rapamycin is to bind the cytosolic protein FK-binding protein 12 (FKBP12), and the rapamycin-FKBP12 complex in turn inhibits the mTOR pathway by directly binding to the mTOR Complex 1 (mTORC1).

[0005] Albumin-based nanoparticle compositions have been developed as a drug delivery system for delivering substantially water insoluble drugs. See, for example, US 5,916,596, 6,506,405, 6,749,868, 6,537,579, 7,820,788, and 7,923,536. Nab-paclitaxel, sold under the trade name ABRAXANE®, an albumin stabilized nanoparticle formulation of paclitaxel, was approved in the United States and various other countries for treating metastatic breast cancer, pancreatic cancer, and lung cancer.

[0006] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

[0007] Described herein are nanoparticle compositions, pharmaceutical compositions, and emulsions containing rapamycin and albumin, along with commercial batches of such nanoparticle compositions, pharmaceutical compositions, and emulsions. Also described are methods of making such nanoparticle compositions, pharmaceutical compositions, and emulsions, as well as methods of using such nanoparticle compositions, pharmaceutical compositions (including, for example, for the treatment of cancer), and emulsions. Additionally, described herein are methods for assessing the suitability of the pharmaceutical compositions for use in a human individual, as well as methods for processing pharmaceutical compositions (and commercial batches of such pharmaceutical compositions) identified as suitable for use in a human individual.

[0008] Parameters of the nanoparticle composition, such as those as described here, can be used to ensure consistency during manufacturing of the composition. The nanoparticles contain rapamycin, a drug known to have efficacy in cancer therapy, and predictable release of the drug is important for reliable treatment. Consistently manufactured nanoparticles are expected to have a consistent drug release profile. Changes to the manufacturing protocol, such as scale up during commercial batch production, can result in changes in the physical and functional parameters of the nanoparticle composition. Described herein are commercial batches of nanoparticle compositions and commercial batches of emulsions used to manufacture commercial batches of

the nanoparticle compositions, with physical and functional parameters that have been determined for such commercial batches.

[0009] A nanoparticle composition described herein can include (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin. In some ~~embodiment~~examples, the nanoparticles comprise a core comprising rapamycin and a coating comprising albumin.

[0010] In a nanoparticle composition, or a commercial batch of such a nanoparticle composition, about 80% to about 95% of the albumin in the composition is in the form of monomeric albumin, about 4% to about 15% of the albumin in the composition is in the form of dimeric albumin, and about 0.5% to about 5% of the albumin in the composition is in the form of polymeric albumin when the percentage of albumin in the composition that is in the form of monomeric albumin, dimeric albumin, or polymeric albumin is determined by subjecting the composition to size-exclusion chromatography (SEC) using a saline mobile phase coupled with a multiple angle light scattering (MALS) detector. In the nanoparticle portion of the composition, about 70% to about 85% of the albumin in the nanoparticle portion is in the form of monomeric albumin, about 9% to about 20% of the albumin in the nanoparticle portion is in the form of dimeric albumin, and about 5% to about 15% of the albumin in the nanoparticle portion is in the form of polymeric albumin when the percentage of albumin in the nanoparticle portion that is in the form of monomeric albumin, dimeric albumin, or polymeric albumin is determined by separating the nanoparticles from the non-nanoparticle portion, re-suspending the nanoparticles in saline, and subjecting the re-suspended nanoparticles to size-exclusion chromatography (SEC) using a saline mobile phase coupled with a multiple angle light scattering (MALS) detector. In the non-nanoparticle portion of the composition, about 80% to about 95% of the albumin in the non-nanoparticle portion is in the form of monomeric albumin, about 4% to about 14% of the albumin in the non-nanoparticle portion is in the form of dimeric albumin, and about 0.5% to about 5% of the albumin in the non-nanoparticle portion is in the form of polymeric albumin when the percentage of albumin in the non-nanoparticle portion that is in the form of monomeric albumin, dimeric albumin, or polymeric albumin is determined by separating the nanoparticles from the non-nanoparticle portion, and subjecting the non-nanoparticle portion to size-exclusion chromatography (SEC) using a saline mobile phase coupled with a multiple angle light scattering (MALS) detector. The nanoparticle composition

may be further characterized in having less than 3% by weight of the sum of seco-rapamycin and rapamycin in the composition being in the form of seco-rapamycin.

[0010a] In one aspect, the present disclosure refers to a nanoparticle composition comprising (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin; wherein about 80% to about 95% of the albumin in the composition is in the form of monomeric albumin, about 4% to about 15% of the albumin in the composition is in the form of dimeric albumin, and about 0.5% to about 5% of the albumin in the composition is in the form of polymeric albumin when the percentage of albumin in the composition that is in the form of monomeric albumin, dimeric albumin, or polymeric albumin is determined by subjecting the composition to size-exclusion chromatography (SEC) using a saline mobile phase coupled with a multiple angle light scattering (MALS) detector.

