permeation as well as reduce the risk of $^{3}H_2O$ vapor inhalation. The second method termed ViVo, was performed by adding the ESR spin probe 4-trimethylammonium TEMPO (CAT1) to a total volume of 1 ml sample and then comparing the concentration of the probe in the supernatant above the centrifuged pellet to the concentration of probe in the total volume using a standard curve.

There is an unmet need for an accurate post-production measurement of the trapped volume of liposomes that can be used to characterize liposomal compositions suitable for clinical use.

SUMMARY

The present disclosure discloses a post-production method of determining the trapped volume and trapped aqueous volume of liposomes, and use thereof in characterization and quality control of batch productions of liposomes for clinical use.

The present disclosure is based, in part, on the discovery of a simple, accurate, and reproducible method of determining the trapped volume and trapped aqueous volume of liposomes. The method utilizes a marker having a molecular weight of 1 kDa or higher that neither penetrates the liposomes nor becomes integrated in or associated with the lipid membrane thereby being present solely in the external aqueous phase. Upon separation of the liposomes from the suspending liquid (e.g. using centrifugation), an increase in the concentration of the marker in the liquid is measured as both the lipid membrane and the intra-liposomal aqueous medium occupy part of the dispersion volume but do not incorporate the marker. This increase in concentration is indicative of the trapped volume. Calculation of the trapped volume is based on the volume of the external aqueous medium deduced from the ratio of the signal induced by the marker in the suspending liquid following separation of the liposomes compared to the total signal before separation. In some examples, calculation may further be based on the determination of the signal induced by the marker in the suspending liquid and in the liposome-containing precipitate following separation compared to the total signal induced by the marker before separation.

Contrary to hitherto known methods which are either not suitable as post-production methods or utilize probes which permeate the lipid bilayer and are dependent on the zeta potential and surface charge of the liposomes, the method of the present disclosure is a post-production method which is sensitive, reproducible, and is less

According to another example, there is provided a method of determining the trapped volume (Vt) and the trapped aqueous volume (Vtaq) of a plurality of liposomes in a liposomal composition, the method comprising the steps of:

(i) obtaining a liposomal composition comprising a plurality of liposomes at a predetermined concentration of lipids which are suspended or dispersed in a fluid medium;

(ii) adding a marker to said liposomal composition, wherein the marker is liposome impermeable and has a molecular weight equal to or higher than 1 kDa;

(iii) measuring a signal induced by the marker in said liposomal composition thereby determining the total volume of the liposomal composition;

(iv) separating the fluid medium from the plurality of liposomes thereby obtaining a fluid medium and a precipitate comprising a plurality of liposomes and an interstitial medium between the liposomes;

(v) measuring a signal induced by the marker in the separated fluid medium of step (iv) thereby determining the volume of the fluid medium and the interstitial medium; and

(vi) calculating the trapped volume (Vt) and the trapped aqueous volume (Vtaq) of the plurality of liposomes based on the volumes determined in steps (iii) and (v) and the predetermined concentration of step (i).

It is to be understood that calculating the trapped volume (Vt) and the trapped aqueous volume (Vtaq) of the plurality of liposomes based on the volumes determined in steps (iii) and (v) comprises calculating the ratio between the signal of the marker in the separated fluid medium following step (iv) and its signal in the liposomal composition prior to step (iv).

According to yet another example, there is provided a method of determining the trapped volume (Vt) and the trapped aqueous volume (Vtaq) of a plurality of liposomes in a liposomal composition, the method comprising the steps of:

(i) obtaining a liposomal composition comprising a plurality of liposomes at a predetermined concentration of lipids which are suspended or dispersed in a fluid medium;

(ii) adding a marker to said liposomal composition, wherein the marker is liposome impermeable and has a molecular weight equal to or higher than 1 kDa;

(iii) measuring a signal induced by the marker in said liposomal composition thereby determining the total volume of the liposomal composition;

(iv) separating the fluid medium from the plurality of liposomes thereby obtaining a fluid medium and a precipitate comprising a plurality of liposomes and an interstitial medium between the liposomes;

(v) measuring a signal induced by the marker in the separated fluid medium of step (iv) thereby determining the volume of the fluid medium and the interstitial medium;

(vi) measuring a signal induced by the marker in the precipitate comprising a plurality of liposomes and an interstitial medium between the liposomes of step (iv) thereby determining the interstitial volume between the liposomes in the precipitate; and

(vii) calculating the trapped volume (Vt) and the trapped aqueous volume (Vtaq) of the plurality of liposomes based on the volumes determined in steps (iii), (v), and (vi) and the predetermined concentration of step (i).

In one example, determination of the trapped volume (Vt) and the trapped aqueous volume (Vtaq) comprises determination of the specific trapped volume and the specific trapped aqueous volume, respectively.

According to some examples, the marker is water soluble. According to other examples, the marker has a molecular weight of at least about 2 kDa. In certain examples, the marker is a radioactive marker. In particular examples, the marker is inulin. In other particular examples, the marker is ^{14}C-carboxy inulin.

