

**FORMULATIONS OF LIPID NANO EMULSIONS WITH CONTROLLED  
PHARMACOKINETIC PROFILES AND METHODS OF MAKING AND USING THE SAME**

**[0001]** This application claims priority to U.S. Provisional Patent Application Serial No. 63/608,271 filed December 10, 2023, and U.S. Provisional Patent Application Serial No. 63/602,622 filed November 26, 2023, which are each incorporated by reference herein in their entirety.

**TECHNICAL FIELD**

**[0002]** This disclosure generally relates to compositions of and methods of making oral liquid emulsions of medium chain triglycerides (MCTs).

**BACKGROUND**

**[0003]** Tricaprilin (which is synonymously also called tricaprylin, trioctanoylglyceride, tricaprylyl glycerol, glycerol trioctanoin, tricapryloyl glycerol, octanoic acid-1,2,3-propanetriylester, glycerin tricaprylate, tricaprylyl glycerin, glycerol tricaprylate, glycerol trioctanoate, glyceryl tricaprylate, caprylic triglyceride, tricaprilin, tricaprylyl glycerol, etc.) is the triglyceride of caprylic acid which is usually manufactured by esterification of caprylic acid with glycerin.

**[0004]** Tricaprilin is used in pharmaceutical preparations, for example as a neutral carrier or excipient, as an absorption promoter and as a solubilizer for active drugs. It is also used as an oily phase to prepare water-in-oil-in-water multiple emulsions for incorporating water-soluble drugs and also for obtaining stable microcapsules. Being readily miscible with natural oils and surfactants, tricaprilin is also used as fat component in two-phase foam baths. It is furthermore used in sunscreen creams and oils because of its compatibility with organic and inorganic filter agents. It is also used as a fixative for perfumes and fragrances. However, as already indicated hereinabove, fatty acid polyol esters, particularly fatty acid glycerol esters, and in particular so-called medium-chain triglycerides (MCTs) and, above all, also tricaprilin have also been used as active ingredients or active compounds in pharmaceutical compositions.

**SUMMARY**

**[0005]** In some aspects, the current disclosure encompasses an emulsion comprising: about 20%-60% by weight of tricaprilin; about 0.1-10% by weight of emulsifier; about 0.1-10% by weight of glycerol; and about 10 mM - 200 mM phosphate buffer (pH 6.0-7.5). Non-limiting examples of emulsifiers include phospholipids, macrogolglycerol hydroxystearate, citric acid ester of mono- and diglycerides, or any combination thereof. In some aspects, the emulsifier is Phospholipon® 90G, Kolliphor® RH40, or a combination thereof. In some aspects, provided herein is an emulsion comprising: 50% by weight of tricaprilin; 4% by weight of Phospholipon®

90G; 2% by weight of Kolliphor RH40; 2.5% by weight of glycerol; and 20 mM - 150 mM phosphate buffer (pH 6.8). In some aspects, the emulsion may further comprise a sweetener. Non-limiting examples of suitable sweeteners include acesulfame potassium, advantame, aspartame, saccharin, sucralose, Luo Han Guo, stevia, or any combination thereof. In some aspects, the sweetener is sucralose, stevia, or any combination thereof. In some aspects, the emulsion comprises about 0.01% to about 1% by weight of sweetener. In an exemplary aspect the sweetener is sucralose and is present in a concentration of about 0.05% by weight. In some aspects, the emulsion further comprises a flavor (flavoring agent). In some aspects, the flavor is oil-soluble. Non-limiting examples of flavors include vanilla, berry and mango. In some aspects, the flavor is vanilla. In some aspects, the emulsion comprises about 0.1% to about 0.3% by weight vanilla flavor. In some aspects, the vanilla flavor is present in a concentration of about 0.1% or about 0.2% by weight. In some aspects, the emulsion comprises 100 mM phosphate buffer, and 0.1% by weight of vanilla flavor. In some aspects, the emulsion comprises 50 mM of phosphate buffer, and the flavor is vanilla present in a concentration of about 0.2% by weight.

**[0006]** In some aspects, disclosed herein is an emulsion comprising: a medium chain triglyceride (MCT); one or more emulsifiers; a buffer; and a sweetener and/or a flavoring agent; wherein the emulsion is an emulsion for oral administration. In some aspects, the MCT is tricaprilin. Non-limiting examples of emulsifiers include phospholipids, macroglycerol hydroxystearate, citric acid ester of mono- and diglycerides, or a combination thereof. In some aspects, the emulsion further comprises a triol. In some aspects, the triol comprises a glycerol. Non-limiting examples of sweeteners include acesulfame potassium, advantame, aspartame, saccharin, sucralose, Luo Han Guo, purified stevia leaf extracts, or a combination thereof. In some aspects, the sweetener is sucralose, stevia, or any combination thereof. In some aspects, the emulsion comprises about 0.01% to about 1% by weight of sweetener. In an exemplary aspect, the sweetener is sucralose and is present in a concentration of about 0.05% by weight. In some aspects, the emulsion comprises an oil-based flavor. In some aspects, the flavor comprises vanilla, mango, berry, or a combination thereof. In some aspects, the flavor is present in an amount of about 0.05% to about 0.5% by weight. In some aspects, the flavor comprises vanilla. In some aspects, the vanilla is present at a concentration of about 0.1% by weight or about 0.2% by weight. In some aspects, the flavor comprises berry. In some aspects, the flavor is present in a concentration of about 0.3% by weight.