[0011] In another characterization of the nanoparticle composition or a commercial batch of the nanoparticle composition, about 42% to about 60% of the albumin in the nanoparticle portion is in the form of polymeric albumin other than oligomeric albumin when the percentage of albumin in the nanoparticle portion that is in the form of polymeric albumin other than oligomeric albumin is determined by separating the nanoparticles from the non-nanoparticle portion, dissolving the nanoparticles, and subjecting the dissolved nanoparticles to size-exclusion chromatography. About 1% to about 4.5% of the albumin in the nanoparticle portion is in the form of oligomeric albumin when the percentage of albumin in the nanoparticle portion that is in the form of oligomeric albumin is determined by separating the nanoparticles from the non-nanoparticle portion, dissolving the nanoparticles, and subjecting the dissolved nanoparticles to size-exclusion chromatography. About 25% to about 50% of the albumin in the nanoparticle portion is in the form of monomeric albumin or about 5% to about 16% of the albumin in the nanoparticle portion is in the form of dimeric albumin when the percentage of albumin in the nanoparticle portion that is in the form of monomeric albumin or dimeric albumin is determined by separating the nanoparticles from the non-nanoparticle portion, dissolving the nanoparticles, and subjecting the dissolved nanoparticles to size-exclusion chromatography. For the non-nanoparticle portion, about 80% to about 95% of the albumin in the non-nanoparticle portion is in the form of monomeric albumin, about 4% to about 14% of the albumin in the non-nanoparticle portion is in the form of dimeric albumin, about 0.5% to about 4% of the albumin in the non-nanoparticle portion is in the form of oligomeric albumin, and/or about 0.5% to about 3% of the albumin in the non-nanoparticle portion is in the form of polymeric albumin other than oligomeric albumin when the percentage of albumin in the non-nanoparticle portion that is in the form of monomeric albumin, dimeric albumin, oligomeric albumin, and/or polymeric albumin other than oligomeric albumin is determined by separating the nanoparticles from the non-nanoparticle portion, and subjecting the non-nanoparticle portion to size-exclusion chromatography. In the composition as a whole, about 80% to about 95% of the total albumin composition is in the form of monomeric albumin, about 4% to about 15% of the total albumin in the composition is in the form of dimeric albumin, about 0.3% to about 3% of the total albumin in the composition is in the form of oligomeric albumin, and/or about 2% to about 7% of the total albumin in the composition is in the form of polymeric albumin other than

oligomeric albumin when the percentage of monomeric albumin, dimeric albumin, oligomeric albumin, and/or polymeric albumin other than oligomeric albumin in the composition is determined by subjecting the composition to size-exclusion chromatography. The nanoparticle composition may be further characterized in having less than 3% by weight of the sum of seco-rapamycin and rapamycin in the composition being in the form of seco-rapamycin.

[0011a] In one aspect, the present disclosure refers to a nanoparticle composition comprising: (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin; wherein about 42% to about 60% of the albumin in the nanoparticles is in the form of polymeric albumin other than oligomeric albumin when the percentage of albumin in the nanoparticles that is in the form of polymeric albumin other than oligomeric albumin is determined by separating the nanoparticles from the non-nanoparticle portion, dissolving the nanoparticles, and subjecting the dissolved nanoparticles to size-exclusion chromatography.

[0011b] In one aspect, the present disclosure refers to a nanoparticle composition comprising: (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin, wherein seco rapamycin is less than 3% by weight of the sum of seco-rapamycin and rapamycin in the composition.

[0011c] In one aspect, the present disclosure refers to a nanoparticle composition comprising: (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin, wherein less than 1% of the rapamycin in the composition is free rapamycin.

[0012] In some ~~embodiment~~examples of the nanoparticle composition, about 70% to about 85% of the albumin in the nanoparticle portion is in the form of monomeric albumin. In some ~~embodiment~~examples, about 5% to about 15% of the albumin in the nanoparticle portion is in the form of polymeric albumin. In some ~~embodiment~~examples, about 9% to about 20% of the albumin in the nanoparticle portion is in the form of dimeric albumin. In some ~~embodiment~~examples, about 0.5% to about 5% of the albumin in the non-nanoparticle portion is in the form of polymeric albumin. In some ~~embodiment~~examples, about 80% to about 95% of the albumin in the non-nanoparticle portion is in the form of monomeric albumin. In some ~~embodiment~~examples, about 4% to about 14% of the albumin in the non-nanoparticle portion is in the form of dimeric albumin. In some ~~embodiment~~examples, about 0.5% to about 5% of total albumin in the composition is in the form of polymeric albumin. In some ~~embodiment~~examples, about 80% to about 95% of total albumin in the composition is in the form of monomeric albumin. In some ~~embodiment~~examples, about 4% to about 15% of total albumin in the composition is in the form of dimeric albumin. In some ~~embodiment~~examples, the percentage of polymeric albumin, dimeric albumin, or monomeric albumin is determined using size-exclusion chromatography.

