agents include, but are not limited to, glutathione and/or dithiothreitol (DTT).

A particle of the present invention may be prepared by removing (e.g., dissolving and/or
25 extracting) water from a hyaluronidase present in a composition (e.g., an aqueous composition), that optionally includes a therapeutic and/or excipient, to provide a solid particle. In some embodiments, a particle of the present invention is prepared by contacting an aqueous phase and a solvent phase to dehydrate hyaluronidase present in one of the phases and form a particle comprising hyaluronidase. A method of the present invention may provide a solid
30 particle comprising hyaluronidase, optionally a therapeutic, and optionally an excipient, wherein the solid particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9. In some embodiments, a method of the present invention comprises removing water from a hyaluronidase present in a composition and providing a solidified hyaluronidase particle (i.e., hyaluronidase in a solid form). Upon

formation of a particle of the present invention, the particle may be separated from any liquid and/or provided in the form of a powder. In some embodiments, a particle of the present invention may be suspended in a solvent (e.g., a non-aqueous solvent).

In some embodiments, a particle of the present invention may be prepared according to a microglassification process. In some embodiments, water may be removed from a composition comprising a hyaluronidase using an aqueous phase and a non-aqueous phase to provide a solid comprising the hyaluronidase. In some embodiments, a composition and/or method (e.g., microglassification method) used herein does not include (i.e., is devoid of) a phase separating agent. In some embodiments, a particle of the present invention is a particle comprising microglassified hyaluronidase. In some embodiments, a particle comprising hyaluronidase may be dissolved back into the initial composition in which it was present (e.g., an aqueous solution), a bodily fluid, and/or into an aqueous composition (e.g., an aqueous buffer). In some embodiments, a method of forming a particle of the present invention may comprise a device, step, and/or composition as described in U.S. Patent No. 8,013,022 and/or U.S. Patent Application Publication No. 2022/0119760, which are incorporated herein by reference in their entirety.

In some embodiments, a composition comprising hyaluronidase (e.g., an aqueous composition) is contacted with a dehydration composition to prepare a particle of the present invention. As used herein "contact", "contacting", "contacted," and grammatical variations thereof, refer to bringing two or more materials (e.g., compositions, compounds, solvents, etc.) together to form a mixture. Contacting the two or more materials may be carried out by pouring, spraying, mixing, flowing, injecting (e.g., microinjecting), and/or the like the two materials or a portion thereof together. For example, contacting may comprise adding a solvent to a composition comprising hyaluronidase or adding a composition comprising hyaluronidase to a solvent. Contacting may be carried out in a device such as, e.g., a mixer, homogenizer, and/or microfluidic device.

In some embodiments, contacting comprises bringing two or more materials (e.g., compositions, compounds, solvents, etc.) together in sufficient proximity such that, under suitable conditions, a desired reaction can be carried out (e.g., water can be removed from a component present in one of the materials). In some embodiments, a contacting step forms a mixture and the mixture may be one phase or two or more (e.g., 2, 3, or more) phases. In some embodiments, the mixture is a multi-phase composition in that the composition has two or more (e.g., 2, 3, 4, or more) phases. In some embodiments, a contacting step forms a two phase composition and/or an emulsion. In some embodiments, a contacting step forms a suspension

(e.g., one or more liquid phases including a solid particle). In some embodiments, an emulsion is formed and the emulsion may be a water-in-oil emulsion, optionally comprising droplets with a diameter and/or smallest dimension of less than about 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 25, 10, 5 or 1 μm . In some embodiments, the mixture is a water-in-oil emulsion comprising droplets having a diameter and/or smallest dimension in a range of about 5, 10, 25, 50, 100, 200, 300, 400, or 500 μm to about 600, 700, 800, 900, or 1000 μm . In some embodiments, the mixture is a water-in-oil emulsion comprising droplets having a diameter and/or smallest dimension in a range of about 0.1, 0.5, 1, 2, 3, 4, or 5 μm to about 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 μm . In some embodiments, a contacting step forms a mixture (e.g., an emulsion) and a component (e.g., hyaluronidase) present in one of the materials is at least partially dehydrated as a result of the contacting step and/or forming the mixture.

A particle of the present invention may be formed by contacting a composition comprising hyaluronidase and a dehydration composition that at least partially dehydrates a component present in the composition (e.g., a hyaluronidase, a therapeutic and/or an excipient present in the composition). Upon contacting the dehydration composition and the composition comprising hyaluronidase, the dehydration composition may be present in a concentration and/or volume sufficient to at least partially dehydrate a component of the composition (e.g., hyaluronidase). In some embodiments, the dehydration composition comprises a solvent (e.g., an organic solvent) that removes water from a component present in the composition (e.g., a hyaluronidase and/or therapeutic). Exemplary organic solvents include, but are not limited to, alcohols such as, e.g., an alcohol having 1-20 carbons (i.e., a C1-C20 alcohol) or a C4-C20 alcohol, ester containing compounds (e.g., a C1-C20 ester containing compound such as, e.g., ethyl acetate, butyl acetate, triacetin, isobutyl acetate, isopropyl acetate, methyl acetate, propyl acetate, and/or butyl lactate), acetic acid, acetone, anisole, tert butyl methyl ether, cumene, dimethyl sulfoxide, diethyl ether, ethyl formate, formic acid, alkanes (e.g., heptane, pentane, etc.), and/or methylethylketone.

In some embodiments, a dehydration composition comprises a C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, and/or C20 alcohol and/or a C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, and/or C20 ester containing compound. In some embodiments, the alcohol is selected from methanol; ethanol; propanol (e.g., 1-propanol; 2-propanol); butanol (e.g., 1-butanol; 2-butanol); 2-methyl-1-propanol; 2-methyl-2-propanol; tert-butanol; pentanol (e.g., 1-pentanol, 3-methyl-1-butanol, 2,2-dimethyl-1-propanol, cyclopentanol); hexanol (e.g., 1-hexanol); cyclohexanol; heptanol (e.g., 1-heptanol); octanol (e.g., 1-octanol); nonanol (e.g., 1-nonanol);

decanol (e.g., 1-decanol); 2-propen-1-ol; benzyl alcohol; phenylmethanol; diphenylmethanol; undecanol; dodecanol; propyldecanol; butadecanol; pentadecanol; hexadecanol (e.g., 1-hexadecanol); and/or triphenylmethanol. In some embodiments, a dehydration composition does not comprise a C10 alcohol (e.g., 1-decanol). In some embodiments, the ester containing
5 compound may be formed from an acid and a C1-C20, C1-C10, C1-C8, C1-C6, or C1-C4 alcohol. In some embodiments, a dehydration composition comprises an isomer of a linear alcohol (e.g., 2-octanol, 3-pentanol, 4-decanol), a derivative of a linear alcohol or their isomer (e.g., octyldodecanol, neopentyl alcohol), a di, tri, or quad hydroxylated materials (e.g., 1,4-butanediol, glycerin), an unsaturated alcohol (e.g., a cyclic, olefinic or alkynyl alcohol such as
10 e.g., cyclohexanol, geraniol, oleic alcohol), and/or an alcohol incorporating an internal and/or external heteroatoms (e.g., polyethylene glycols, polypropylene glycols, lactates, etc.).

One or more (e.g., 1, 2, 3, 4, or more) solvents may be present in a dehydration composition of the present invention. For example, in some embodiments, at least two alcohols are present in a dehydration composition (e.g., 1-pentanol and 1-hexadecanol), at least two
15 ester containing compounds (e.g., ethyl acetate and triacetin), or at least one alcohol (e.g., 1-pentanol) and one ester containing compound (e.g., ethyl acetate). When two or more solvents are present in a dehydration composition, they may be present in any suitable ratio. In some embodiments, a dehydration composition comprises two solvents that are present in the composition in a ratio (by volume or weight) of about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9,
20 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, or 1:20.

A solvent (e.g., organic solvent) present in a dehydration composition of the present invention may have a solubility for water of about 0.05%, 1%, 2%, or 5% to about 10%, 12%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% w/w. In some embodiments, the organic solvent has a solubility for water of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%,
25 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50% w/w. A solvent (e.g., an organic solvent) may have an interfacial tension with water and/or an aqueous composition less than about 55 mN/m. In some embodiments, a solvent present in a dehydration composition of the present invention
30 has an interfacial tension with water and/or an aqueous composition that is less than about 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 mN/m. In some embodiments, a solvent present in a dehydration composition of the present invention has an interfacial tension with water and/or an aqueous composition in a range of about 1, 2, 3, 4, or 5 to about 6, 7, 8, 9, or 10.

In some embodiments, a dehydration composition of the present invention consists of one or more (e.g., 1, 2, 3, 4, 5, or more) different organic solvent(s) (e.g., an alcohol). In some embodiments, the dehydration composition consists of two or more C1-C20 alcohols. In some embodiments, a dehydration composition of the present invention comprises an organic solvent
5 in an amount of about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%,
10 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% by weight of the dehydration composition.

A dehydration composition may comprise one or more (e.g., 1, 2, 3, 4, 5, or more) additives (e.g., stabilizers, surfactants, tonicity agents, salts, sugars, antimicrobial agents, antioxidants, etc.). Exemplary additives (e.g., surfactants, antimicrobial agents, antioxidants,
15 etc.) include, but are not limited to, those described above. In some embodiments, an additive is present in a dehydration composition at a concentration of about 0.01%, 0.1%, or 0.5% to about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, or 35% by weight of the dehydration composition. In some embodiments, the
20 dehydration composition comprises a surfactant and/or a sugar. In some embodiments, a surfactant may be present in a dehydration composition in an amount of about 30% or less by weight of the dehydration composition such as, e.g., about 25%, 20%, 15%, 10%, 5%, or less by weight of the dehydration composition. In some embodiments, an antimicrobial agent may be present in a dehydration composition in an amount of about 1% or less by weight of the
25 dehydration composition such as, e.g., about 0.5%, 0.1%, or less by weight of the dehydration composition. In some embodiments, an antioxidant may be present in a dehydration composition in an amount of about 2.5% or less by weight of the dehydration composition such as, e.g., about 2%, 1.5%, 1%, 0.5%, 0.1%, or less by weight of the dehydration composition.