**[0007]** In some aspects, the emulsions disclosed herein are stable over a period of at least about 3 months, 6 months, 9 months, 12 months or more at about 2 °C to about 8 °C. In some aspects, the emulsions are stable over a period of 3 months, 6 months, 9 months, 12 months

or more at about 25 °C. In some aspects, the emulsions are stable over a period of 3 months, 6 months, 9 months, 12 months or more at about 40 °C.

**[0008]** In some aspects, also disclosed herein is a method of making an emulsion comprising tricaprilin, wherein the method comprises the steps of: a) feeding ingredients comprising a buffer, tricaprilin, and one or more emulsifiers to a vessel; b) high shear mixing the buffer, tricaprilin, and one or more emulsifiers in the vessel to form a course emulsion; c) optionally sterilizing the course emulsion to form a sterile emulsion; and d) high pressure homogenizing (HPH) the sterile emulsion to form the emulsion comprising tricaprilin. In some aspects, the step a may also comprise feeding glycerol to the vessel. In some aspects, step (c) comprises a ultra-heat-treatment (UHT) step, an irradiation step, or a combination thereof. In some aspects step (c) comprises a UHT step. In some aspects, the UHT step (step c) is conducted at a temperature of about 130 °C to about 145 °C and for about 3 seconds to about 45 seconds. In some aspects, the temperature is about 135 °C and the hold time is about 2-3 seconds. In some aspects, step (c) does not comprise a irradiation step. In some aspects, the method further comprises mixing a flavor with the tricaprilin in step (a). In some aspects, the method further comprises mixing a sweetener with the tricaprilin in step (a). In some aspects, the one or more emulsifiers comprise Phospholipon® 90G, Kolliphor® RH40, or a combination thereof. In some aspects, the method does not comprise a gamma irradiation step for sterilization. In some aspects, the emulsion is the emulsion as disclosed herein above. In some aspects, the emulsion made by the disclosed method is stable for at least 3 months, at least 6 months, or at least 12 months. In some aspects, the emulsion has a particle size of 0.01-10 µm in size. In some aspects of the disclosed method, the high pressure homogenization is done at a pressure of about 5,000 PSI to about 10,000 PSI. In some aspects, high pressure homogenization is done at a pressure of about 7,500 PSI. In some aspects, the method further comprises a high shear mixing step with recirculation.

**[0009]** In some aspects, also disclosed herein is a method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of a disclosed emulsion. In some aspects, also disclosed herein is a method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of an emulsion comprising: about 20%-60% by weight of tricaprilin; about 0.1-10% by weight of emulsifier; about 0.1-10% by weight of glycerol; and about 10 mM - 200 mM phosphate buffer (pH 6.0-7.5). In some aspects, the emulsion comprises: 50% by weight of tricaprilin; 4% by weight of Phospholipon® 90G; 2% by weight of Kolliphor® RH40; 2.5% by weight of glycerol; and 20 mM - 100 mM phosphate buffer (pH 6.8). Non-limiting examples of disease or disorder that may be treated by using the disclosed emulsions comprise Age-Associated Memory Impairment (AAMI), Alzheimer's Disease (AD),

Parkinson's Disease, Friedreich's Ataxia (FRDA), GLUT1-deficient Epilepsy, Leprechaunism, and Rabson-Mendenhall Syndrome, Coronary Arterial Bypass Graft (CABG) dementia, anesthesia-induced memory loss, Huntington's Disease, infantile Spasms, migraine and related headaches.

#### **BRIEF DESCRIPTION OF THE FIGURES**

**[0010]** The following figures form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. Aspects of the present disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific aspects presented herein:

**[0011]** FIG. 1 shows the manufacturing method used to prepare un-flavored emulsions for AC-OLE-01.

**[0012]** FIG. 2 shows manufacturing method with potential points of flavor addition as marked by the red stars. The sucralose was either mixed with the buffer and emulsifiers (1) at the start of the process or added along with the vanilla flavor just before filling (2) as highlighted in gray writing.

**[0013]** FIG. 3 shows manufacturing method with point of flavor addition marked by the gray star. The sucralose is mixed with the buffer and emulsifiers at the start of the process as highlighted in gray writing.

**[0014]** FIG. 4 shows particle size distributions after high shear mixing (top) and after seven passes of high-pressure homogenisation (bottom) for AC-OLE-01-VA, active formulation flavored with (0.2% Intense Vanilla Flavor and 0.05% Sucralose).

**[0015]** FIGS. 5A-5I show pH evolution of formulation screening DOE formulations ordered from most stable to least stable at 25 °C as indicated as batch on each graph.

**[0016]** FIGS. 6A-6D show pH evolution of active formulations with varying levels of flavor and buffer concentration as indicated on each graph. FIG. 6A provides pH of active formulation with 50 mM buffer and 0.2% vanilla. FIG. 6B provides pH of active formulation with 100 mM buffer and 0.2% vanilla. FIG. 6C provides pH of active formulation with 100 mM buffer and 0.1% vanilla. FIG. 6D provides pH of active formulation with 100 mM buffer, unflavored.

**[0017]** FIG. 7 shows manufacturing process including UHT treatment.

**[0018]** FIGS. 8A-8B show in process particle size measurements after high shear mixing (FIG. 8A) and after pass 5 of high-pressure homogenization (FIG. 8B).