[0013] In some ~~embodiment~~examples of the nanoparticle composition, the volume-weighted mean particle size of the nanoparticles is about 200 nm or less. In some ~~embodiment~~examples, the volume-weighted mean particle size of the nanoparticles is about 50 nm to about 200 nm. In some ~~embodiment~~examples, the Z-average particle size of the nanoparticles is about 200 nm or less. In some ~~embodiment~~examples, the Z-average particle size of the nanoparticles is about 50 nm to about 200 nm.

[0014] In some ~~embodiment~~examples of the nanoparticle composition, the polydispersity index of the nanoparticles is less than 0.2. In some ~~embodiment~~examples, the polydispersity index of the nanoparticles is about 0.03 to about 0.2. In some ~~embodiment~~examples, the span of particle size distribution ((Dv95-Dv5)/Dv50) of the nanoparticles is about 0.8 to about 1.2.

[0015] In some ~~embodiment~~examples of the nanoparticle composition, the weight percentage of the albumin in the nanoparticle portion is about 25% to about 45%. In some ~~embodiment~~examples, the

weight percentage of rapamycin in the nanoparticle portion is about 55% to about 75%. In some ~~embodiment~~examples, the weight ratio of the albumin to the rapamycin in the nanoparticle portion is about 1:1 to about 1:4.

[0016] In some ~~embodiment~~examples of the nanoparticle composition, the weight ratio of the albumin to the rapamycin in the composition is about 1:1 to about 10:1.

[0017] In some ~~embodiment~~examples, about 90% or more of the albumin in the composition is in the non-nanoparticle portion. In some ~~embodiment~~examples, about 90% or more of the rapamycin in the composition is in the nanoparticles.

[0018] In some ~~embodiment~~examples of the nanoparticle composition, the nanoparticle composition is a nanoparticle suspension. In some ~~embodiment~~examples, the nanoparticle composition is a dried composition, such as a powder. In some ~~embodiment~~examples, the nanoparticles had been resuspended from the dried composition.

[0019] In some ~~embodiment~~examples of the nanoparticle composition, the concentration of albumin in the composition is about 30 mg/mL to about 100 mg/mL. In some ~~embodiment~~examples, the concentration of albumin in the composition that is in the non-nanoparticle portion is about 30 mg/mL to about 100 mg/mL. In some ~~embodiment~~examples, the concentration of albumin in the nanoparticle composition that is in the nanoparticle portion is about 1 mg/mL to about 5 mg/mL. In some ~~embodiment~~examples, the concentration of rapamycin in the nanoparticle composition is about 1 mg/mL to about 100 mg/mL. In some ~~embodiment~~examples, the concentration of rapamycin in the composition that is in the non-nanoparticle portion is about 20 µg/mL to about 55 µg/mL. In some ~~embodiment~~examples, the concentration of rapamycin in the composition that is in the nanoparticle portion is about 1 mg/mL to about 15 mg/mL.

[0020] In some ~~embodiment~~examples of the nanoparticle composition, the osmolality of the composition is about 300 mOsm/kg to about 350 mOsm/kg. In some ~~embodiment~~examples, the viscosity of the composition is about 1.2 cP to about 1.5 cP. In some ~~embodiment~~examples, the pH of the composition is about 6.0 to about 7.5.

[0021] In some ~~embodiment~~examples of the nanoparticle composition, the composition is stable at 25 °C for at least 24 hours. In some ~~embodiment~~examples, the composition is stable at 4 °C for at least 24 hours.

[0022] In some ~~embodiment~~examples of the nanoparticle composition, the composition comprises less than 10 µg/mL tert-butanol. In some ~~embodiment~~examples, the composition comprises tert-butanol. In

some ~~embodiment~~examples, the composition comprises less than 5 µg/mL chloroform. In some ~~embodiment~~examples, the composition comprises chloroform.

[0023] In some ~~embodiment~~examples of the nanoparticle composition, the zeta potential of the nanoparticles is about -25 mV to about -50 mV.

[0024] In some ~~embodiment~~examples of the nanoparticle composition, the composition has an amorphous morphology as determined by measuring crystallinity of a lyophilized form of the composition by X-ray diffraction. In some ~~embodiment~~examples, the nanoparticles have an amorphous morphology as determined by separating the nanoparticles from the composition, lyophilizing the separated nanoparticles, and measuring crystallinity of the separated and lyophilized nanoparticles by X-ray diffraction.

[0025] In some ~~embodiment~~examples of the nanoparticle composition, the rapamycin in nanoparticles has an amorphous morphology as determined by Raman spectroscopy, polarized light microscopy, differential scanning calorimetry (DSC), modulated differential scanning calorimetry (mDSC), Fourier transform infrared (FTIR) spectroscopy, or nuclear magnetic resonance (NMR) spectroscopy.