In some embodiments, a dehydration composition comprises at least one alcohol
30 selected from benzyl alcohol, 1-propanol, 1-butanol, tert-butyl, sec-butyl, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, 1-decanol, 1-undecanol, 1-dodecanol, propyldecanol, butadecanol, pentadecanol, and hexadecanol, and/or at least one solvent selected from water, triacetin, benzyl alcohol, acetic acid, acetone, anisole, 2-butanol, butyl acetate, tert butyl methyl ether, cumene, dimethyl sulfoxide, ethanol, ethyl acetate, diethyl

ether, ethyl formate, formic acid, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethylketone, 2-methyl-1-propanol, pentane, 2-propanol, propyl acetate, and tert-butanol. Further exemplary solvents that may be present in a dehydration composition include, but are not limited to, alkanes such as, e.g., an alkane having 1-20 carbon atoms (i.e., a C1-C20 alkane) or a C4-C20 alkane (e.g., propane, pentane, hexane, etc.); 5 alcohols (e.g., a C5-C14 alcohol); acetates; esters; ethers; carboxylic acids; (poly)heteroatomic cyclic, acyclic, linear or branched molecules; and/or lactates. In some embodiments, a dehydration composition includes a solvent that includes a carbon, nitrogen, and/or sulfur atom.

A method of the present invention may comprise contacting a composition comprising 10 hyaluronidase and one or more (e.g., 1, 2, 3, 4, or more) dehydration compositions. Accordingly, the method may comprise contacting a composition comprising hyaluronidase and one or more (e.g., 1, 2, 3, 4, or more) solvents such as, e.g., a first organic solvent, second organic solvent, etc. When two or more dehydration compositions are used, the solvent present in the compositions may be the same or different. In some embodiments, a solvent present in 15 a dehydration composition may be more volatile than a solvent in an immediately prior dehydration composition and/or may be a volatile organic compound (e.g., has a boiling point from about 0, 50, or 100°C to about 150, 200, or 260°C). In some embodiments, a dehydration composition and an at least partially dehydrated component may be contacted one or more (e.g., 1, 2, 3, 4, 5, or more) times.

20 A batch or inline process may be used to contact a composition comprising hyaluronidase with one or more dehydration composition(s) to prepare a particle of the present invention. In some embodiments, a method comprises contacting a composition comprising hyaluronidase and a first dehydration composition to form a mixture, then contacting the mixture and a second dehydration composition to form a particle of the present invention. In 25 some embodiments, a method comprises contacting a composition comprising hyaluronidase and a first dehydration composition to form a mixture, separating an at least partially dehydrated hyaluronidase from the mixture, and contacting the at least partially dehydrated hyaluronidase with a second dehydration composition, thereby forming a particle of the present invention.

30 A solvent present in a dehydration composition may form an interface with a composition comprising hyaluronidase. In some embodiments, a solvent present in a dehydration composition is not miscible with water. In some embodiments, a solvent present in a dehydration composition that is used first to dehydrate a component (i.e., a first dehydration composition or first organic solvent) is not miscible with water and/or forms an interface with

water, and the solvent is at least partially water soluble. In some embodiments, a solvent present in a dehydration composition that is used after a first dehydration composition (e.g., a second or third dehydration composition or second or third organic solvent) is not miscible with water.

5 Contacting a dehydration composition and a composition comprising hyaluronidase and/or forming a mixture may comprise reducing the water content of a component (e.g., a hyaluronidase and/or therapeutic) present in the mixture by at least about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%,
10 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the water content of the component is reduced by about 5%,
15 10%, 15%, 20%, 25%, 30%, or 35% to about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% after a contacting step of the present invention.

 A mixture comprising a dehydration composition and a component (e.g., a hyaluronidase and/or therapeutic) may have a water activity of less than about 0.99, 0.98, 0.95, 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, or 0.1. In some
20 embodiments, the mixture has a water activity in a range of about 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, or 0.5 to about 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, or 0.95. In some embodiments, the mixture has a fractional water saturation of less than about 0.95, 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, or 0.1. In some embodiments, the mixture has a fractional water saturation in a range of about 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4,
25 0.45, or 0.5 to about 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, or 0.95. For example, in some embodiments, a mixture comprising a protein may have a fractional water saturation of about 0.95 or less. In some embodiments, a mixture comprising a salt may have a fractional water saturation of about 0.5 or less.

 An at least partially dehydrated component (e.g., a hyaluronidase and/or therapeutic)
30 may have a water content of about 99%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 1%, or 0% after being contacted with one or more dehydration compositions. In some embodiments, an at least partially dehydrated component (e.g., a hyaluronidase) has a water content in a range of about 0%, 0.5%, 1%, 5%, 10%, 15%, 20%, or 25% to about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,

or 75% after being contacted with one or more dehydration compositions. In some embodiments, an at least partially dehydrated component (e.g., a hyaluronidase) has a water content in a range of about 0%, 0.5%, 1%, 2%, 3%, or 4% to about 5%, 6%, 7%, 8%, 9%, or 10% after being contacted with one or more dehydration compositions. In some embodiments, an at least partially dehydrated component (e.g., a hyaluronidase) has a water activity of about 0.986, 0.98, 0.95, 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1, 0.05, or 0 after being contacted with one or more dehydration compositions. In some embodiments, an at least partially dehydrated component (e.g., a hyaluronidase) has a water activity of in a range of about 0, 0.05, 0.1, 0.15, 0.2, 0.25, or 0.3 to about 0.35, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 after being contacted with one or more dehydration compositions. In some embodiments, an at least partially dehydrated component (e.g., a hyaluronidase) has a water activity of less than about 0.75 after being contacted with one or more dehydration compositions. In some embodiments, an at least partially dehydrated component (e.g., a hyaluronidase) has a water activity of less than about 0.5 after being contacted with one or more dehydration compositions.

In some embodiments, after contacting an initial dehydration composition and a composition comprising hyaluronidase, an at least partially dehydrated component (e.g., hyaluronidase) has a water content of less than about 60% and/or in a range of about 1%, 5%, 10%, 15%, 20%, or 25% to about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70%. In some embodiments, after contacting an at least partially dehydrated component and a subsequent (e.g., second) dehydration composition, which may be the same as the initial dehydration composition or different, the at least partially dehydrated component has a water content of less than about 45% and/or in a range of about 0.5%, 1%, 5%, 10%, 15%, or 20% to about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70%. In some embodiments, an at least partially dehydrated component is a particle of the present invention.

The contacting step may be carried out at a temperature in a range of about -50, -40, -30, -20, -10, 0, 4, 10, 15, or 20°C to about 25, 30, 35, 40, 45, or 50°C. In some embodiments, the contacting step in a method of the present invention is carried out at room temperature and/or atmospheric pressure.

In some embodiments, upon contacting a dehydration composition (e.g., an organic solvent) and a composition comprising hyaluronidase and/or forming a mixture comprising the dehydration composition and a composition comprising hyaluronidase, a suspension may be formed. In some embodiments, the suspension comprises hyaluronidase in the form of a solid that is suspended in a liquid (e.g., a non-aqueous organic phase). Contacting a dehydration

composition and a composition comprising hyaluronidase to provide a mixture and forming an at least partially dehydrated component in the mixture may be performed substantially simultaneously. "Substantially simultaneously" as used herein in reference to at least partially dehydrating a component refers to at least partially dehydrating a component immediately upon
5 contact with a dehydration composition or in less than about 1 minute from initial contact with the dehydration composition. In some embodiments, forming an at least partially dehydrated component in the mixture is achieved within about 100 minutes or less (e.g., about 90, 60, 30, 15, 10, 5, or 1 or less) after initial contact of a dehydration composition and a composition comprising hyaluronidase.

10 In some embodiments, an at least partially dehydrated component (e.g. a hyaluronidase) having a water content of less than about 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% may be achieved within less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 minutes or less than about 50, 40, 30, 20, 10, 5, 1, 0.5, or 0.1 seconds. In some embodiments, an at least partially dehydrated component (e.g. a hyaluronidase) having a water content of less than
15 about 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% may be achieved within about 0.01, 0.05, 0.1, or 0.5 seconds to about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 seconds.

According to some embodiments, contacting two different compositions (e.g., a dehydration composition and a composition comprising hyaluronidase, or a solvent and a composition comprising an at least partially dehydrated component) comprises mixing them
20 together. Mixing may be accomplished by any methods known in the art such as, e.g., vortexing, stirring, static mixing, homogenizing, extruding, pumping, injecting (e.g., microinjecting), adding one composition to another, spraying one composition into another, and/or spraying the compositions together to form a mixture. Mixing may form an emulsion. In some embodiments, air may be incorporated into the mixture. One composition may be
25 added to another using a slow addition, fast addition, or total addition and mixing may be accomplished with low shear and/or high shear (e.g., about 1-10000000/s). When the two different compositions comprise an aqueous composition and a hydrophobic composition (e.g., a composition comprising an organic solvent) the aqueous composition may be added to the hydrophobic composition or vice versa. In some embodiments, the two different compositions
30 are contacted prior to mixing and/or are contacted in a mixer. Exemplary mixers include, but are not limited to, high pressure homogenizers, rotor-stator homogenizers, impellers, pipeline mixers (turbulent and laminar), static mixers, in-line mixers, and/or microfluidic devices (e.g., a microfluidic device including a cross junction). In some embodiments, the mixer is inline such as, e.g., an inline homogenizer. In some embodiments, the mixer is a device (e.g., a

microfluidic device) and two different compositions may be added or injected into the device to thereby mix the two different compositions together and form a mixture. Forming an at least partially dehydrated component may comprise mixing (e.g., homogenizing) the two different compositions and/or mixture.

5 In some embodiments, two different compositions that form a mixture and/or a composition comprising an at least partially dehydrated component may be mixed, such as in a batch process, at a ratio that is a volume ratio of the two different compositions. For example, for a first composition and a second composition that are to be contacted in a batch process to form a mixture, the volume ratio may be a volume to be mixed for the first composition to a
10 volume to be mixed of the second composition. In some embodiments, two different compositions that form a mixture and/or a composition comprising an at least partially dehydrated component may be mixed, such as in an inline process, at a ratio based on the feed rate of the two different compositions. For example, for a first composition and a second composition that are to be contacted in an inline process to form a mixture, the ratio may be a
15 feed rate (e.g., flow rate) for the first composition during the contacting step to a feed rate (e.g., flow rate) for the second composition during the contacting step.