**[0019]** FIG. 9 shows manufacturing process used to prepare vanilla flavored emulsions.

[0020] FIGS. 10A-10B show in process particle size measurements after high shear mixing (FIG. 10A) and after pass 2 (FIG. 10B) of high-pressure homogenization for one of the active formulations.

[0021] FIG. 11 shows an alternate manufacturing process which uses a mixture of dihydrogen phosphate and di-sodium hydrogen phosphate as buffering agents, high shear mixing with recirculation, and use of 7,500 PSI for high pressure homogenization instead of 10,000 PSI.

#### **DETAILED DESCRIPTION**

[0022] The following detailed description references the accompanying drawings that illustrate various aspects of the present disclosure. The drawings and description are intended to describe aspects of the present disclosure in sufficient detail to enable those skilled in the art to practice the present disclosure. Other components can be utilized and changes can be made without departing from the scope of the present disclosure. The following description is, therefore, not to be taken in a limiting sense.

#### **I. Emulsion formulation**

[0023] The present disclosure relates to an emulsion comprising an active pharmaceutical ingredient, one or more emulsifiers, and a buffer. In some aspects, the emulsion may include an alcohol, a sweetener, a flavor, or a combination thereof.

[0024] In some aspects the active pharmaceutical ingredient may be an oil. In some aspects, the active pharmaceutical ingredient is a medium chain triglyceride (MCT).

[0025] In some aspects, the pharmaceutical ingredient is tricaprilin. The amount of active pharmaceutical ingredient in the emulsion may range from about 10 percent by weight (wt%) to about 60 wt%. For example, the active pharmaceutical ingredient may be from about 10 wt% to about 15 wt%, from about 15 wt% to about 20 wt%, from about 20 wt% to about 25 wt%, about 25 wt% to about 30 wt%, from about 30 wt% to about 35 wt%, from about 35 wt% to about 40 wt%, from about 40 wt% to about 45 wt%, from about 45 wt% to about 50 wt%, from about 50 wt% to about 55 wt%, or from about 55 wt% to about 60 wt%.

[0026] In some aspects, the emulsion, comprises, consists essentially of, consists of tricaprilin, one or more emulsifiers, a buffer, a sweetener and/or a flavoring agent and optionally an alcohol,

[0027] In some aspects, the buffer comprises a physiologically safe buffer. In some aspects, the buffer maintains the pH stability without interfering with cellular functions, and enzyme activities. In some aspects, the buffer may comprise, consists essentially of, consist of a phosphate buffer (mixture of sodium dihydrogen phosphate and di-sodium hydrogen phosphate in an amount to get a desired pH, or pH adjusted with sodium hydroxide), a citrate

buffer, an acetate buffer, MOPS buffer (3-(N-morpholino)propanesulfonic acid), PIPES (Piperazine-N,N'-bis(2-ethanesulfonic acid)), MES (2-(N-morpholino)ethanesulfonic acid), or any combination thereof.

**[0028]** The buffer may have a concentration of from about 50 mM to about 200 mM, or about 50 mM to about 150 mM. For example, the concentration may range from about 50 mM to about 60 mM, from about 60 mM to about 70 mM, from about 70 mM to about 80 mM, from about 80 mM to about 90 mM, from about 90 mM to about 100 mM, or from about 110 mM to about 120 mM, or from about 120 mM to about 130 mM, or from about 130 mM to about 140 mM, or from about 140 mM to about 150 mM. The pH of the buffer may range from about 6.5 to about 8.0. For example, the pH may range from about 6.5 to about 6.6, from about 6.6 to about 6.7, from about 6.7 to about 6.8, from about 6.8 to about 6.9, from about 6.9 to about 7.0, from about 7.0 to about 7.1, from about 7.1 to about 7.2, from about 7.2 to about 7.3, from about 7.3 to about 7.4, from about 7.4 to about 7.5, from about 7.5 to about 7.6, from about 7.6 to about 7.7, from about 7.7 to about 7.8, from about 7.8 to about 7.9, or from about 7.9 to about 8.0. In some aspects, the buffer is a phosphate buffer. In some aspects, the phosphate buffer has a concentration of about 50 mM to about 200 mM, or about 50 mM to about 110 mM. For example, the concentration may range from about 50 mM to about 60 mM, from about 60 mM to about 70 mM, from about 70 mM to about 80 mM, from about 80 mM to about 90 mM, from about 90 mM to about 100 mM, or from about 110 mM to about 120 mM. The pH of the phosphate buffer may range from about 6.5 to about 8.0. For example, the pH may range from about 6.5 to about 6.6, from about 6.6 to about 6.7, from about 6.7 to about 6.8, from about 6.8 to about 6.9, from about 6.9 to about 7.0, from about 7.0 to about 7.1, from about 7.1 to about 7.2, from about 7.2 to about 7.3, from about 7.3 to about 7.4, from about 7.4 to about 7.5, from about 7.5 to about 7.6, from about 7.6 to about 7.7, from about 7.7 to about 7.8, from about 7.8 to about 7.9, or from about 7.9 to about 8.0.

**[0029]** In some aspects, the one or more emulsifiers comprise phospholipids, macroglycerol hydroxystearate, citric acid ester of mono- and diglycerides, sodium salt of oleic acid, or a combination thereof. In some aspects, the one or more emulsifiers may include Phospholipon® 90G, Kolliphor® RH40, citrem, sodium oleate, or a combination thereof. In some aspects, the emulsifier comprises Phospholipon® 90G.