[0026] In some ~~embodiment~~examples of the nanoparticle composition, the vinyl chain of the rapamycin in the nanoparticle portion interacts with the albumin in the nanoparticles.

[0027] In some ~~embodiment~~examples of the nanoparticle composition, at least a portion of the nanoparticles are non-spherical. In some ~~embodiment~~examples, at least 20% of the nanoparticles in the composition are non-spherical.

[0028] In some ~~embodiment~~examples of the nanoparticle composition, the nanoparticle composition comprises less than about 3% seco-rapamycin in the nanoparticles compared to the sum of seco-rapamycin and rapamycin in the nanoparticles, by weight. In some ~~embodiment~~examples, the nanoparticle composition comprises more than about 0.2% seco-rapamycin in the nanoparticles compared to the sum of seco-rapamycin and rapamycin in the nanoparticles, by weight.

[0029] In some ~~embodiment~~examples of the nanoparticle composition, about 3% or less of the rapamycin in the nanoparticle composition is free rapamycin.

[0030] In some ~~embodiment~~examples of the nanoparticle composition, the albumin is human albumin.

[0031] In some ~~embodiment~~examples of the nanoparticle composition, the nanoparticle composition is sterile.

[0032] In some ~~embodiment~~examples of the nanoparticle composition, the nanoparticle composition has been sterilized by filtration.

[0033] In some ~~embodiment~~examples of the nanoparticle composition, the nanoparticle composition is contained within a sealed container. In some ~~embodiment~~examples, the sealed container is a sealed vial or a sealed bag.

[0034] In some ~~embodiment~~examples of the nanoparticle composition, the nanoparticle composition is a pharmaceutical composition.

[0035] Also described herein is an emulsion (for example, an emulsion in a commercial batch), comprising: a dispersed organic phase comprising nanodroplets comprising rapamycin dissolved in an organic solvent comprising chloroform and tert-butanol, and a continuous aqueous phase comprising albumin.

[0035a] In one aspect, the present disclosure refers to a commercial batch of the nanoparticle composition disclosed herein.

[0035b] In one aspect, the present disclosure refers to a batch of an emulsion, comprising: a dispersed organic phase comprising nanodroplets comprising rapamycin dissolved in an organic solvent comprising chloroform and *tert*-butanol, and a continuous aqueous phase comprising albumin; wherein the batch of the emulsion comprises at least 20 grams of rapamycin.

[0036] In some ~~embodiment~~examples of the emulsion, the organic solvent comprises about 10% to about 50% tert-butanol by volume. In some ~~embodiment~~examples, the organic solvent comprises about 50% to about 90% chloroform by volume. In some ~~embodiment~~examples, the organic solvent comprises chloroform and tert-butanol at a volumetric ratio of about 1:1 to about 9:1.

[0037] In some ~~embodiment~~examples of the emulsion, the concentration of rapamycin in the organic phase is about 20 mg/mL to about 500 mg/mL. In some ~~embodiment~~examples, the concentration of rapamycin in the emulsion is about 2 mg/mL to about 50 mg/mL. In some ~~embodiment~~examples, the concentration of albumin in the aqueous phase is about 10 mg/mL to about 200 mg/mL. In some ~~embodiment~~examples, the concentration of albumin in the emulsion is about 8 mg/mL to about 200 mg/mL.

[0038] In some ~~embodiment~~examples of the emulsion, the phase fraction of the organic phase in the emulsion is about 1% to about 20%.

[0039] In some ~~embodiment~~examples of the emulsion, the nanodroplets have a Z-average particle size of about 200 nm or less. In some ~~embodiment~~examples, the nanodroplets have a Z-average particle size of about 50 nm to about 200 nm.

[0040] In some ~~embodiment~~examples of the emulsion, the Z-average particle size of the nanodroplets does not increase by more than 30% after storing the emulsion for about 4 hours at 4 °C.

[0041] In some ~~embodiment~~examples of the emulsion, the albumin is human albumin.

[0042] Also described herein is a method of making a nanoparticle suspension. The method may include removing organic solvent from an emulsion to make the nanoparticle suspension, the emulsion comprising: a dispersed organic phase comprising nanodroplets comprising rapamycin dissolved in an organic solvent comprising chloroform and tert-butanol, and a continuous aqueous phase comprising albumin. In some ~~embodiment~~examples, the organic solvent is

removed using a wiped film evaporator or a rotary evaporator. The evaporator can be a continuous evaporator or a batch evaporator. A continuous evaporator is an evaporator in which the feed and product streams are continuous and their concentrations remain generally constant with a consistent input to the feed stream. A batch evaporator is an evaporator in which the feed and product streams are discontinuous. In some ~~embodiment~~examples, the organic solvent is removed with a continuous evaporator. In some ~~embodiment~~examples, the organic solvent is removed with a batch evaporator. In some ~~embodiment~~examples, the method further comprises forming the emulsion by homogenizing the organic phase and the aqueous phase.