 In some embodiments, two different compositions that form a mixture and/or a composition comprising an at least partially dehydrated component may be mixed (e.g., homogenized) at a feed rate (e.g., flow rate) in a range of about 1, 5, 10, 25, 50, 75, or 100
20 mL/min to about 150, 250, 500, 1,000, 1,500, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, or 8,000 mL/min, or in a range of about 5, 10, 25, 50, 75, or 100 L/min to about 150, 250, 500, 750, 1000, 1,500, 2,000, 3,000, 4,000, or 5,000 L/min. In some embodiments, two different compositions that form a mixture and/or a composition comprising an at least partially dehydrated component may be mixed (e.g., homogenized) at a feed rate in a range of about 1,
25 5, 10, 25, 50, 75, or 100 L/hour to about 150, 250, 500, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, or 4,000 L/hour, or in a range of about 10,000, 15,000, 20,000, 25,000, or 30,000 L/hour to about 50,000, 75,000, 100,000, 125,000, or 150,000 L/hour. The feed rate for each composition may be the same or different.

 In some embodiments, two different compositions that form a mixture and/or a
30 composition comprising an at least partially dehydrated component may be contacted and/or combined with a given feed volume ratio. The two different compositions may include an aqueous composition (e.g., a composition comprising hyaluronidase and/or composition comprising water) that provides an aqueous phase, and a solvent containing composition (e.g., a hydrophobic composition) that provides a solvent phase. In some embodiments, the two

different compositions may be contacted and/or combined with a feed volume ratio of about 0.1:100, 0.2:100, 0.3:100, 0.4:100, 0.5:100, 0.6:100, 0.7:100, 0.8:100, 0.9:100, 1:100, 1.5:100, 2:100, 2.5:100, 3:100, 3.5:100, 4:100, 4.5:100, 5:100, 5.5:100, 6:100, 6.5:100, 7:100, 7.5:100, 8:100, 8.5:100, 9:100, 9.5:100, 10:100, 11:100, 12:100, 13:100, 14:100, 15:100, 16:100, 17:100, 18:100, 19:100, 20:100, 21:100, 22:100, 23:100, 24:100, or 25:100 (aqueous composition:solvent containing composition). In some embodiments, the two different compositions that form a mixture and/or a composition comprising an least partially dehydrated component may be contacted and/or combined with a feed volume ratio in a range of about 0.1:100, 0.3:100, 0.5:100, 0.7:100, or 1:100 to about 2:100, 4:100, 6:100, 8:100, 10:100, 12:100, 14:100, 16:100, 18:100, 20:100, 22:100, or 25:100.

An at least partially dehydrated component and/or particle of the present invention may be separated from a mixture and/or liquid phase using methods known to those of skill in the art. For example, an at least partially dehydrated component and/or particle of the present invention may be separated from a liquid phase by filtration (e.g., pressure filtration, tangential flow filtration, rotary filtration, centrifugal filtration, disc stack filtration, etc.), cyclone separation, sedimentation (e.g., sedimentation by acoustic resonance fields), evaporation, centrifugation, fluid bed drying, evaporative drying, thermal drying, freeze drying, atmospheric freeze drying, spray drying, spray freeze drying, microwave/IR drying, and/or sieving. In some embodiments, an at least partially dehydrated component and/or particle of the present invention may be separated from a liquid phase by a washing step and/or solvent exchange method, sedimentation and/or filtration step, and/or further dried (e.g., to remove water and/or a solvent) using air, vacuum, and/or, freeze drying step, and/or any combination thereof.

In some embodiments, a method of the present invention comprises washing an at least partially dehydrated component and/or particle of the present invention with a wash composition. A wash composition may comprise an organic solvent such as, e.g., an alcohol (e.g., a C1-C20 alcohol), alkane (e.g., a C4-C20 alkane), ester, and/or ether. Such organic solvents include, but are not limited to those, described above and/or an organic solvent may have solubility for water as described above. In some embodiments, an organic solvent in a wash composition is miscible with water. An organic solvent in a wash composition may be the same as or different than an organic solvent in a dehydration composition. In some embodiments, an organic solvent present in a wash composition may be a Class II or Class III residual solvent as classified by the U.S. Food & Drug Administration.

In some embodiments, an organic solvent present in a wash composition may be more volatile than an organic solvent a dehydration composition. In some embodiments, an organic

solvent in a wash composition is a volatile organic compound (e.g., has a boiling point from about 0, 50, or 100°C to about 150, 200, or 260°C). In some embodiments, a wash composition may be used to wash away and/or remove a dehydration composition, organic solvent, and/or impurity. In some embodiments, an organic solvent present in a wash composition has a
5 solubility for a solvent present in a dehydration composition.

According to some embodiments, a method of the present invention is a one-step method in which a composition comprising hyaluronidase is contacted to a dehydration composition to provide a mixture and a particle of the present invention. The contacting step in a one-step method may be repeated one or more times (e.g., 1, 2, 3, 4, 5, or more times) with
10 the same or a different dehydration composition. The one step method may further comprise contacting the at least partially dehydrated component or a composition comprising the same with a wash composition that optionally includes an organic solvent that is more volatile than prior organic solvent(s) used in the method.

A method of the present invention may contact a dehydration composition or wash
15 composition with a composition comprising hyaluronidase, an at least partially dehydrated component, or a composition comprising an at least partially dehydrated component for a period of time. The contact time for each of these steps may be for about 1, 5, 15, 30, 45, or 60 minutes or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours or more. In some embodiments, the contact time is about 1, 5, or 15 minutes to
20 about 30, 45, or 60 minutes or about 1, 2, 3, 4, or 5 hours to about 6, 7, 8, 9, or 10 hours or more.

In some embodiments, a composition comprising an at least partially dehydrated component (e.g., a particle of the present invention) has a pH of greater than 7 such as, e.g., about 7.5, 8, 8.5, 9, 10, or more. In some embodiments, a composition comprising an at least
25 partially dehydrated component has a pH of less than 7 such as, e.g., about 6.5, 6, 5.5, 5, 4.5, 4, or less. In some embodiments, a composition comprising an at least partially dehydrated component has a pH that is at least ± 0.5 , 1, 1.5, or 2 pH units away from the isoelectric point (pI) of the desired isolated component (e.g., hyaluronidase). In some embodiments, the pH of composition comprising an at least partially dehydrated component in a method of the present
30 invention varies by less than about ± 2 , 1.5, 1, or 0.5 pH units from the initial pH of the composition.

According to some embodiments of the present invention provided is a composition comprising a plurality of solid particles (e.g., microparticles, nanoparticles) as described herein. In some embodiments, the composition is non-aqueous. The plurality of solid particles

comprised in the composition may be amorphous and/or crystalline. In some embodiments, a plurality of solid particles comprises amorphous particles. In some embodiments, a plurality of solid particles may comprise crystalline particles such as, e.g., when small molecules (e.g., salts) are present that can be crystallized. In some embodiments, the composition may be provided and/or obtained following separation of the solid particles from a liquid phase. The plurality of solid particles may be uniform in size or may be polydisperse. In some embodiments, at least a portion of the plurality of solid particles have a size that is within about $\pm 5\%$, 10%, 15%, 20%, 25%, 50%, 75%, 100%, 150%, 200% or more of the average particle size. In some embodiments, the composition comprises discrete particles. In some embodiments, the composition comprises aggregations of a biologic (e.g., hyaluronidase and/or a protein) in an amount less than about 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% by weight of the biologic.

In some embodiments, a particle of the present invention has a size configured to pass through a pore created in a hyaluronan network as hyaluronidase (e.g., hyaluronidase present in and/or from a particle of the present invention) breaks down the hyaluronan network and/or a size capable of passing through a pore in a hyaluronan network, which can allow a particle of the present invention to move away from a site at which the particle was administered to a subject (e.g., an injection site). In some embodiments, a particle of the present invention has a size of about 50 nm to about 500 nm or about 100 nm to about 300 nm or about 100 nm to about 200 nm or about 200 nm to about 400 nm. In some embodiments, a plurality of particles of the present invention have an average size of about 50 nm to about 500 nm or about 100 nm to about 300 nm or about 100 nm to about 200 nm. In some embodiments, a particle of the present invention moves and/or disperses an increased distance from an administration site compared to the same compound(s) present in the particle that are not administered in the form of a particle of the present invention. In some embodiments, a particle of the present invention does not disperse from an administration site (e.g., an injection site) and/or stays local to an administration site. Upon dissolution of an administered particle of the present invention the components of the particle (e.g., hyaluronidase and/or a therapeutic) may disperse from the administration site. In some embodiments, a particle of the present invention has a size of about 300 nm or more and the particle does not disperse from an administration site (e.g., an injection site) and/or stays local to an administration site.

In some embodiments, at least a portion of the plurality of solid particles in a composition of the present invention are solid particles of the present invention comprising a solidified hyaluronidase. In some embodiments, at least a portion of the plurality of solid

particles in a composition of the present invention are solid particles comprising a solidified therapeutic that optionally have a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9. In some embodiments, at least a portion of the plurality of solid particles in a composition of the present invention comprise both a solidified
5 hyaluronidase and a solidified therapeutic comprised in the same particle or in different particles. In some embodiments, a composition comprising a plurality of solid particles comprises at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 100% solid particles comprising solidified hyaluronidase by weight of the composition. In some embodiments, the solid particles
10 comprising solidified hyaluronidase may be present in the composition in an amount of about 1, 5, 10, 25, 50, 75, 100, 150, or 200 mg/mL to about 300, 400, 500, 600, or 700 mg/mL. In some embodiments, a composition comprising a plurality of solid particles comprises at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 100% solid particles comprising a solidified therapeutic by
15 weight of the composition. In some embodiments, the plurality of solid particles have a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9. In some embodiments, the plurality of solid particles have a water content of less than about 10% by weight of the particle and/or a water activity of less than about 0.5.

Provided according to embodiments of the present invention is a method of increasing
20 dissolution of a therapeutic in a liquid, the method comprising combining the liquid, the therapeutic, and a particle comprising hyaluronidase of the present invention. In some embodiments, the particle comprising the hyaluronidase dissolves into the liquid. In some embodiments, the liquid is an aqueous liquid (e.g., a bodily fluid and/or aqueous buffer). The therapeutic may be present in the particle comprising hyaluronidase or may be separate from
25 the particle comprising hyaluronidase. In some embodiments, the therapeutic may be in a different particle than the particle comprising hyaluronidase, and the particle comprising the therapeutic may have a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9 and may be a solid particle. In some embodiments, the therapeutic may be separate from the particle comprising hyaluronidase and may be present in
30 (e.g., dissolved and/or suspended in) a composition in which the particle is present. In some embodiments, the particle comprising hyaluronidase is present in a solvent that is different than the liquid. In some embodiments, the therapeutic is present in a non-aqueous composition and the particle is present in a non-aqueous composition, optionally wherein the therapeutic and particle are present in the same composition or different compositions. In some embodiments,

the therapeutic is present in an aqueous composition. In some embodiments, the therapeutic is present in an aqueous composition and the particle is present in a non-aqueous composition.