In further examples, the signal induced by the marker is measured using at least one technique selected from radiation detection, calorimetry, fluorescence, High Performance Liquid Chromatography (HPLC), Liquid Chromatography combined with mass spectrometry (LC/MS), Gas-Chromatography (GC), GC-lined Mass-Spectrometry (GC-MS), Infrared spectroscopy, Raman spectroscopy, NMR, colorimetry, UV-VIS spectroscopy, and combinations thereof. Each possibility represents a separate example.

In various examples, the liposomal composition comprises a plurality of liposomes selected from the group consisting of unilamellar vesicles (SUV), large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV), oligolamellar vesicles (OLV), multilamellar vesicles (MLV), multivesicular liposomes (MVL), and a mixture or combination thereof. Each possibility represents a separate example.

In certain examples, the liposomal composition comprises a plurality of liposomes in sizes ranging from about 0.3 μm to about 50 μm, including each value within the specified range. In other examples, the liposomal composition comprises a plurality of liposomes in sizes ranging from about 1 μm to about 40 μm, including each value within the specified range.

In various examples, the liposomal composition comprises a plurality of liposomes having a lipid concentration of about 50 mM to about 200 mM, including each value within the specified range. In particular examples, the liposomal composition comprises a plurality of liposomes having a lipid concentration of about 100 mM to about 150 mM, including each value within the specified range.

In some examples, the liposomal composition comprises a plurality of empty liposomes essentially free of an active pharmaceutical ingredient. In other examples, the liposomal composition comprises a plurality of liposomes encapsulating an active pharmaceutical ingredient. In accordance with the latter examples, the method of the present disclosure further comprises determining the concentration of the active pharmaceutical ingredient encapsulated by the plurality of liposomes.

In various examples, separating the fluid medium from the plurality of liposomes is performed by centrifugation, filtration, or decantation. Each possibility represents a separate example. In one example, separating the fluid medium from the plurality of liposomes provides a fluid medium which is substantially devoid of liposomes.

In a currently preferred example, separating the fluid medium from the plurality of liposomes is performed by centrifugation.

In some examples, centrifugation of the liposomal composition is performed at a relative centrifugal force of about 10,000 to about 30,000 (×g), including each value within the specified range.

In further examples, centrifugation of the liposomal composition is performed at a temperature of about 2°C to about 25°C, including each value within the specified range.

In additional examples, centrifugation of the liposomal composition is performed for a time period in the range of about 10 minutes to about 100 minutes, including each value within the specified range.

In various examples, the method disclosed herein is useful in controlling the quality of a liposomal composition. In accordance with these examples, the method further comprises comparing the determined trapped volume (Vt) and trapped aqueous volume (Vtaq) with reference trapped volume (Vt) and trapped aqueous volume (Vtaq) values, and rejecting said liposomal composition if the determined trapped volume (Vt) and trapped aqueous volume (Vtaq) differ from the reference values by more than plus or minus 10%. In one aspect, the present disclosure refers to a method for controlling the quality of a liposomal composition, the method comprising: (i) determining the trapped volume (Vt) and the trapped aqueous volume (Vtaq) of a plurality of liposomes in a liposomal composition as disclosed herein; (ii) comparing the determined trapped volume (Vt) and trapped aqueous volume (Vtaq) with reference trapped volume (Vt) and trapped aqueous volume (Vtaq) values; and (iii) rejecting said liposomal composition if the determined trapped volume (Vt) and trapped aqueous volume (Vtaq) differ from the reference values by more than plus or minus 10%.

In other examples, the method disclosed herein is useful in controlling the quality of a liposomal composition comprising an active pharmaceutical ingredient (API) encapsulated within a plurality of liposomes, the method comprising determining the concentration of API encapsulated within a plurality of liposomes in a liposomal composition from the trapped volume (Vt) and trapped aqueous volume (Vtaq) as disclosed herein, comparing the determined API concentration with a reference API concentration, and rejecting said liposomal composition if the determined API concentration differs from the reference API concentration by more than plus or minus 10%. In one aspect, the present disclosure refers to a method for controlling the quality of a liposomal composition comprising an active pharmaceutical ingredient encapsulated within a plurality of liposomes, the method comprising: (i) determining the concentration of the active pharmaceutical ingredient encapsulated within a plurality of liposomes in a liposomal composition by determining the trapped volume (Vt) and the trapped aqueous volume (Vtaq) of a plurality of liposomes in a liposomal composition as disclosed herein; (ii) comparing the determined active pharmaceutical ingredient concentration with a reference active pharmaceutical ingredient concentration; and (iii) rejecting said liposomal composition if the determined active pharmaceutical ingredient concentration differs from the reference active pharmaceutical ingredient concentration by more than plus or minus 10%.