[0042a] In one aspect, the present disclosure refers to a method of making a commercial batch of a nanoparticle suspension, comprising removing organic solvent from the batch of the emulsion disclosed herein to make the nanoparticle suspension.

[0043] In some ~~embodiment~~examples of making the nanoparticle suspension, the organic solvent comprises about 10% to about 50% tert-butanol by volume. In some ~~embodiment~~examples, the organic solvent comprises about 50% to about 90% chloroform by volume. In some ~~embodiment~~examples, the organic solvent comprises chloroform and tert-butanol at a volumetric ratio of about 1:1 to about 9:1. In some ~~embodiment~~examples, the concentration of rapamycin in the organic phase is about 20 mg/mL to about 500 mg/mL. In some ~~embodiment~~examples, the concentration of rapamycin in the emulsion is about 2 mg/mL to about 50 mg/mL. In some ~~embodiment~~examples, the concentration of albumin in the aqueous phase is about 10 mg/mL to about 200 mg/mL. In some ~~embodiment~~examples, the concentration of albumin in the emulsion is about 8 mg/mL to about 200 mg/mL. In some ~~embodiment~~examples, the phase fraction of the organic phase in the emulsion is about 1% to about 20%. In some ~~embodiment~~examples, the nanodroplets have a Z-average particle size of about 200 nm or less. In some ~~embodiment~~examples, the nanodroplets have a Z-average particle size of about 50 nm to about 200 nm. In some ~~embodiment~~examples, the albumin is human albumin.

[0044] In some ~~embodiment~~examples of making the nanoparticle suspension, the emulsion is stored between about 2 °C and about 8 °C before removing the organic solvent. In some ~~embodiment~~examples, the emulsion is stored for about 4 hours or for about 24 hours.

[0045] In some ~~embodiment~~examples of making the nanoparticle suspension, the method further comprises filtering the organic phase, the aqueous phase, or both, prior to forming the emulsion.

[0046] In some ~~embodiment~~examples of making the nanoparticle suspension, the organic phase and the aqueous phase are homogenized using a high pressure homogenizer.

[0047] In some ~~embodiment~~examples of making the nanoparticle suspension, the method further comprises adding a solution comprising albumin to the nanoparticle suspension. In some ~~embodiment~~examples, adding the solution comprising albumin adjusts the weight ratio of albumin to rapamycin in the nanoparticle suspension to between about 1:1 and about 10:1.

[0048] In some ~~embodiment~~examples of making the nanoparticle suspension, the method further comprises filtering the nanoparticle suspension.

[0049] In some ~~embodiment~~examples of making the nanoparticle suspension, the method further comprises lyophilizing the nanoparticle suspension. In some ~~embodiment~~examples of making the nanoparticle suspension, the method further adding the nanoparticle suspension into one or more vials. In some ~~embodiment~~examples, the method comprises lyophilizing the nanoparticle suspension after adding the nanoparticle suspension into the one or more vials.

[0050] Also described herein is a method of assessing suitability of a pharmaceutical composition for medical use in a human individual, wherein the pharmaceutical composition comprises (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin, the method comprising measuring a quality control parameter for the pharmaceutical composition; and assessing the suitability of the pharmaceutical composition for medical use in a human individual, wherein a measured quality control parameter within a quality control threshold is indicative of suitability of the pharmaceutical composition for medical use. In some ~~embodiment~~examples, the method further comprises separating the nanoparticles from the non-nanoparticle portion, wherein the quality control parameter comprises a quality control parameter for the nanoparticles or the non-nanoparticle portion. In some ~~embodiment~~examples, the nanoparticles comprise a core comprising rapamycin and a coating comprising albumin. The quality control parameter can be any or a combination of the multiple parameters described herein for the nanoparticle compositions, for example, as assessed by corresponding methods described herein.

[0050a] In one aspect, the present disclosure refers to a method of assessing suitability of a pharmaceutical composition for medical use in a human individual, wherein the pharmaceutical composition comprises (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin, the method comprising: measuring a quality control parameter for the pharmaceutical composition; and assessing the suitability of the pharmaceutical composition for medical use in a human individual, wherein a measured quality control parameter within a quality control threshold is indicative of suitability of the pharmaceutical composition for medical use.

[0051] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of monomeric albumin in the nanoparticle portion of the total albumin in the nanoparticle portion; and a weight percentage of albumin in the form of monomeric albumin in the nanoparticle portion of the total albumin in the nanoparticle portion being about 70% to about 85% is indicative of suitability of the pharmaceutical composition for medical use.

[0052] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of polymeric albumin in the nanoparticle portion of the total albumin in the nanoparticle portion; and a weight percentage of albumin in the form of polymeric albumin in the nanoparticle portion of the total

albumin in the nanoparticle portion being about 5% to about 15% is indicative of suitability of the pharmaceutical composition for medical use.