In some embodiments, a method of increasing dissolution of a therapeutic in a liquid (e.g., *in vitro* and/or *in vivo*) may comprise separately combining the therapeutic and a particle comprising a hyaluronidase with the liquid. For example, the therapeutic and the particle comprising hyaluronidase may be sequentially combined with (e.g., added to) the liquid. In some embodiments, the combining comprises combining a particle comprising hyaluronidase and the therapeutic in the liquid at the same time, optionally wherein the hyaluronidase and the therapeutic are present in the same particle or in different particles. Prior to and/or at the time of combining a liquid and a particle comprising hyaluronidase, the liquid and/or a composition comprising the particle may be devoid of hyaluronidase in solution (e.g., hyaluronidase dissolved in water) and/or free hyaluronidase. Upon combining the liquid and the particle comprising hyaluronidase, the hyaluronidase and/or particle may dissolve in the liquid.

A method of the present invention may increase dissolution of a therapeutic in a liquid by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60% or more over a period of time (e.g., about 1 minute to about 2 hours) compared to dissolution of the therapeutic in the same liquid in the absence of a particle of the present invention over the same period of time and at the same conditions (e.g., temperature and/or pressure). In some embodiments, at least about 90%, 95%, 99%, or 100% of a therapeutic is dissolved within about 10, 20, 30, or 40 minutes to about 50, 60, 70, 80, 90, 100, 110, or 120 minutes following initial combining of a particle comprising hyaluronidase of the present invention, the therapeutic, and a liquid. In some embodiments, at least about 90%, 95%, 99%, or 100% of a therapeutic is dissolved within about 10, 20, 30, or 40 minutes to about 50 or 60 minutes following initial combining of a particle comprising hyaluronidase of the present invention, the therapeutic, and a liquid.

Provided according to some embodiments of the present invention is a method of improving tolerance and/or increasing absorption of a therapeutic upon administration to a subject, the method comprising administering to the subject a particle comprising hyaluronidase of the present invention. In some embodiments, the method comprises subcutaneously, intramuscularly, and/or intradermally administering to the subject the therapeutic and/or the particle comprising hyaluronidase. In some embodiments, the therapeutic and/or particle may be administered to a subject via subcutaneous administration, intramuscular administration, intradermal administration, or the like using methods and/or devices known in the art. Exemplary devices include, but are not limited to, syringes (e.g., pre-filled syringes with staked needles or removable needles, dual-chamber syringes, plastic

syringes that are filled from a vial by a subject or caregiver), cartridges, and/or vials. In some embodiments, syringes or cartridges may be used with an auto injector or pen device. Exemplary needle gauges for a device (e.g., syringe) include, but are not limited to, 27G, 27G thin wall, 27G ultra thin wall, 21G, 23G, 25G, 26G, 29G, and/or 30G. In some embodiments, a therapeutic and/or a particle of the present invention are subcutaneously injected into a subject. In some embodiments, the method reduces irritation at the site of the administration to the subject (e.g., at the injection site).

In some embodiments, a therapeutic and a particle comprising hyaluronidase are separately administered to a subject, optionally at the same time or at different times (e.g., sequentially). In some embodiments, a therapeutic is first administered to a subject and then a particle comprising hyaluronidase is administered to the subject. In some embodiments, a particle comprising hyaluronidase is first administered to a subject and then a therapeutic is administered to the subject. In some embodiments, when a therapeutic and a particle comprising hyaluronidase are separately administered, the time period between the two administrations may be less than about 24 hours such as less than about 20, 15, 12, 8, 6, 4, 2, or 1 hour(s) or less than about 45, 30, 15, 10, 5, or 2 minutes. In some embodiments, a therapeutic and a particle comprising hyaluronidase are concurrently administered to a subject. In some embodiments, a therapeutic and a particle comprising hyaluronidase are present in the same composition that is administered to a subject. In some embodiments, the therapeutic is present in a non-aqueous composition and the particle is present in a non-aqueous composition, optionally wherein the therapeutic and particle are present in the same composition or different compositions. In some embodiments, a therapeutic and a hyaluronidase are present in the same particle that is administered to a subject or are present in separate particles that are administered to a subject. In some embodiments, a therapeutic and a particle comprising hyaluronidase are both subcutaneously administered to a subject. In some embodiments, a therapeutic and a particle comprising hyaluronidase are both intradermally administered to a subject. In some embodiments, a therapeutic is subcutaneously administered to a subject and a particle comprising hyaluronidase is intradermally administered to the subject. In some embodiments, a therapeutic is intradermally administered to a subject and a particle comprising hyaluronidase is subcutaneously administered to the subject. In some embodiments, a therapeutic and/or a particle comprising hyaluronidase are intramuscularly administered to a subject.

A particle of the present invention and/or therapeutic may be administered to a subject in a volume of about 0.1, 0.2, 0.3, 0.4, or 0.5 mL to about 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mL. In some embodiments, a composition comprising particle of

the present invention and/or a therapeutic may be administered to a subject, the composition having a volume of about 0.1, 0.2, 0.3, 0.4, or 0.5 mL to about 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mL. In some embodiments, a composition of the present invention that is administered to a subject has a volume of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 5 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mL. In some embodiments, a particle of the present invention and a therapeutic are administered to a subject in a total administered volume (i.e., the sum of the volume of the composition comprising the particle and the volume of the composition comprising the therapeutic) of less than about 10 mL such as less than about 9, 8, 7, 6, 5, 4, 3, 2, or 1 mL. In some embodiments, a particle of the present 10 invention and a therapeutic are administered to a subject in a total volume of less than about 2 mL.

A method of the present invention may be devoid of administering hyaluronidase in solution (e.g., hyaluronidase dissolved in water) and/or free hyaluronidase. In some embodiments, a particle of the present invention and/or hyaluronidase is in the form of a solid 15 at the time of administration (e.g., subcutaneous and/or intradermal administration) to a subject. In some embodiments, hyaluronidase comprised in a particle of the present invention and/or the particle dissolves upon administration to the subject such as upon contact with a bodily fluid of the subject. Hyaluronidase may be administered to a subject in an amount of about 0.1%, 0.5%, or 1% to about 2%, 3%, 4%, or 5% by weight of the particle. In some 20 embodiments, a method of the present invention may comprise administering to a subject a composition comprising a particle including hyaluronidase and hyaluronidase may be administered to the subject and/or present in the composition in an amount of about 0.05%, 0.1%, 0.5%, or 1% to about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% by weight of the composition. In some embodiments, hyaluronidase may be administered to a subject in an 25 amount of about 100 activity units to about 50,000 activity units (e.g., about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,250, 1,500, 1,750, 2,000, 2,250, 2,500, 2,750, 3,000, 3,250, 3,500, 3,750, 4,000, 4,250, 4,500, 4,750, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, or 50,000 activity units). In some embodiments, the hyaluronidase activity in a composition of the present invention is in the 30 range of about 1, 2, 3, 4, 5, units per mg of therapeutic to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 units per mg of therapeutic. In some embodiments, the hyaluronidase activity in a composition of the present invention is in the range of about 10, 11, 12, 13, 14, or 15 units per mg of therapeutic to about 16, 17, 18, 19, or 20 units per mg of therapeutic. In some embodiments, hyaluronidase is present in a composition of the present invention in an amount

of about 1,000, 2,000, 3,000, or 4,000 units per mL of the composition to about 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, or 15,000 units per mL of the composition. In some embodiments, hyaluronidase is present in a composition of the present invention in an amount of about 1,000, 1,500, or 2,000 units per mL of the composition to about 2,500, 3,000, 3,500, or 4,000 units per mL of the composition. In some embodiments, upon administering a therapeutic and a particle comprising hyaluronidase to a subject, at least about 90%, 95%, 99%, or 100% of the therapeutic, hyaluronidase, and/or particle is dissolved (e.g., in a bodily fluid) within about 1, 5, 10, 20, 30, or 40 minutes to about 50, 60, 70, 80, 90, 100, 110, or 120 minutes or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours or more following administration to the subject. In some embodiments, upon administering a therapeutic and a particle comprising hyaluronidase to a subject, at least about 90%, 95%, 99%, or 100% of the therapeutic, hyaluronidase, and/or particle is dissolved within about 5, 10, 20, 30, or 40 minutes to about 50 or 60 minutes following administration to the subject. In some embodiments, upon administering a therapeutic and a particle comprising hyaluronidase to a subject, at least about 90%, 95%, 99%, or 100% of the therapeutic, hyaluronidase, and/or particle has dissipated and/or dispersed from the administration site within about 5, 10, 20, 30, or 40 minutes to about 50, 60, 70, 80, 90, 100, 110, or 120 minutes or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours or more following administration to the subject.

In some embodiments, upon administering a therapeutic and a particle comprising hyaluronidase to a subject, the method increases the dispersion of the particle, therapeutic, and/or composition administered in the subject. For example, in some embodiments, the method increases the dispersion of the particle, therapeutic, and/or composition under the skin of the subject. This may be determined by methods known in the art such as by measuring a length of a lump, bleb, and/or wheal under the skin following administration. In some embodiments, a therapeutic that is administered to a subject according to a method of the present invention may have increased absorption, bioavailability, distribution, metabolism, and/or excretion in the subject compared to absorption, bioavailability, distribution, metabolism, and/or excretion of the therapeutic in the subject following administration in the absence of a particle of the present invention.

In some embodiments, a method of the present invention comprises administering a therapeutically effective amount of a therapeutic, hyaluronidase, particle, and/or composition of the present invention to a subject. As used herein, the term "therapeutically effective amount" refers to an amount of a therapeutic, hyaluronidase, particle, and/or composition of the present invention that elicits a therapeutically useful response in a subject. Those skilled

in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

"Treat," "treating" or "treatment of" (and grammatical variations thereof) as used herein refer to any type of treatment that imparts a benefit to a subject and may mean that the severity of the subject's condition (e.g., disease or disorder) is reduced, at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one clinical symptom associated with the subject's condition is achieved and/or there is a delay in the progression of the symptom. In some embodiments, the severity of a symptom associated with a subject's condition may be reduced in a subject compared to the severity of the symptom in the absence of a method of the present invention. In some embodiments, pain, swelling, and/or irritation at an injection site in a subject are reduced in a method of the present invention.

In some embodiments, a therapeutic, hyaluronidase, particle, and/or composition of the present invention may be administered in a treatment effective amount. A "treatment effective" amount as used herein is an amount that is sufficient to treat (as defined herein) a subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject. In some embodiments, a treatment effective amount may be achieved by administering a therapeutic, hyaluronidase, particle, composition of the present invention.