According to some examples, there is provided a liposomal composition formulated for intra-articular administration comprising a polyol tonicity agent; and a plurality of liposomes consisting essentially of 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC) at a ratio of about 45:55, wherein the composition is essentially free of an active pharmaceutical agent, and wherein the plurality of liposomes are characterized by a specific trapped aqueous volume in the range of about 1 to about 4 µl/mg lipid, including each value within the specified range, as determined by any one of the methods disclosed herein.

According to another example, there is provided a liposomal composition formulated for intra-articular administration comprising a fluid medium; a polyol tonicity

agent; and a plurality of liposomes consisting essentially of 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC) at a ratio of about 45:55, wherein the composition is essentially free of an active pharmaceutical agent, and wherein the plurality of liposomes are characterized by a specific trapped aqueous volume in the range of about 1 to about 4 μl/mg lipid, including each value within the specified range, as determined by a method comprising the steps of:

(i) adding a marker to said liposomal composition, wherein the marker is liposome impermeable and has a molecular weight equal to or higher than 1 kDa;

(ii) measuring a signal induced by the marker in the liposomal composition thereby determining the total volume of the liposomal composition;

(iii) determining the volume of the fluid medium and the interstitial medium between liposomes obtained by separating the fluid medium from the plurality of liposomes and measuring a signal induced by the marker in the fluid medium following separation; and

(iv) calculating the trapped volume (Vt) and trapped aqueous volume (Vtaq) of the plurality of liposomes based on the volumes determined in steps (ii) and (iii) and the concentration of the lipids forming the liposomes.

In another aspect, the present disclosure refers to a liposomal composition formulated for intra-articular administration comprising a fluid medium; a polyol tonicity agent; and a plurality of liposomes consisting essentially of 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC) at a ratio of about 45:55, wherein the composition is essentially free of an active pharmaceutical agent, and wherein the plurality of liposomes are characterized by a specific trapped aqueous volume in the range of about 1 to about 4 μl/mg lipid as determined by a method comprising the steps of:

(i) adding a marker to said liposomal composition, wherein the marker is liposome impermeable and has a molecular weight equal to or higher than 1 kDa;

(ii) measuring a signal induced by the marker in said liposomal composition thereby determining the total volume of the liposomal composition;

(iii) determining the volume of the fluid medium and the interstitial medium between liposomes obtained by separating the fluid medium from the plurality of liposomes and measuring a signal induced by the marker in the fluid medium following separation; and

(iv) calculating the trapped volume (Vt) and trapped aqueous volume (Vtaq) of the plurality of liposomes based on the volumes determined in steps (ii) and (iii) and the concentration of the lipids forming the liposomes.

According to various examples, step (iii) of determining the volume of the fluid medium and the interstitial medium between liposomes further comprises determining the interstitial volume between liposomes in a precipitate by measuring a signal induced by the marker in the precipitate following separation from the fluid medium. In one example, separation is performed by centrifugation.

According to some examples, the fluid medium comprises a histidine buffer. According to other examples, the polyol is a linear polyol. According to yet other examples, the polyol is selected from the group consisting of mannitol, dextrose, lactose, trehalose, and combinations thereof. Each possibility represents a separate example. According to particular examples, the polyol is mannitol. According to various examples, the weight ratio between the plurality of liposomes and the polyol tonicity agent ranges from about 6:1 to about 2:1, including all iterations of ratios within the specified range.

According to further examples, the plurality of liposomes consists essentially of a combination of DMPC and DPPC, wherein DMPC is present in a weight percent ranging from about 1% (w/w) to about 10% (w/w) and DPPC is present in a weight percent ranging from about 2% (w/w) to about 12% (w/w), including each value within the specified ranges. According to certain examples, the liposomal composition has a pH in the range of about 5 to about 8, including each value within the specified range. According to other examples, the plurality of liposomes are characterized by a specific trapped aqueous volume of about 1.5 to about 3.5 μl/mg lipid, including each value within the specified range. According to yet other examples, the plurality of liposomes are characterized by a specific trapped aqueous volume of about 2 to about 3 μl/mg lipid, including each value within the specified range. According to further examples, the plurality of liposomes are characterized by a trapped aqueous volume of about 10% to about 35%, including each value within the specified range. According to additional examples, the plurality of liposomes are characterized by a trapped aqueous volume of about 20% to about 30%, including each value within the specified range.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of examples of the disclosure, exemplary methods and/or materials are described below. These exemplary methods and/or materials are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of an Eppendorf vial following centrifugation indicating the different volumes used for calculating the trapped volume according to certain embodiments of the present invention.

Figure 2 is a calibration curve of the optical density of ferricyanide at a wavelength of 420 nm in water and HMB.

Figure 3 is a diagram of the trapped volume and turbidity of the upper phase of different samples as determined using a ferricyanide dye. A- 3mM lipid; B- 52 mM lipid; C- 122 mM lipid; D- 160 mM lipid; E- 206 mM lipid; F- 305 mM lipid; G- 10 mM NaCl;