[0053] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of dimeric albumin in the nanoparticle portion of the total albumin in the nanoparticle portion; and a weight percentage of albumin in the form of dimeric albumin in the nanoparticle portion of the total albumin in the nanoparticle portion being about 9% to about 20% is indicative of suitability of the pharmaceutical composition for medical use.

[0054] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of polymeric albumin in the non-nanoparticle portion of the total albumin in the non-nanoparticle portion; and a weight percentage of albumin in the form of polymeric albumin in the non-nanoparticle portion of the total albumin in the non-nanoparticle portion being about 0.5% to about 5% is indicative of suitability of the pharmaceutical composition for medical use.

[0055] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of monomeric albumin in the non-nanoparticle portion of the total albumin in the non-nanoparticle portion; and a weight percentage of albumin in the form of monomeric albumin in the non-nanoparticle portion of the total albumin in the non-nanoparticle portion being about 80% to about 95% is indicative of suitability of the pharmaceutical composition for medical use.

[0056] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of dimeric albumin in the non-nanoparticle portion of the total albumin in the non-nanoparticle portion; and a weight percentage of albumin in the form of dimeric albumin in the non-nanoparticle portion of the total albumin in the non-nanoparticle portion being about 4% to about 15% is indicative of suitability of the pharmaceutical composition for medical use.

[0057] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of polymeric albumin in the composition of the total albumin in the composition; and a weight percentage of albumin in the form of polymeric albumin in the composition of the total albumin in the composition being about 0.5% to about 5% is indicative of suitability of the pharmaceutical composition for medical use.

[0058] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of monomeric albumin in the composition of the total albumin in the composition; and a weight percentage of albumin in the form of monomeric albumin in the composition of the total albumin in the composition being about 80% to about 95% is indicative of suitability of the pharmaceutical composition for medical use.

[0059] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of albumin in the form of dimeric albumin in the composition of the total albumin in the composition; and a weight percentage of albumin in the form of dimeric albumin in the composition of the total albumin in the composition being about 4% to about 15% is indicative of suitability of the pharmaceutical composition for medical use.

[0060] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the percentage of polymeric albumin, dimeric albumin, or monomeric albumin is determined using size-exclusion chromatography.

[0061] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a volume weighted mean particle size of the nanoparticles; and a volume weighted mean particle size of the nanoparticles being about 200 nm or less is indicative of suitability of the pharmaceutical composition for medical use.

[0062] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a volume-weighted mean particle size of the nanoparticles; and a volume weighted mean particle size of the nanoparticles being about 50 nm to about 200 nm is indicative of suitability of the pharmaceutical composition for medical use.

[0063] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a Z-average particle size of the nanoparticles; and a Z-average particle size of the nanoparticles being about 200 nm or less is indicative of suitability of the pharmaceutical composition for medical use.

[0064] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a Z-average particle size of the nanoparticles; and a Z-average particle size being about 50 nm to about 200 nm is indicative of suitability of the pharmaceutical composition for medical use.

[0065] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a polydispersity index of the nanoparticles; and a polydispersity index of the nanoparticles being less than 0.3 is indicative of suitability of the pharmaceutical composition for medical use.

[0066] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a polydispersity index of the nanoparticles; and a polydispersity index of the nanoparticles being about 0.03 to about 0.3 is indicative of suitability of the pharmaceutical composition for medical use.

[0067] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a span of particle size distribution ((Dv95 - Dv5)/Dv50) of the nanoparticles; and a span of particle size distribution of the nanoparticles being about 1.2 or less is indicative of suitability of the pharmaceutical composition for medical use.

[0068] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of the albumin in the nanoparticle portion; and a weight percentage of the albumin in the nanoparticle portion being about 25% to about 45% is indicative of the suitability of the pharmaceutical composition for medical use.

[0069] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight percentage of the rapamycin in the nanoparticle portion; and a weight percentage of the rapamycin in the nanoparticle portion being about 55% to about 75% is indicative of the suitability of the pharmaceutical composition for medical use.

[0070] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight ratio of the albumin to the rapamycin in the nanoparticle portion; and a weight ratio of the albumin to the rapamycin in the nanoparticle portion being about 1:1 to about 1:4 is indicative of the suitability of the pharmaceutical composition for medical use.

[0071] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a weight ratio of the albumin to the rapamycin in the composition; and a weight ratio of the albumin to the rapamycin in the composition being about 1:1 to about 10:1 is indicative of the suitability of the pharmaceutical composition for medical use.

[0072] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a percentage of albumin in the composition that is in the

non-nanoparticle portion; and a percentage of albumin in the composition that is in the non-nanoparticle portion being about 95% or more is indicative of the suitability of the pharmaceutical composition for medical use.