The terms "prevent," "preventing" and "prevention" (and grammatical variations thereof) refer to avoidance, reduction and/or delay of the onset of a symptom associated with a condition (e.g., disease and/or disorder) and/or a reduction in the severity of the onset of symptom associated with a condition relative to what would occur in the absence of a method of the present invention. The prevention can be complete, e.g., the total absence of the symptom. The prevention can also be partial, such that the occurrence of the symptom in the subject and/or the severity of onset is less than what would occur in the absence of a method of the present invention. In some embodiments, a method of the present invention prevents or avoids pain, swelling, and/or irritation at an injection site in a subject.

In some embodiments, a therapeutic, hyaluronidase, particle, and/or composition of the present invention may be administered in a prevention effective amount. A "prevention effective" amount as used herein is an amount that is sufficient to prevent (as defined herein) a symptom associated with a condition in a subject. Those skilled in the art will appreciate that the level of prevention need not be complete, as long as some benefit is provided to the subject. In some embodiments, a prevention effective amount may be achieved by administering a therapeutic, hyaluronidase, particle, and/or composition of the present invention.

The present invention finds use in both veterinary and medical applications. Subjects suitable to be treated with a method of the present invention include, but are not limited to, mammalian subjects. Mammals of the present invention include, but are not limited to, canines, felines, bovines, caprines, equines, ovines, porcines, rodents (e.g., rats and mice),
5 lagomorphs, primates (e.g., simians and humans), non-human primates (e.g., monkeys, baboons, chimpanzees, gorillas), and the like, and mammals in utero. Any mammalian subject in need of being treated according to the present invention is suitable. Human subjects of both genders and at any stage of development (i.e., neonate, infant, juvenile, adolescent, adult) may be treated according to the present invention. In some embodiments of the present invention,
10 the subject is a mammal and in certain embodiments the subject is a human. Human subjects include both males and females of all ages including fetal, neonatal, infant, juvenile, adolescent, adult, and geriatric subjects as well as pregnant subjects. In particular embodiments of the present invention, the subject is a human adolescent and/or adult.

A method of the present invention may also be carried out on animal subjects,
15 particularly mammalian subjects such as mice, rats, dogs, cats, livestock and horses for veterinary purposes, and/or for drug screening and drug development purposes.

In some embodiments, the subject is "in need of" or "in need thereof" a method of the present invention, for example, the subject has findings typically associated with a condition, is suspected to have a condition, and/or the subject has a condition.

20 In some embodiments, a method of the present invention may provide increased dispersion, dissolution, and/or dissipation of a therapeutic, composition comprising a therapeutic, vehicle, and/or injected particle in the presence of hyaluronidase at a reduced (lower) concentration than the concentration of hyaluronidase not in accordance with the present invention (e.g., hyaluronidase that is not administered in the form of a particle of the
25 present invention). For example, a method of the present invention that comprises administering a particle of the present invention comprising hyaluronidase in a first concentration may allow for a composition comprising a therapeutic and/or the therapeutic to be administered to a subject and to be dissipated and/or dissolved in about 1 hour following administration of the therapeutic, whereas a method not in accordance with the present
30 invention (e.g., a method that administers hyaluronidase not in a particle of the present invention and/or administers hyaluronidase dissolved in an aqueous solution and/or a method that does not include and/or administer hyaluronidase) has to use a concentration of hyaluronidase that is greater than the first concentration in order to get the same composition comprising the therapeutic and/or the therapeutic to be dissipated and/or dissolved in the same

time. In some embodiments, a method of the present invention may use (e.g., administer to a subject) a concentration of hyaluronidase that is reduced (e.g., by at least about 5% or more) compared to a method not in accordance with the present invention, optionally to obtain a similar result (e.g., a measurable value within $\pm 20\%$) as the method of the present invention.

5 In some embodiments, a method of the present invention may use (e.g., administer to a subject) a concentration of hyaluronidase that is reduced compared to a method that administers free hyaluronidase and/or that administers hyaluronidase that is present in an aqueous composition, optionally to obtain a similar result (e.g., a measurable value within $\pm 20\%$) as the method of the present invention.

10 In some embodiments, a method of the present invention may provide for an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) volume of a therapeutic and/or a composition comprising a therapeutic to be administered to a subject compared to a method not in accordance with the present invention (e.g., a method that administers hyaluronidase not in a particle of the present invention and/or administers hyaluronidase dissolved in an aqueous solution and/or a method that does not include and/or administer hyaluronidase), optionally to obtain a similar result (e.g., a measurable value within $\pm 20\%$) as the method of the present invention or an improved result. The similar result may be a similar dissolution profile, dispersion, bioavailability, swelling amount, adverse effects, pain, irritation at the injection site, etc.

15 In some embodiments, a method of the present invention may administer an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) volume of a therapeutic or composition comprising the therapeutic compared to a method that administers free hyaluronidase and/or that administers hyaluronidase that is present in an aqueous composition, optionally to obtain a similar result (e.g., a measurable value within $\pm 20\%$) as the method of the present invention or an improved result.

20 In some embodiments, a method of the present invention may administer an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) total administered volume to a subject compared to a method that administers free hyaluronidase and/or that administers hyaluronidase that is present in an aqueous composition, optionally to obtain a similar result (e.g., a measurable value within $\pm 20\%$) as the method of the present invention or an improved result.

25 In some embodiments, a method of the present invention may administer an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) total administered volume to a subject compared to a method that administers free hyaluronidase and/or that administers hyaluronidase that is present in an aqueous composition, optionally to obtain a similar result (e.g., a measurable value within $\pm 20\%$) as the method of the present invention or an improved result.

30 In some embodiments, a method of the present invention may administer an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) volume of a therapeutic or composition comprising the therapeutic

compared to a method that does not administer hyaluronidase. In some embodiments, a method of the present invention may administer an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) total administered volume to a subject compared to a method that does not administer hyaluronidase.

5 In some embodiments, a method of the present invention may provide for an increased rate of dispersion, dissolution, and/or dissipation (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) of a therapeutic, composition comprising a therapeutic, vehicle, and/or injected particle compared to a method not in accordance with the present invention (e.g., a method that administers
10 hyaluronidase not in a particle of the present invention and/or administers hyaluronidase dissolved in an aqueous solution and/or a method that does not include and/or administer hyaluronidase). In some embodiments, a composition comprising particles of the present invention may provide for an increased rate of dispersion, dissolution, and/or dissipation (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%,
15 70%, 80%, 90%, 100%, or more) of a therapeutic, composition comprising a therapeutic, vehicle, and/or injected particle compared to a composition not comprising particles of the present invention (e.g., a method that administers hyaluronidase not in a particle of the present invention and/or administers hyaluronidase dissolved in an aqueous solution).

In some embodiments, a method of the present invention may administer (e.g., via
20 injection into a subject) an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) total administered volume of a composition (e.g., a non-aqueous formulation) to a subject compared to a method that administers free hyaluronidase and/or that administers hyaluronidase dissolved in an aqueous solution, optionally to obtain a similar result (e.g., a measurable value within $\pm 20\%$)
25 as the method of the present invention. In some embodiments, a composition comprising particles of the present invention may allow for administration of an increased (e.g., by at least about 5%; e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) total administered volume of a composition (e.g., a non-aqueous formulation) to a subject compared to a composition not comprising particles of the present
30 invention.

In some embodiments, a composition of the present invention that comprises a hyaluronidase particle of the present invention may provide for an increased injection volume of the composition than compared to the injection volume of a non-aqueous composition that is devoid of a particle of the present invention. In some embodiments, a composition of the

present invention (e.g., a composition comprising a particle of the present invention that comprises a hyaluronidase) may provide for a lower injection force and/or lower inline pressure during injection of the composition than compared to the injection force and/or inline pressure of a non-aqueous composition that is devoid of a particle of the present invention. In some
5 embodiments, a composition of the present invention that comprises a hyaluronidase particle of the present invention may provide an increased dispersion and/or absorption of one or more component(s) of the composition (e.g., increased dispersion and/or absorption of a solvent, therapeutic, and/or particle) compared to the dispersion and/or absorption of one or more component(s) in a non-aqueous composition that is devoid of a particle of the present invention.
10 In some embodiments, a composition of the present invention that comprises a hyaluronidase particle of the present invention may provide an increased stability for one or more component(s) in the composition compared to the stability of one or more component(s) in a non-aqueous composition that is devoid of a particle of the present invention.

The present invention is now discussed in the following non-limiting examples.

15

Examples

Example 1

Using a small-scale microglassification method, particles were prepared containing either hyaluronidase type 5 (SIGMA H6254-500MG lot SLCK4168) and sucrose (SIGMA
20 1.00892.1003 lot K52895792 03) in 17-mM Trizma buffer (SIGMA T1503-500G lot 068K5451) with and without the addition of bovine gamma globulin (RMBIO BGG-BBZ-01K lot 20150424IG). Three stocks were used. The first contained no hyaluronidase, but did contain 21 mg/g sucrose and 77 mg/g bovine gamma globulin. The second stock contained 21 mg/g sucrose, 71 mg/g bovine gamma globulin, and 10 mg/g hyaluronidase. The third stock
25 contained 20 mg/g sucrose, no bovine gamma globulin, and 80 mg/g hyaluronidase. The particles were produced by mixing 10 to 12 microliters of the hyaluronidase stock with one milliliter of dry n-octanol (f-value = 0.3). The particles were formed by shearing for 30 seconds with a homogenizer (IKA T-10) at setting “6.” Once formed, a small sample of the suspension was taken for particle analysis. The suspensions were centrifuged at 14,000 rcf for 10 minutes
30 and the octanol was carefully pipetted off. The particles were washed or decanted three times with n-pentanol by addition, mixing, centrifugation (using the same settings as above), and then carefully removing the supernatant. The particles were dried under vacuum overnight and then sealed and stored at 5°C. Particles were spherical in morphology and will be tested to determine enzyme activity.

Example 2

Dry, dense particles comprising hyaluronidase, sucrose, and bovine gamma globulin (BGG) were produced using a microglassification process. Three batches were produced. The
5 resulting particles contained approximately 2% hyaluronidase by weight of the particle.