**[0030]** Phospholipon® 90G is a highly purified phospholipid derived from soybean lecithin, primarily composed of phosphatidylcholine with a content of at least 94%. It is available in a granulated form and is commonly used in various pharmaceutical and cosmetic applications due to its emulsifying, solubilizing, and stabilizing properties. Phospholipon® 90G is identified by the CAS number 97281-47-5. Alternate chemical names include phosphatidylcholine (soy), lecithin (soy), lecithinon, phospholutein, PtdCho.

**[0031]** In some aspects, the emulsifier comprises Kolliphor® RH40. Kolliphor® RH40 is a nonionic solubilizer and emulsifying agent produced by BASF. It is derived from the reaction of hydrogenated castor oil with ethylene oxide, specifically 1 mole of hydrogenated castor oil combined with 40 moles of ethylene oxide. Alternate chemical names include: polyoxyl 40 hydrogenated Castor Oil (USP), macrogolglycerol hydroxystearate and has a CAS Number: 61788-85-0. It forms a white to yellowish paste at 20 °C, has a HLB value: Between 14 and 16 and is generally odorless and tasteless in aqueous solutions.

**[0032]** In some aspects, the emulsifier comprises citrem. Citrem (short for citrate ester of mono- and diglycerides) is an emulsifier commonly used in food and pharmaceutical industries. It is derived from the esterification of citric acid with mono- and diglycerides of fatty acids, which are typically derived from vegetable oils. The CAS number for Citrem is sourced by various entries due to the diversity of possible formulations and ester compositions.

**[0033]** In some aspects, the sweetener may be acesulfame potassium, advantame, aspartame, saccharin, sucralose, Luo han guo, purified stevia leaf extracts, or a combination thereof. In some aspects, the sweetener is sucralose, stevia, or any combination thereof.

**[0034]** In some aspects, the alcohol may be a triol. Suitable triols include but are not limited to glycerol, 1,2,3-butanetriol, trimethylolpropane, erythritol, and triethanolamine. In some aspects, the triol is glycerol.

**[0035]** The flavor may be oil-based. In some aspects, the flavor may include vanilla, mango, or berry. In some aspects, the flavor may be vanilla. In some aspects, the flavor may be Intense Vanilla Flavor (Sensient®). In some aspects, the flavor may be berry. In some aspects, the flavor may be Sensient® Berry.

**[0036]** The total amount of one or more emulsifiers in the emulsion may range from about 0.1 wt% to about 10 wt% or about 2 wt% to about 8 wt%. For example, the of one or more emulsifiers may be from about 2 wt% to about 2.5 wt%, from about 2.5 wt% to about 3.0 wt%, from about 3.0 wt% to about 3.5 wt%, from about 4.0 wt% to about 4.5 wt%, from about 4.5 wt% to about 5.0 wt%, from about 5.0 wt% to about 5.5 wt%, from about 5.5 wt% to about 6.0 wt%, from about 6.5 wt% to about 7.0 wt%, from about 7.0 wt% to about 7.5 wt%, or from about 7.5 wt% to about 8.0 wt%.

**[0037]** The amount of phosphate buffer in the emulsion may range from about 40 percent by wt% to about 80 wt%. For example, the phosphate buffer may be from about 40 wt% to about 45 wt%, from about 45 wt% to about 50 wt%, from about 50 wt% to about 55 wt%, about 55 wt% to about 60 wt%, from about 60 wt% to about 65 wt%, from about 65 wt% to about 70 wt%, from about 70 wt% to about 75 wt%, or from about 75 wt% to about 80 wt%.

**[0038]** In some aspects, the flavor may be present an amount of about 0.1 wt% to about 0.4 wt%. For example, the flavor may be present in an amount of about 0.1 wt%, about 0.15 wt%, about 0.2 wt%, about 0.25 wt%, about 0.3 wt%, about 0.35 wt%, about 0.4 wt%.

**[0039]** In some aspects, the vanilla may be present in an amount of about 0.01 wt% to about 0.2 wt%. For example, the vanilla may be present in an amount of about 0.1 wt%, about 0.11 wt%, about 0.12 wt%, about 0.13 wt%, about 0.14 wt%, about 0.15 wt%, about 0.16 wt%, about 0.17 wt%, about 0.18 wt%, about 0.19 wt%, or about 0.2 wt%.

**[0040]** In some aspects, the sweetener may be present in an amount of about 0.01 wt% to about 0.1 wt%. For example, the sweetener may be present in an amount of about 0.01 wt%, about 0.02 wt%, about 0.03 wt%, about 0.04 wt%, about 0.05 wt%, about 0.06 wt%, about 0.07 wt%, about 0.08 wt%, about 0.09 wt%, or about 0.1 wt%.

**[0041]** In some aspects, the emulsion may be stable over a period of 3 months at about 2 °C to about 8 °C, about 25 °C, or about 40 °C.