[0073] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a percentage of rapamycin in the composition that is in the nanoparticle portion; and a percentage of rapamycin in the composition that is in the nanoparticle portion being about 98% or more is indicative of the suitability of the pharmaceutical composition for medical use.

[0074] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the pharmaceutical composition is a nanoparticle suspension.

[0075] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the pharmaceutical composition is reconstituted from a dried nanoparticle composition, such as a powder.

[0076] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a concentration of albumin in the composition; and a concentration of albumin in the composition being about 30 mg/mL to about 100 mg/mL is indicative of the suitability of the pharmaceutical composition for medical use.

[0077] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a concentration of albumin in the composition that is in the non-nanoparticle portion; and a concentration of albumin in the composition that is in the non-nanoparticle portion being about 30 mg/mL to about 100 mg/mL is indicative of the suitability of the pharmaceutical composition for medical use.

[0078] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a concentration of albumin in the composition that is in the nanoparticle portion; and a concentration of albumin in the composition that is in the nanoparticle portion being about 1.8 mg/mL to about 15 mg/mL is indicative of the suitability of the pharmaceutical composition for medical use.

[0079] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a concentration of rapamycin in the composition; and a concentration of rapamycin in the composition being about 1 mg/mL to about 15 mg/mL is indicative of the suitability of the pharmaceutical composition for medical use.

[0080] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a concentration of rapamycin in the composition that is in the non-nanoparticle portion; and a concentration of rapamycin in the composition that is in the non-nanoparticle portion being about 20 µg/mL to about 55 µg/mL is indicative of the suitability of the pharmaceutical composition for medical use.

[0081] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a concentration of rapamycin in the composition that is in the nanoparticle portion; and a concentration of rapamycin in the composition that is in the nanoparticle portion being about 1 mg/mL to about 15 mg/mL is indicative of the suitability of the pharmaceutical composition for medical use.

[0082] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises an osmolality of the composition; and an osmolality of the composition being about 300 mOSm/kg to about 350 mOSm/kg is indicative of the suitability of the pharmaceutical composition for medical use.

[0083] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a viscosity of the composition; and a viscosity of the composition being about 1.2 cP to about 1.5 cP is indicative of the suitability of the pharmaceutical composition for medical use.

[0084] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a stability of the composition; and the composition being stable at 25 °C for at least 24 hours is indicative of the suitability of the pharmaceutical composition for medical use.

[0085] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a stability of the composition; and the composition being stable at 4 °C for at least 24 hours is indicative of the suitability of the pharmaceutical composition for medical use.

[0086] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a pH of the composition; and a pH of the composition being about 6.0 to about 7.5 is indicative of the suitability of the pharmaceutical composition for medical use.

[0087] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the composition is made using tert-butanol, and wherein the quality control parameter comprises a

concentration of tert-butanol; and a concentration of tert-butanol being less than 10 µg/mL tert-butanol is indicative of the suitability of the pharmaceutical composition for medical use.

[0088] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the composition is made using chloroform, and wherein: the quality control parameter comprises a concentration of chloroform; and a concentration of tert-butanol being less than 5 µg/mL chloroform is indicative of the suitability of the pharmaceutical composition for medical use.

[0089] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a zeta potential of the nanoparticles; and a zeta potential of the nanoparticles being about -25 mV to about -50 mV is indicative of the suitability of the pharmaceutical composition for medical use.

[0090] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a morphology of the composition, wherein the morphology is measured by measuring crystallinity of a lyophilized form of the composition by X-ray diffraction; and an amorphous morphology of the composition is indicative of the suitability of the pharmaceutical composition for medical use.

[0091] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a morphology of the composition, wherein the morphology is measured by separating the nanoparticles from the composition, lyophilizing the separated nanoparticles, and measuring crystallinity of the separated and lyophilized nanoparticles by X-ray diffraction; and an amorphous morphology of the composition is indicative of the suitability of the pharmaceutical composition for medical use.

[0092] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a morphology of the composition, wherein the morphology is measured by Raman spectroscopy differential scanning calorimetry (DSC), modulated differential scanning calorimetry (mDSC), Fourier transform infrared (FTIR) spectroscopy, or nuclear magnetic resonance (NMR) spectroscopy; and an amorphous morphology of the composition is indicative of the suitability of the pharmaceutical composition for medical use.

[0093] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises an interaction of the vinyl chain of the rapamycin in the nanoparticle portion with the albumin in the nanoparticle portion; and an identified interaction between the vinyl chain of the rapamycin in the nanoparticle portion with the albumin in the

nanoparticle portion is indicative of the suitability of the pharmaceutical composition for medical use.

[0094] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a portion of the nanoparticles that are non-spherical; and identification of at least a portion of the nanoparticles as non-spherical is indicative of the suitability of the pharmaceutical composition for medical use.

[0095] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a portion of the nanoparticles that are non-spherical; and identification of at least 20% of the nanoparticles as non-spherical is indicative of the suitability of the pharmaceutical composition for medical use.