For the first batch, a stock solution of the protein was prepared at approximately 100 mg/ml total solids concentration. Octanol and the aqueous stock were fed into an inline homogenizer (IKA T-25) with a custom feed at a rate of 176 ml/min total flow, 2.228 ml/min of which was aqueous feed. Approximately 10 ml of aqueous stock solution was processed.
10 The resulting suspension was simultaneously fed into a 0.4-micron filter to collect the particles. The particles were then washed with pentanol while on the filter and dried under nitrogen. The powder contained 1.1% residual moisture, 0.1% residual pentanol, and 0.5% residual octanol by weight. A sample of the unfiltered suspension was kept for particle size analysis. 11,321 particles were imaged for particle size analysis. The average particle size was 2.5 microns with
15 the largest particle measured at 8.5 microns.

For the second batch, a stock solution of the protein was prepared at approximately 102 mg/ml total solids concentration. Octanol and the aqueous stock were fed into an inline homogenizer (IKA T-25) with a custom feed at a rate of 156 and 1.963 ml/min, respectively. Approximately 99 ml of aqueous stock solution was processed. The resulting suspension was
20 then fed into a 0.4-micron filter to collect the particles. Filtration was performed at 10 atmospheres of pressure supplied by compressed nitrogen. The particles were then washed with pentanol while on the filter and dried under nitrogen. The powder contained 0.9% residual moisture, 0.1% residual pentanol, and 0.7% residual octanol by weight. A sample of the unfiltered suspension was kept for particle size analysis. 13,135 particles were imaged for
25 particle size analysis. The average particle size was 2.4 microns with the largest particle measured at 9.9 microns.

For the third batch, a stock solution of the protein was prepared at approximately 103 mg/ml total solids concentration. Octanol and the aqueous stock were fed into an inline homogenizer (IKA T-25) with a custom feed at a rate of 155 and 1.946 ml/min, respectively.
30 Approximately 110 ml of aqueous stock solution was processed. The resulting suspension was then fed into a 0.4-micron filter to collect the particles. Filtration was performed at 10 atmospheres of pressure supplied by compressed nitrogen. The particles were then washed with pentanol while on the filter and dried under nitrogen. The powder contained 1.5% residual moisture, 0.1% residual pentanol, and 0.7% residual octanol by weight. A sample of the

unfiltered suspension was kept for particle size analysis. 12,816 particles were imaged for particle size analysis. The average particle size was 2.6 microns with the largest particle measured at 10.1 microns.

5 **Example 3**

The *in vitro* release of a suspension of microglassified protein particles into a release media of either phosphate-buffered saline (PBS) including 0.25 mg/mL hyaluronidase (PBS-Hyal media) or PBS including 0.5% hyaluronic acid and hyaluronidase (PBS-HA-Hyal media) at a concentration of 0.25 mg/mL was tested and compared. Release media of PBS and 0.25
10 mg/mL hyaluronidase was also included as a control. The suspension of particles contained bovine gamma globulin (BGG) and sucrose and was made in benzyl benzoate at a concentration of 500 mg/mL total solids. Approximately 10 μ L of this suspension was added to a 5 mL tube including either PBS-Hyal release media or PBS-HA-Hyal release media.

The release media including the suspension of particles was sampled over time and the
15 absorbance of the sampled release media at 280 nm was measured to determine the release of BGG from the suspension over time (e.g., the concentration of BGG released from the particles and/or suspension and/or dissolved in the release media over time following contact with the release media). As can be seen from **Fig. 3**, the sample containing the release media PBS-HA exhibited slow release of the BGG from the suspension over time, while the sample containing
20 the PBS-HA-Hyal release media as seen in **Fig. 1** exhibited fast release of the BGG from the suspension over time. The results of this experiment demonstrate the feasibility of using hyaluronidase in an aqueous phase to increase the dissolution rate of a solidified protein from a nonaqueous suspension such as in a subcutaneous space.

25 **Example 4**

For three separate samples, the *in vitro* release of a suspension of protein particles into a solution of either PBS (PBS media-1, PBS media-2, or PBS media-3) or 0.5% hyaluronic acid (HA) in PBS (PBS-HA media-1, PBS-HA media-2, or PBS HA media-3) was tested and compared. The *in vitro* release of the suspension of protein particles in PBS or HA was also
30 compared to the *in vitro* release of a suspension including particles including the same protein and hyaluronidase in PBS or HA.

A suspension of microglassified particles containing bovine gamma globulin (BGG) and sucrose was made at a concentration of 500 mg/mL total solids in benzyl benzoate.

Approximately 10 μ L of this suspension was added to a 5 mL tube along with either PBS or HA as the release media. The release media including the suspension of BGG particles was sampled over time, and the absorbance of the sampled release media at 280 nm was measured to determine the protein concentration in the release media over time. Triplicate samples were prepared for each release media used.

Fig. 2 shows the measured absorbance for each of the three PBS media samples over time, and **Fig. 3** shows the measured absorbance for each of the three PBS-HA media samples over time. In **Fig. 2** and **Fig. 3**, the maximum absorbance measured indicates the time for complete release of the protein (BGG) from the suspension. As shown in **Fig. 2**, the suspension added to the PBS media exhibited quick release of the protein over time, with complete release of the protein within one hour. The suspension added to the 0.5% hyaluronic acid (HA) release media exhibited slow release, with complete release of the protein occurring on the order of days, as shown in **Fig. 3**.

A suspension of microglassified particles that comprised hyaluronidase and BGG were prepared from a solution including hyaluronidase in an amount of 2% by mass of the dissolved solids and BGG in an amount of about 98% by mass of the dissolved solids. The particles were provided in the suspension at a concentration of approximately 500 mg/mL total solids in benzyl benzoate. The same experiment was conducted as above, wherein approximately 10 μ L of the suspension including the particles comprising BGG and hyaluronidase was added to a 5 mL tube along with PBS or HA as the release media. Assuming complete release of the hyaluronidase from the particles, 0.025 mg of hyaluronidase would be present per mL of the release media, which is 10X less than the amount of hyaluronidase in Example 3. The release media was sampled over time in the same manner as above, wherein the absorbance of the sampled release media at 280 nm was measured to determine the protein concentration in the release media over time. Triplicate samples were prepared for each release media used.

Fig. 4 shows the measured absorbance for each of the PBS samples (PBS media-1, PBS media-2, or PBS media-3) including particles comprising BGG and hyaluronidase over time, and **Fig. 5** shows the measured absorbance for each of the three PBS-HA media samples (PBS-HA media-1, PBS-HA media-2, or PBS HA media-3) including particles comprising BGG and hyaluronidase over time. In **Fig. 4** and **Fig. 5**, the maximum absorbance measured indicates the time for complete release of the protein from the suspension. As shown in **Fig. 4** and **Fig. 5**, the release of the protein from the suspension was complete within one hour for both the PBS release media and the PBS-HA release media. These results indicate that the hyaluronidase, contained within the solid protein particles, was able to efficiently break down

the hyaluronic acid in the HA release media and allow more rapid dissolution of the protein from the suspension into the aqueous phase of the release media. In addition, the amount of hyaluronidase in the particles prepared in Example 4 was approximately 25 times lower than the amount of hyaluronidase that was added to the media in Example 3, indicating that a lesser amount of hyaluronidase in the form of a particle is required to increase protein release from the suspension compared to hyaluronidase free in solution.

Example 5

The protein release from suspensions including solid particles with different amounts of hyaluronidase will be compared. Particles having different amounts of hyaluronidase in the particle (e.g., hyaluronidase in an amount of 0.1%, 0.5%, 1%, 3%, or 4% by weight of the particle) will be prepared and may be prepared as described in one or more of Examples 1-4.

Example 6

The protein release from suspensions containing hyaluronidase particles of the present invention and separate solid protein particles will be compared. Particles having different amounts of hyaluronidase in the particle (e.g., hyaluronidase in an amount of 0.1%, 0.5%, 1%, 3%, or 4% by weight of the particle) will be prepared and may be prepared as described in one or more of Examples 1-4. The hyaluronidase particles will be provided in a suspension with particles that do not include hyaluronidase, and suspensions with different concentrations of hyaluronidase particles will be prepared. For example, suspensions including hyaluronidase particles that are present at 0.1%, 0.5%, 1%, 3%, or 4% by weight of the total solids content of the suspension and including particles that do not include hyaluronidase may be prepared.

Example 7

The enzymatic activity of solid protein particles containing hyaluronidase was measured over time (e.g., using the method in Dorfman, A. (1955) Methods in Enzymology, Volume I, 166-173 for hyalurodinase EC 3.2.1.35, Hase, turbidity measurements and comparing the turbidity to a standard curve) and compared to the activity of the hyaluronidase in a stock formulation. Particles containing hyaluronidase were prepared in the formulations as shown in **Table 1**.

Table 1: Hyaluronidase Particle Formulations.

Formulation ID	Sugar type	Amino acid (AA)	Hyaluronidase:Sugar:AA:Buffer
F1	NA	NA	98:0:0:2
F2	Trehalose	NA	60:38:0:2
F3	Sucrose	NA	60:38:0:2
F4	Sucrose	Proline	40:29:29:2

Powders of the particle formulations were placed on stability at 2-8 °C or 25 °C for 3 months. At predetermined time points (initial (t=0), after 30 days, and 90 days) the powders were rehydrated (using 20 mM Sodium Phosphate pH 7.0 at 37°C with 77 mM Sodium Chloride and 0.01% (w/v) Bovine Serum Albumin) and the activity of the enzyme was measured as described in Dorfman, A. (1955) Methods in Enzymology, Volume I, 166-173. The results are shown in (Fig. 6 and Fig. 7).

10 **Example 8**

Using a Göttingen minipig model, injection site reactions and pharmacokinetics will be compared for injected suspensions with and without hyaluronidase particles of the present invention in a nonaqueous vehicle and for an injected suspension comprising microglassified particles comprising a therapeutic with or without hyaluronidase.

15 Formulations were prepared according to the following table (BB = benzyl benzoate; EO = ethyl oleate):

Group	HYAL (% particle by weight)	Total Particle Concentration in Suspension (mg/ml)	Suspension Vehicle
1	2	500	BB
2	2	500	EO
3	2	500	BB:EO (50:50)
4	2	500	BB:EO (25:75)
5	2	300	BB:EO (25:75)
6	0	500	BB:EO (25:75)
7	0	300	BB:EO (25:75)

8	0	500	BB:EO (25:75)
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Animals used were male Göttingen Minipigs, 7-8 months of age. 2 ml of each formulation was injected into the flank (4 groups per animal, 4 animals per group, 8 animals total) using a 27G needle over ~ 10s. Local changes (warmth, redness, swelling, bleb size) at the injection sites were scored directly after injection, 1h, 2h, 3h, 4h and 6h after injection and once daily for 14 days.