**[0042]** In some aspects, the emulsion includes about 50 wt% of tricaprilin, about 4 wt% of Phospholipon® 90G, about 2 wt% of Kolliphor® RH40, about 2.5 wt% of glycerol, and about 50 mM phosphate buffer. In some aspects, the emulsion includes about 50 wt% of tricaprilin, 4 wt% of Phospholipon® 90G, about 2 wt% of Kolliphor® RH40, about 2.5 wt% of glycerol, about 50 mM phosphate buffer, and about 0.01 wt% to about 0.1 wt% of sweetener. For example, the amount of sweetener may be about 0.01 wt%, about 0.02 wt%, about 0.03 wt%, about 0.04 wt%, about 0.05 wt%, about 0.06 wt%, about 0.07 wt%, about 0.08 wt%, about 0.09 wt%, or about 0.1 wt%. The sweetener may be sucralose, stevia, or any combination thereof. In some aspects, the emulsion includes about 50 wt% of tricaprilin, about 4 wt% of Phospholipon® 90G, about 2 wt% of kolliphor RH40, about 2.5 wt% of glycerol, about 50 mM phosphate buffer, about 0.01 wt% to about 0.1 wt% of sweetener, and about 0.1 wt% to about 0.3 wt% of a flavor. For example, the flavor may be about 0.1 wt%, about 0.11 wt%, about 0.12 wt%, about 0.13 wt%, about 0.14 wt%, about 0.15 wt%, about 0.16 wt%, about 0.17 wt%, about 0.18 wt%, about 0.19 wt%, about 0.20 wt%, about 0.21 wt%, about 0.22 wt%, about 0.23 wt%, about 0.24 wt%, about 0.25 wt%, about 0.26 wt%, about 0.27 wt%, about 0.28 wt%, about 0.29 wt%, or about 0.30 wt%. The flavor may be oil-soluble.

**[0043]** In some aspects, the emulsion includes about 50 wt% of tricaprilin, about 4 wt% of Phospholipon® 90G, about 2 wt% of Kolliphor® RH40, about 2.5 wt% of glycerol, and about 100 mM phosphate buffer. In some aspects, the emulsion includes about 50 wt% of tricaprilin, about 4 wt % of Phospholipon® 90G, about 2 wt % of Kolliphor® RH40, about 2.5 wt% of glycerol, about 100 mM phosphate buffer, and about 0.01 wt% to about 0.1 wt% of sweetener. For example, the amount of sweetener may be about 0.01 wt%, about 0.02 wt%, about 0.03

wt%, about 0.04 wt%, about 0.05 wt%, about 0.06 wt%, about 0.07 wt%, about 0.08 wt%, about 0.09 wt%, or about 0.1 wt%. The sweetener may be sucralose, stevia, or any combination thereof. In some aspects, the emulsion includes about 50 wt% of tricaprilin, about 4 wt% of Phospholipon® 90G, about 2 wt% of Kolliphor® RH40, about 2.5 wt% of glycerol, about 100 mM phosphate buffer, about 0.01 wt% to about 0.1 wt% of sweetener, and about 0.1 wt% to about 0.3 wt% of a flavor. For example, the flavor may be about 0.1 wt%, about 0.11 wt%, about 0.12 wt%, about 0.13 wt%, about 0.14 wt%, about 0.15 wt%, about 0.16 wt%, about 0.17 wt%, about 0.18 wt%, about 0.19 wt%, about 0.20 wt%, about 0.21 wt%, about 0.22 wt%, about 0.23 wt%, about 0.24 wt%, about 0.25 wt%, about 0.26 wt%, about 0.27 wt%, about 0.28 wt%, about 0.29 wt%, or about 0.30 wt%. The flavor may be oil-soluble.

**[0044]** In some aspects, the phosphate buffer is 100 mM phosphate buffer and the flavor is vanilla present in a concentration of about 0.1 wt%.

**[0045]** In some aspects, the phosphate buffer is 50 mM phosphate buffer and the flavor is vanilla present in a concentration of about 0.2 wt%.

**[0046]** Table 1 and Table 2 show the composition of two different emulsions.

**Table 1:** Emulsion 1

| <b>Ingredient</b>                | <b>Amount per g</b> |
|----------------------------------|---------------------|
| Tricaprilin                      | 500 mg              |
| Phospholipon® 90G                | 40 mg               |
| Kolliphor® RH40                  | 20 mg               |
| Glycerol                         | 25 mg               |
| Sucralose                        | 0.5 mg              |
| Vanilla Flavor                   | 1 mg                |
| Phosphate Buffer, 100 mM, pH 6.8 | 413.5 mg            |

**Table 2:** Emulsion 2

| <b>Ingredient</b>               | <b>Amount per g</b> |
|---------------------------------|---------------------|
| Tricaprilin                     | 500 mg              |
| Phospholipon 90G                | 40 mg               |
| Kolliphor® RH40                 | 20 mg               |
| Glycerol                        | 25 mg               |
| Sucralose                       | 0.5 mg              |
| Phosphate Buffer, 50 mM, pH 6.8 | 412.5 mg            |

**[0047]** The percent of flavor in the active or control may affect the stability of the emulsion. In some aspects, lowering the percent of flavor (e.g. from about 0.2 wt% to about 0.1 wt% for

active and from 0.4 wt% to 0.2 wt% for control) may improve the stability of the emulsion. The concentration of phosphate buffer may affect the stability of the emulsion. In some aspects, the emulsions prepared with 100 mM buffer strength may display improved long-term stability than emulsions prepared with 50 mM buffer strength but otherwise equivalent composition.