[0096] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a percentage of seco-rapamycin compared to the sum of seco-rapamycin and rapamycin, by weight, in the nanoparticle portion; and a percentage of seco-rapamycin compared to the sum of seco-rapamycin and rapamycin, by weight, in the nanoparticle portion being less than 2.5% seco-rapamycin is indicative of the suitability of the pharmaceutical composition for medical use.

[0097] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the quality control parameter comprises a percentage of rapamycin in the pharmaceutical composition that is free rapamycin; and a percentage of rapamycin in the pharmaceutical composition that is free rapamycin being less than 3% is indicative of the suitability of the pharmaceutical composition for medical use.

[0098] In some ~~embodiment~~examples of assessing the suitability of the pharmaceutical composition, the albumin is human albumin.

[0099] Also provided herein is a method of releasing a commercial batch of a pharmaceutical composition comprising (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin, the method comprising: assessing the suitability of the pharmaceutical composition for medical use in a human individual using a sample of the commercial batch, and releasing the commercial batch if the pharmaceutical composition is suitable for medical use.

[0099a] In one aspect, the present disclosure refers to a method of releasing a commercial batch of a pharmaceutical composition comprising (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin, the method comprising: assessing the suitability of the pharmaceutical composition for medical use in a human individual using a sample of the commercial batch, wherein the suitability of the pharmaceutical composition is assessed according to the method disclosed herein; and releasing the commercial batch if the pharmaceutical composition is suitable for medical use.

[0100] Further provided herein is a method of processing a sample of a pharmaceutical composition to validate the sample as suitable for medical use in a human individual, the pharmaceutical composition comprising (a) nanoparticles comprising rapamycin and albumin,

and (b) a non-nanoparticle portion comprising albumin and rapamycin, the method comprising: obtaining the sample from a commercial batch; and assessing the suitability of the pharmaceutical composition for medical use in a human individual using a sample of the commercial batch.

[0100a] In one aspect, the present disclosure refers to a method of processing a sample of a pharmaceutical composition to validate the sample as suitable for medical use in a human individual, the pharmaceutical composition comprising (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin, the method comprising: obtaining the sample from a commercial batch; and assessing the suitability of the pharmaceutical composition for medical use in a human individual using a sample of the commercial batch, wherein the suitability of the pharmaceutical composition is assessed according to the method disclosed herein.

[0101] Also described is a method of preparing a pharmaceutical composition comprising (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin for release, the method comprising: assessing the suitability of the pharmaceutical composition for medical use in a human individual; identifying the pharmaceutical composition as suitable for medical use in a human individual; and packaging the pharmaceutical composition for release. In some ~~embodiment~~examples, packaging the pharmaceutical composition comprises lyophilizing the pharmaceutical composition. In some ~~embodiment~~examples, packaging the pharmaceutical composition comprises filling the pharmaceutical composition in a container. In some ~~embodiment~~examples, the method comprises sealing the container.

[0101a] In one aspect, the present disclosure refers to a method of preparing a pharmaceutical composition comprising (a) nanoparticles comprising rapamycin and albumin, and (b) a non-nanoparticle portion comprising albumin and rapamycin for release, the method comprising: assessing the suitability of the pharmaceutical composition for medical use in a human individual according to the method disclosed herein; identifying the pharmaceutical composition as suitable for medical use in a human individual; and packaging the pharmaceutical composition for release.

BRIEF DESCRIPTION OF THE FIGURES

[0102] FIG. 1 depicts Cryo-TEM images at 52,000x magnification (0.21 nm/pixel) of lot #11 (top left), lot #1 (top right), lot #3 (middle left), human albumin alone (middle right), or lot #2 (bottom).

[0103] FIG. 2 depicts a Cryo-TEM image at 21,000x magnification of lot #11 with an observed irregularly shaped particles with nonuniform internal density (bottom arrow), a spherical particle with uniform density (top left arrow), and a small, round particle (top right arrow).

[0104] FIG. 3 depicts a Cryo-TEM image at 21,000x magnification of lot #1 with an observed irregularly shaped particle with nonuniform internal density (top arrow), a spherical particle (bottom arrow) with uniform density, and a small round particle (middle arrow).

[0105] FIG. 4 depicts a representative chromatograph of the pharmaceutical composition comprising rapamycin and albumin (lot #1 after 12 months of storage at 5 °C) as measured by size exclusion chromatography (SEC). Peaks corresponding to monomer, dimer, polymer, and oligomers (which can be identified by suitable techniques such as mass spectrometry) are indicated on the chromatograph.

[0106] FIG. 5 depicts light scattering intensity (kcps) as a function of rapamycin concentration from a reconstituted suspension of the pharmaceutical composition comprising nanoparticles comprising rapamycin and albumin. When the suspensions were diluted to concentrations below