Example 9

Dry, dense particles comprising hyaluronidase, sucrose and bovine serum albumin (BSA) were produced using a microglassification process and compared to particles of BSA without hyaluronidase. Two batches were produced. The resulting particles with the enzyme contained approximately 0.37% hyaluronidase by weight of the particle.

For the first batch, a stock solution of the BSA and sucrose was prepared at approximately 85 mg/ml total solids concentration. Octanol and the aqueous stock were fed into an inline homogenizer (IKA T-25) to provide discrete, spherical, solid particles. Approximately 33 ml of aqueous stock solution was processed. The resulting suspension was washed with a solvent, filtered, and dried under nitrogen. The powder contained 3.2% residual moisture, 0.3% residual wash solvent, and 5.2% residual octanol by weight. A sample of the unfiltered suspension was kept for particle size analysis. 8,559 particles were imaged for particle size analysis. The average particle size was 2.7 microns with the largest particle measured at 11.9 microns.

For the second batch, a stock solution of the BSA, sucrose, and hyaluronidase was prepared at approximately 85 mg/ml total solids concentration. Octanol and the aqueous stock were fed into an inline homogenizer (IKA T-25) to provide discrete, spherical, solid particles. Approximately 33 ml of aqueous stock solution was processed. The resulting suspension was washed with a solvent, filtered, and dried under nitrogen. The powder contained 4.2% residual moisture, 0.2% residual wash solvent, and 5.3% residual octanol by weight. A sample of the unfiltered suspension was kept for particle size analysis. 12,583 particles were imaged for particle size analysis. The average particle size was 2.5 microns with the largest particle measured at 10.7 microns.

Powders were placed on stability at 2-8 °C, 25 °C, and 40 °C for 1 month. At predetermined time points, the powder was rehydrated, enzyme activity measured as described

in Dorfman, A. (1955) Methods in Enzymology, Volume I, 166-173 (**Fig. 8**), and BSA aggregation measured by SEC-HPLC (**Fig. 9**).

Example 10

5 Dry, dense particles comprising hyaluronidase, trehalose, and a monoclonal antibody (mAb) were produced using a microglassification process and compared to particles of the mAb without hyaluronidase. Two batches were produced. The resulting particles with the enzyme contained approximately 0.3% hyaluronidase by weight of the particle.

10 For the first batch, a stock solution of the mAb and trehalose was prepared at approximately 101 mg/ml total solids concentration. Octanol and the aqueous stock were fed into an inline homogenizer (IKA T-25) to provide discrete, spherical, solid particles. Approximately 10 ml of aqueous stock solution was processed. The resulting suspension was washed with a solvent, filtered, and dried under nitrogen. The powder contained 1.8% residual moisture, 0.1% residual wash solvent, and 0.5% residual octanol by weight. A sample of the
15 unfiltered suspension was kept for particle size analysis. 12,779 particles were imaged for particle size analysis. The average particle size was 2.6 microns with the largest particle measured at 8.9 microns.

20 For the second batch, a stock solution of the mAb, hyaluronidase, and trehalose was prepared at approximately 100 mg/ml total solids concentration. Octanol and the aqueous stock were fed into an inline homogenizer (IKA T-25) to provide discrete, spherical, solid particles. Approximately 10 ml of aqueous stock solution was processed. The resulting suspension was washed with a solvent, filtered, and dried under nitrogen. The powder contained 0.6% residual moisture, 0.1% residual wash solvent, and 0.4% residual octanol by weight. A sample of the
25 unfiltered suspension was kept for particle size analysis. 11,742 particles were imaged for particle size analysis. The average particle size was 2.7 microns with the largest particle measured at 16.1 microns (**Fig. 10**).

30 Powders were placed on stability at 2-8 °C, 25 °C, and 40 °C for 60 days. At 0, 15, and 30 days, the powders were rehydrated and monomer percent of the mAb measured via SEC-HPLC (**Fig. 11, Fig. 12, Fig. 13, Fig. 14, Fig. 15, and Fig. 16**) and enzyme activity measured as described in Dorfman, A. (1955) Methods in Enzymology, Volume I, 166-173 (**Fig. 17**).

Example 11

 In contrast to Example 10, particles were formed using a microbatch microglassification process in which approximately 12 µl of aqueous stock solution was

- processed for each sample. Particle formulations with a mAb, hyaluronidase, and various excipients (**Table 2**) were formed and placed on stability at 40°C. After 12 days, the powders were rehydrated, enzyme activity was assayed as described in Dorfman, A. (1955) Methods in Enzymology, Volume I, 166-173, and aggregation of the mAb measured via SEC-HPLC (**Table 3**).

Table 2: Microbatches of additional hyaluronidase formulations

Formulation ID	Percentage composition (HYAL : mAb : Trehalose : Buffer)	Buffer type
F5	98 : 0 : 0 : 2	a
F6	98 : 0 : 0 : 2	b
F7	0.34 : 70 : 28 : 2	c
F8	0 : 70 : 28 : 2	c
F9	0.34 : 70 : 28 : 2	a
F10	0.34 : 70 : 28 : 2	c (+ 0.1% P188)
F11	0.34 : 60 : 38 : 2	c (+0.1% P188, + 0.3% Methionine)

a = 10mM Phosphate at pH 7.2, b = Phosphate buffer saline, c = 10mM Histidine at pH 6.0

- 10 **Table 3:** Hyaluronidase (HYAL) activity of microbatches (F5 to F7 and F9 to F11) and change in percent monomer in mAb (for microbatches F7 to F11) after 12 days of storage at 40 °C

Formulation ID	HYAL's percentage activity at		Change in % monomer in mAb after 12 days
	T=0	T=12 days in 40 °C	
F5	76 %	< 25%	NA
F6	80 %	< 25%	NA
F7	&	87 %	8.8 ± 0.7
F8	NA	NA	3.5 ± 0.7
F9	&	50 %	38.7 ± 0.7
F10	86 %	59 %	12.4 ± 0.8
F11	97 %	48 %	13.4 ± 1.0

& Data not available

15 **Example 12**

Nanoparticles of hyaluronidase (e.g., particles having an average size of about 100 nm to about 500 nm) will be produced by adding an aqueous feed including about 1-5 mg/ml of hyaluronidase to a dehydration solvent using a method described above. The powder will be assayed for activity using one of the methods mentioned above.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein. All publications, patent applications, patents, patent
5 publications, and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

THAT WHICH IS CLAIMED IS:

1. A particle comprising:
hyaluronidase; and
a therapeutic,
5 wherein the particle has a water content of less than about 15% by weight of the
particle and/or a water activity of less than about 0.9.
2. The particle of claim 1, wherein the therapeutic is a biologic, optionally wherein the
biologic is selected from the group consisting of an amino acid, peptide, protein, nucleotide,
10 polynucleotide, and any combination thereof.
3. The particle of claim 1, wherein the therapeutic is a small molecule (e.g., a small
organic molecule).
- 15 4. The particle of any preceding claim, further comprising a stabilizer, optionally
wherein the stabilizer is sugar (e.g., a sucrose, glucose, trehalose, mannitol, sorbitol, dextrose,
maltose, and/or lactose), an amino acid, and/or a protein (e.g., an albumin such as a serum
albumin).
- 20 5. The particle of claim 4, wherein the stabilizer is present in the particle in an amount of
about 0.1%, 5%, 10%, 15%, or 20% to about 25%, 30%, 35%, 40%, 45%, or 50% by weight
of the particle.
6. The particle of any one of claims 4 or 5, wherein the stabilizer is not covalently bound
25 to the hyaluronidase.
7. The particle of any one of claims 4-6, wherein the stabilizer is an amino acid,
optionally wherein the amino acid is selected from the group consisting of histidine, arginine,
proline, glycine, leucine, and any combination thereof.
- 30 8. The particle of any one of claims 4-7, wherein the stabilizer is present in the particle
in an amount of about 5% to about 30% by weight of the particle.

9. The particle of any preceding claim, wherein the hyaluronidase is present in the particle in an amount of about 0.1%, 0.5%, or 1% to about 2%, 3%, 4%, or 5% by weight of the particle.
- 5 10. The particle of any preceding claim, wherein the therapeutic is present in the particle in an amount of about 50%, 55%, 60%, 65%, or 70% to about 75%, 80%, 85%, 90%, 95%, or 99% by weight of the particle, optionally wherein the therapeutic is present in the particle in an amount of about 75% or 80% to about 85%, 90%, or 95% by weight of the particle.
- 10 11. The particle of any preceding claim, wherein the hyaluronidase and the therapeutic are not covalently bound.
12. A particle comprising:
hyaluronidase; and
15 a stabilizer,
wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9.
13. The particle of claim 12, wherein the stabilizer is a sugar (e.g., a sucrose, glucose,
20 trehalose, mannitol, sorbitol, dextrose, maltose, and/or lactose), an amino acid, and/or a protein (e.g., an albumin such as a serum albumin), optionally wherein the sugar is selected from the group consisting of sucrose, glucose, trehalose, mannitol, sorbitol, dextrose, maltose, lactose, and any combination thereof.
- 25 14. The particle of claim 12, wherein the stabilizer is an amino acid, optionally wherein the amino acid is selected from the group consisting of histidine, arginine, proline, glycine, leucine, and any combination thereof.
15. The particle of claim 12, wherein the stabilizer is an albumin, optionally a serum
30 albumin.
16. The particle of any one of claims 12-15, wherein the stabilizer is present in the particle in an amount of about 0.1%, 0.5%, 1%, 5%, 10%, 15%, or 20% to about 25%, 30%, 35%, 40%, 45%, or 50% by weight of the particle.

17. The particle of any one of claims 12-16, wherein the hyaluronidase and the stabilizer are not covalently bound.
- 5 18. The particle of any one of claims 12-18, wherein the hyaluronidase is present in the particle in an amount of about 50%, 55%, 60%, 65%, or 70% to about 75%, 80%, 85%, 90%, 95%, or 99% by weight of the particle.
19. The particle of any preceding claim, wherein the particle is spherical in shape.
- 10 20. The particle of any preceding claim, wherein the particle has a density of at least about 0.5, 0.6, 0.7, 0.8, 0.9, or 1 g/cm³ to about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 g/cm³.
- 15 21. The particle of any preceding claim, wherein the particle has a size (e.g., a diameter) of about 0.1 microns to about 30 microns, optionally wherein the particle has a size of about 2 microns to about 5 microns, about 5 microns to about 20 microns, or about 10 microns to about 30 microns.
- 20 22. The particle of any preceding claim, wherein the particle has a total moisture content of less than about 3% of the total particle mass, optionally wherein the particle has a total moisture content of less than about 2% of the total particle mass.
23. The particle of any preceding claim, wherein the particle has a residual (e.g. octanol and/or pentanol) content in an amount less than about 3% by weight of the total particle mass.
- 25 24. The particle of any preceding claim, wherein the particle is amorphous and/or wherein the hyaluronidase in the particle is amorphous.
- 30 25. The particle of any preceding claim, wherein the particle is a microparticle or a nanoparticle.
26. The particle of any preceding claim, wherein the enzymatic activity of the hyaluronidase, following dissolution of the particle in an aqueous composition, is within

about $\pm 50\%$ of the enzymatic activity of free hyaluronidase, optionally wherein the enzymatic activity of the hyaluronidase, following dissolution of the particle in an aqueous composition, is within about $\pm 40\%$ of the enzymatic activity of free hyaluronidase.