**[0048]** The appearance of the emulsion may be white to off white with no or easily reversible phase separation. The pH of the emulsion may be from about 5.5 to about 7.5. For example, the pH may be about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, or about 7.5. The total aerobic microbial count of the emulsion may be less than 1000 cfu/g. The total combined yeasts and molds count may be less than 100 cfu/g. The total impurities in the emulsion, as measured by gas chromatography may be less than 3.5% by weight. The impurities include but are not limited to glyceryl-dioctanoate, glyceryl-monohexanoate-dioctanoate, glyceryl-monoheptanoate-dioctanoate, glyceryl-monononanoate-dioctanoate, glyceryl-monodecanoate-dioctanoate, glyceryl-didecanoate-monooctanoate, glyceryl-tridecanoate, 1,1-diglyceryl-tetraoctanoate, 1,2-diglyceryl-tetraoctanoate, 2,2-diglyceryl-tetraoctanoate, or a combination thereof.

**[0049]** Table 3 shows the release specification of an exemplary emulsion.

**Table 3:** Release and Stability Specification

| TEST                                        | METHOD | ACCEPTANCE CRITERIA                                                                                               |
|---------------------------------------------|--------|-------------------------------------------------------------------------------------------------------------------|
| Appearance                                  | Visual | White to off white emulsion with no or easily reversible phase separation                                         |
| Identification                              | GC     | Retention time of principal peak from sample preparation is within 2% from that of reference standard preparation |
| Assay                                       | GC     | 90% to 110% of label claim                                                                                        |
| Impurities and Related Substances (% area)  | GC     | NMT 1.5%                                                                                                          |
| Glyceryl-dioctanoate (Imp 1)                |        |                                                                                                                   |
| Glyceryl-monohexanoate-dioctanoate (Imp 2)  |        |                                                                                                                   |
| Glyceryl-monoheptanoate-dioctanoate (Imp 3) |        |                                                                                                                   |
| Glyceryl-monononanoate-dioctanoate (Imp 4)  |        |                                                                                                                   |
|                                             |        | NMT 3.0%                                                                                                          |

|                                                                                                                                                                                                                                                                                                                |                             |                                                     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|-----------------------------------------------------|
| Glyceryl-monodecanoate-dioctanoate (Imp 5)<br>Glyceryl-didecanoate-monooctanoate (Imp 6)<br>Glyceryl-tridecanoate (Imp 7)<br><br>Imp 8 - Sum of:<br>1, 1-Diglyceryl-tetraoctanoate<br>1,2-Diglyceryl-tetraoctanoate<br>2,2-Diglyceryl-tetraoctanoate<br><br>Any unspecified impurities<br><br>Total impurities |                             | NMT 0.4%<br><br>NMT 0.2%<br><br>NMT 3.5%            |
| pH                                                                                                                                                                                                                                                                                                             | USP <791><br>Ph Eur 2.2.3   | 5.5-7.5                                             |
| Particle Size                                                                                                                                                                                                                                                                                                  | USP <429>                   | Report results for $d_{10}$ , $d_{50}$ and $d_{90}$ |
| Microbial Limits:<br>Total Aerobic Microbial Count (TAMC)<br><br>Total Combined Yeasts and Molds Count (TYMC)                                                                                                                                                                                                  | USP <61> /<br>Ph Eur 2.6.12 | $\leq 1000$ cfu/g<br><br>$\leq 100$ cfu/g           |
| Specified Microorganisms:<br><br><i>E. coif</i>                                                                                                                                                                                                                                                                | USP <62> /<br>Ph Eur 2.6.13 | Absent in 1 g                                       |

**[0050]** In some aspects, the emulsion formulation starting with a pH of 6.5 at 25 °C may be more stable. In other aspects, the emulsion formulation starting with a pH of 7.8 at 25 °C may be the least stable.

**[0051]** In some aspects, the emulsion formulation starting with a pH of 6.5 at 40 °C may start to drop off at 3 months. This drop off may be most likely as a result of the pH moving away from the pKa of the phosphate buffer ( $pK_a \approx 6.8$ ) where the buffer may be most effective. In some aspects, the optimal pH may be slightly higher than 6.8 to optimize pH stability and avoid the drop off observed at 3 months.

**[0052]** In some aspects, the most stable emulsion formulations may have a low flavor level. In other aspects, the least stable emulsion formulations may have a high flavor level.

**[0053]** In some aspects, vanilla and berry flavored formulations may behave similarly and there may be no clear difference in pH stability with respect to flavor type.

**[0054]** In some aspects, the most stable emulsion formulations may have a high buffer strength. In some aspects, the least stable formulations may have a low buffer strength. In some aspects, both buffer strength and flavor may be important to pH stability.

**[0055]** Emulsion formulations with higher buffer strength, lower flavor levels, and a starting pH of 6.8 may improve pH stability.

**[0056]** Some exemplary emulsions are listed below:

**[0057]** In an aspect 1, disclosed herein is an emulsion comprising: about 20%-60% by weight of tricaprilin; about 0.1-10% by weight of emulsifier; about 0.1-10% by weight of glycerol; and about 10 mM - 200 mM phosphate buffer (pH 6.0-7.5).

**[0058]** In an aspect 2, the emulsion comprises: about 50% by weight of tricaprilin; about 4% by weight of Phospholipon® 90G; about 2% by weight of Kolliphor® RH40; about 2.5% by weight of glycerol; and about 20 mM - 100 mM phosphate buffer (pH 6.8).

**[0059]** In an aspect 3, the emulsion comprises: about 500 mg tricaprilin; about 40 mg Phospholipon® 90G; about 20 mg Kolliphor® RH40; about 25 mg glycerol; and about 415 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0060]** In an aspect 4, the emulsion comprises: about 500 mg tricaprilin; about 40 mg Phospholipon® 90G; about 20 mg Kolliphor® RH40; and about 440 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0061]** In an aspect 5, the emulsion comprises: about 400 mg tricaprilin; about 21.3 mg Phospholipon® 90G; about 10.7 mg Kolliphor® RH40; about 25 mg glycerol; and about 543 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0062]** In an aspect 6, the emulsion comprises: about 400 mg tricaprilin; about 32 mg Phospholipon® 90G; about 16 mg Kolliphor® RH40; and about 552 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0063]** In an aspect 7, the emulsion comprises: about 400 mg tricaprilin; about 21.3 mg Phospholipon® 90G; about 10.7 mg Kolliphor® RH40; and about 568 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0064]** In an aspect 8, the emulsion comprises: about 400 mg tricaprilin; about 32 mg Phospholipon® 90G; about 16 mg citrem; about 25 mg glycerol; and about 527 mg phosphate buffer (50 mM, pH 7 per gram of the emulsion).

**[0065]** In an aspect 9, the emulsion comprises: about 200 mg tricaprilin; about 16 mg Phospholipon® 90G; about 8 mg Kolliphor® RH40; about 25 mg glycerol; and about 751 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0066]** In an aspect 10, the emulsion comprises: about 200 mg tricaprilin; about 24 mg Phospholipon® 90G; about 2 mg Kolliphor® RH40; about 5 mg sodium oleate; about 25 mg glycerol; and about 744 mg phosphate buffer (50 mM, pH 8.0) per gram of the emulsion.

**[0067]** In an aspect 11, the emulsion comprises: about 400 mg tricaprilin; about 32 mg Phospholipon® 90G; about 16 mg Kolliphor® RH40; about 25 mg glycerol; and about 527 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0068]** In an aspect 12, the emulsion comprises: about 200 mg tricaprilin; about 16 mg Phospholipon® 90G; about 8 mg Kolliphor® RH40; and about 776 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0069]** In an aspect 13, the emulsion of any one of aspects 1-12, further comprises: 0.1% to about 1% by weight of sweetener, wherein the amount in grams of phosphate buffer is reduced to accommodate the amount of sweetener added.

**[0070]** In an aspect 14, the emulsion of aspects 1-13, further comprises: 0.1%-0.3% of a flavoring agent, wherein the amount in grams of phosphate buffer is reduced to accommodate the amount of flavoring agent added.

**[0071]** In an aspect 15, the emulsion comprises: about 500 mg tricaprilin; about 40 mg Phospholipon® 90G; about 20 mg Kolliphor® RH40; about 25 mg glycerol; about 0.5 mg of sucralose; about 2 mg of vanilla flavor; and about 412.5 mg phosphate buffer (50 mM, pH 6.8) per gram of the emulsion.

**[0072]** In an aspect 16, the emulsion comprises: about 500 mg tricaprilin; about 40 mg Phospholipon® 90G; about 20 mg Kolliphor® RH40; about 25 mg glycerol; about 0.5 mg of sucralose; about 1 mg of vanilla flavor; and about 413.5 mg phosphate buffer (100 mM, pH 6.8) per gram of the emulsion.

**[0073]** In an aspect 17, the emulsion comprises: about 500 mg tricaprilin; about 40 mg Phospholipon® 90G; about 20 mg Kolliphor® RH40; about 25 mg glycerol; about 0.5 mg of sucralose; about 1 mg of vanilla flavor; and about 413.5 mg phosphate buffer (50 mM, pH 7.0) per gram of the emulsion.

## II. Method of making emulsion formulation

**[0074]** The present disclosure also relates to a method of making an emulsion. The emulsion may include tricaprilin. The method includes feeding a buffer, tricaprilin, and one or more emulsifiers to a vessel; high shear mixing the phosphate buffer, tricaprilin, and one or more emulsifiers in the vessel to form a course emulsion; sterilizing the course emulsion to obtain a sterile emulsion; and high pressure homogenizing (HPH) the sterile emulsion to form the emulsion comprising tricaprilin. In some aspects, the sterilizing of the emulsion may comprise, consists of, or exclude an irradiation process, for example gamma radiation or X-ray irradiation. In some aspects, the sterilization process may comprise, consist of, or exclude a ultra-high temperature (UTH) treatment.

**[0075]** FIGs. 1-3, 7, 9 and 11 illustrate some processes of making emulsions of the present disclosure. The first step of the process includes high shear mixing the phosphate buffer, tricaprilin, and one or more emulsifiers in the vessel to form a course emulsion. In some aspects, the high shear mixing may be accomplished by adding water, sodium dihydrogen phosphate dihydrate into a vessel. The pH of the vessel may be adjusted to about 6.8 using an about 2M solution of sodium hydroxide. Next, one or more emulsifiers may be added to the phosphate buffer. In some aspects, alcohol may be added to the phosphate buffer. The alcohol may be a triol. In some aspects, the triol may be glycerin. The active pharmaceutical or control ingredient may be added to the vessel. The mixture may be mixed in-line by high shear mixing or by an overhead high shear mixer at about 10,000 rpm resulting in a course emulsion. The temperature at which the high shear mixing may be performed ranges from about 15 °C to about 45 °C. The particle size of the resulting course emulsion may be from about 1 µm to about 10 µm. In some aspects, the particle size may range from about 1 µm to about 5 µm