5 27. The particle of any preceding claim, wherein the enzymatic activity of the hyaluronidase following storage at about 4°C to about 40°C for at least about 3 months is within about $\pm 50\%$ of the enzymatic activity of free hyaluronidase, optionally wherein the enzymatic activity of the hyaluronidase following storage at about 4°C to about 40°C for at least about 3 months is within about $\pm 40\%$ of the enzymatic activity of the free
10 hyaluronidase.

28. The particle of any preceding claim, wherein the hyaluronidase is hyaluronidase V.

29. A composition comprising the particle of any preceding claim.

15

30. The composition of claim 29, wherein the composition is a suspension.

31. The composition of claim 29 or 30, wherein the composition has a viscosity of about 20 centipoise (cP) to about 200 cP, when measured at a temperature of about 20 degrees
20 Celsius to about 25 degrees Celsius and at a shear rate of about $1,000\text{ s}^{-1}$ to about $3,000\text{ s}^{-1}$.

32. The composition of any one of claims 29-31, wherein the particle is present in the composition in an amount of about 1, 10, 50, 100, or 200 mg/mL to about 300, 400, 500, 600, or 700 mg/mL.

25

33. The composition of any one of claims 29-32, wherein the composition is non-aqueous.

34. The composition of any one of claims 29-33, further comprising a solvent, optionally
30 wherein the solvent is an ester (e.g., an alkyl ester or aryl ester).

35. The composition of claim 34, wherein the solvent is selected from the group consisting of benzyl benzoate, ethyl oleate, a triglyceride ester (e.g., MIGLYOL® 812), ethyl lactate, sesame oil, and any combination thereof.

36. The composition of any one of claims 29-35, wherein the hyaluronidase is not soluble in the solvent and/or composition.
- 5 37. The composition of any one of claims 29-36, wherein the hyaluronidase is in the form of a solid in the composition.
38. The composition of any one of claims 29-37, further comprising a therapeutic that is dissolved or suspended in the composition.
- 10 39. A composition comprising:
a solvent; and
a particle comprising hyaluronidase, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9.
- 15 40. The composition of claim 39, further comprising a therapeutic, optionally wherein the therapeutic is dissolved or suspended in the composition.
41. The composition of claim 39 or 40, wherein the composition is non-aqueous.
- 20 42. The composition of any one of claims 39-41, wherein the hyaluronidase is not soluble in the solvent and/or composition.
43. The composition of any one of claims 39-42, wherein the hyaluronidase is in the form
25 of a solid in the composition.
44. The composition of any one of claims 39-43, wherein the composition is a suspension.
- 30 45. The composition of any one of claims 39-44, wherein the composition has a viscosity of about 20 centipoise (cP) to about 200 cP, when measured at a temperature of about 20 degrees Celsius to about 25 degrees Celsius and at a shear rate of about 1,000 s⁻¹ to about 3,000 s⁻¹.

46. The composition of any one of claims 39-45, wherein the particle is present in the composition in an amount of about 1, 10, 50, 100, or 200 mg/mL to about 300, 400, 500, 600, or 700 mg/mL.
- 5 47. The composition of any one of claims 29-46, further comprising a detergent (e.g., polysorbate-20, polysorbate-80, and/or Poloxamer 188).
48. A method of increasing dissolution of a therapeutic in a liquid, the method comprising:
- 10 combining the liquid, the therapeutic, and a particle comprising hyaluronidase, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9, thereby increasing dissolution of the therapeutic in the liquid.
- 15 49. The method of claim 48, wherein the therapeutic is separate from the particle, optionally wherein the therapeutic and/or particle is present in a solvent that is different than the liquid.
50. The method of claim 48, wherein the particle comprises the therapeutic, optionally
- 20 wherein the particle is present in a solvent that is different than the liquid.
51. The method of claim 48, wherein the particle is the particle of any one of claims 1-28.
52. The method of claim 48, wherein the particle is present in a composition, optionally
- 25 wherein the composition is the composition of any one of claims 29-47.
53. The method of any one of claims 48-52, wherein the liquid is an aqueous liquid.
54. The method of any one of claims 48-53, wherein the combining comprises separately
- 30 combining the hyaluronidase and the therapeutic with the liquid, optionally wherein the hyaluronidase and the therapeutic are sequentially combined with (e.g., added to) the liquid.

55. The method of any one of claims 48-53, wherein the combining comprises combining the hyaluronidase and the therapeutic in the liquid at the same time, optionally wherein the hyaluronidase and the therapeutic are present in the same particle or in different particles.

5 56. The method of any one of claims 48-55, wherein dissolution of the therapeutic is increased by at least about 10%, 20%, 30%, 40%, 50%, 60% or more over a period of time of about 5 minutes to about 2 hours compared to dissolution of the therapeutic in the absence of the particle.

10 57. The method of any one of claims 48-56, wherein at least about 90%, 95%, 99%, or 100% of the therapeutic is dissolved within about 20, 30, or 40 minutes to about 100, 110, or 120 minutes following combining of the particle, therapeutic, and the liquid, optionally wherein at least about 90%, 95%, 99%, or 100% of the therapeutic is dissolved within about 20, 30, or 40 minutes to about 50, 60, 70, or 80 minutes following combining of the particle,
15 therapeutic, and the liquid.

58. A method of improving tolerance and/or increasing absorption of a therapeutic upon subcutaneous, intramuscular, and/or intradermal administration to a subject, the method comprising:

20 subcutaneously, intramuscularly, and/or intradermally administering to the subject a particle comprising hyaluronidase, wherein the particle has a water content of less than about 15% by weight of the particle and/or a water activity of less than about 0.9, thereby improving tolerance and/or increasing absorption of the therapeutic upon subcutaneous, intramuscular, and/or intradermal administration to the subject.

25

59. The method of claim 58, wherein the therapeutic is separate from the particle.

60. The method of claim 58, wherein the particle comprises the therapeutic.

30 61. The method of claim 58, wherein the particle is the particle of any one of claims 1-28.

62. The method of claim 58, wherein the subcutaneously, intramuscularly, and/or intradermally administering comprises subcutaneously, intramuscularly, and/or intradermally

administering a composition comprising the particle to the subject, optionally wherein the composition is the composition of any one of claims 29-47.

63. The method of any one of claims 58-62, wherein the method reduces irritation at the injection site of the subcutaneous, intramuscular, and/or intradermal administration to the subject.

64. The method of any one of claims 58-63, wherein the method increases dispersion of the particle or composition under the skin of the subject following the subcutaneous, intramuscular, and/or intradermal administration.

65. The method of any one of claims 58-64, wherein the method increases absorption, bioavailability, distribution, metabolism, and/or excretion of the therapeutic in the subject following subcutaneous, intramuscular, and/or intradermal administration compared to absorption, bioavailability, distribution, metabolism, and/or excretion of the therapeutic in the subject following subcutaneous, intramuscular, and/or intradermal administration in the absence of the particle or composition.

66. The method of any one of claims 58-65, wherein the subcutaneously, intramuscularly, and/or intradermally administering comprises subcutaneously, intramuscularly, and/or intradermally administering the particle or composition to the subject in a volume of about 0.1 or 0.5 mL to about 1, 2, 5, or 10 mL.

67. The method of any one of claims 58-66, wherein the method is devoid of administering hyaluronidase in solution (e.g., hyaluronidase dissolved in water) and/or free hyaluronidase.

68. The method of any one of claims 58-67, wherein the particle and/or hyaluronidase is in the form of a solid at the time of subcutaneous, intramuscular, and/or intradermal administration to the subject, optionally wherein the hyaluronidase in the particle dissolves upon subcutaneous, intramuscular, and/or intradermal administration.

69. The method of any one of claims 58-68, wherein the subcutaneously, intramuscularly, and/or intradermally administering comprises separately subcutaneously, intramuscularly, and/or intradermally administering the hyaluronidase and the therapeutic.
- 5 70. The method of claim 69, wherein the subcutaneously, intramuscularly, and/or intradermally administering comprises subcutaneously, intramuscularly, and/or intradermally administering the hyaluronidase to the subject and then subcutaneously, intramuscularly, and/or intradermally administering the therapeutic to the subject.
- 10 71. The method of claim 69, wherein the subcutaneously, intramuscularly, and/or intradermally administering comprises subcutaneously, intramuscularly, and/or intradermally administering the therapeutic to the subject and then subcutaneously, intramuscularly, and/or intradermally administering the hyaluronidase to the subject.
- 15 72. The method of any one of claims 58-68, wherein the subcutaneously, intramuscularly, and/or intradermally administering comprises concurrently administering the hyaluronidase and the therapeutic to the subject, optionally wherein the hyaluronidase and the therapeutic are present in the same particle or the same composition.
- 20 73. The method of any one of claims 58-72, wherein at least about 90%, 95%, 99%, or 100% of the therapeutic is dissolved within about 20, 30, or 40 minutes to about 100, 110, or 120 minutes following the subcutaneous, intramuscular, and/or intradermal administration to the subject, optionally wherein at least about 90%, 95%, 99%, or 100% of the therapeutic is dissolved within about 20, 30, or 40 minutes to about 50, 60, 70, or 80 minutes following the
25 subcutaneous, intramuscular, and/or intradermal administration to the subject.
74. The method of any one of claims 58-73, wherein the hyaluronidase is administered to the subject in an amount of about 0.1%, 0.5%, or 1% to about 2%, 3%, 4%, or 5% by weight of the particle.
- 30 75. The method of any one of claims 58-74, wherein the subcutaneously, intramuscularly, and/or intradermally administering comprises administering the particle and therapeutic in a total volume of less than about 10 mL, optionally in a total volume of less than about 2 mL.