**[0076]** In some aspects, the next step of the process may include a sterilization step. In some aspects, the sterilization step is a ultra-heat treatment (UHT) step. The UHT conditions may include heating the course mixture at a temperature of about 125 °C to about 150 °C for about 5 seconds to about 60 seconds. In some aspects, the temperature may be from about 125 °C to about 130 °C, from about 130 °C to about 135 °C, from about 135 °C to about 140 °C, from about 140 °C to about 145 °C, or from about 145 °C to about 150 °C. In some aspects, the time duration may be from about 5 seconds to about 10 seconds, from about 10 seconds to about 15 seconds, from about 15 seconds to about 20 seconds, from about 20 seconds to about 25 seconds, from about 25 seconds to about 30 seconds, from about 30 seconds to about 35 seconds, from about 35 seconds to about 40 seconds, from about 40 seconds to about 45 seconds, from about 45 seconds to about 50 seconds, from about 50 seconds to about 55 seconds, or from about 55 seconds to about 60 seconds. In some aspects, UHT

treatment is performed at 135 °C for 2.35 seconds. In some aspects, UHT treatment is performed at 135 °C for 5 seconds.

**[0077]** In some aspects, the next step of the process includes high pressure homogenizing (HPH) the ultra-heat-treated course emulsion to form a fine emulsion. In some aspects, the high pressure may be between 5,000 PSI to about 10,000 PSI. In the some aspects, the high pressure may be at least 5,000, at least 5,500, at least 6,000, at least 6,500, at least 7,000, at least 7,500, at least 8,000, at least 8,500, at least 9,000, at least 9,500 or at least 10,000 PSI. In some aspects the high pressure may be around 7,500 PSI. The particle size of the fine emulsion may range from about 0.05 µm to about 1 µm. In some aspects, the particle size of the fine emulsion may be less than 0.4 µm. HPH may be performed one or more times until the desired particle size may be achieved. For example, the number of HPH passes may be from 1 pass to about 10 passes.

**[0078]** In some aspects, the last step of the process includes filling the fine emulsions into suitable glass vials. The glass vials include but are not limited to 30 mL bottles or 100 mL amber glass bottles. In some aspects, the vials may be filled in a bio cabinet to avoid microbial contamination. In some aspects, gamma radiation may be performed to the emulsion. In other aspects, gamma radiation may not be performed to the emulsion. Gamma radiation may have a negative impact on the taste of the resulting emulsion.

**[0079]** In some aspects, the glass vials may be filled under laminar flow. In some aspects, the bottles and caps may undergo gamma radiation and the glass vials may be filled in a bio cabinet. In some aspects, the bottles and caps may undergo gamma radiation and the glass vials may be filled under laminar flow.

**[0080]** In some aspects, the method also encompasses adding one or more sweeteners and/or flavoring agents during the manufacturing process, In some aspects, the one or more sweeteners and/or flavoring agents may be added at any step during the manufacturing process. In some aspects, the one or more sweeteners and/or flavoring agents may be added during, or before the feeding step, the high sheer mixing step, or the high pressure homogenizing step. In some aspects, the one or more sweeteners and/or flavoring agents may be added prior to the bottling step. In such aspects, the one or more sweeteners and/or flavoring agents may be independently sterilized.

### **III. Method of using the emulsion**

**[0081]** In certain aspects, the current disclosure relates to methods of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition of the disclosure in an amount effective to elevate ketone body concentrations in said subject to thereby treat said disease or disorder. In certain aspects, the

pharmaceutical composition of the disclosure may be administered outside of the context of a ketogenic diet. For instance, in the context of the present disclosure, carbohydrates may be consumed at the same time as pharmaceutical compositions disclosed herein.

**[0082]** In accordance with certain aspects of the disclosure, the disease or disorder is a neurological disease or disorder, for example a disease associated with reduced cognitive function, reduced neuronal metabolism, or infantile spasms.

**[0083]** In some aspects, the diseases and disorders may include diseases and disorders associated with reduced cognitive function; for example Age-Associated Memory Impairment (AAMI), Alzheimer's Disease (AD), Parkinson's Disease, Friedreich's Ataxia (FRDA), GLUT1-deficient Epilepsy, Leprechaunism, and Rabson-Mendenhall Syndrome, Coronary Arterial Bypass Graft (CABG) dementia, anesthesia-induced memory loss, Huntington's Disease, migraine and related headaches, and many others. In another aspect, the patient has or is at risk of developing disease-related reduced cognitive function caused by reduced neuronal metabolism, for example, reduced cognitive function associated with Alzheimer's Disease (AD), Parkinson's Disease, Friedreich's Ataxia (FRDA), GLUT1-deficient Epilepsy, Leprechaunism, and Rabson-Mendenhall Syndrome, Coronary Arterial Bypass Graft (CABG) dementia, anesthesia-induced memory loss, Huntington's Disease, and many others.

**[0084]** As used herein, reduced neuronal metabolism refers to all possible mechanisms that could lead to a reduction in neuronal metabolism. Such mechanisms include, but are not limited to mitochondrial dysfunction, free radical attack, generation of reactive oxygen species (ROS), ROS-induced neuronal apoptosis, defective glucose transport or glycolysis, imbalance in membrane ionic potential, dysfunction in calcium flux, and the like.

**[0085]** According to the present invention, high blood ketone levels will provide an energy source for brain cells that have compromised glucose metabolism, leading to improved performance in cognitive function. As used herein, "subject" and "patient" are used interchangeably, and refer to any mammal, including humans that may benefit from treatment of disease and conditions associated with or resulting from reduced neuronal metabolism.

**[0086]** "Effective amount" refers to an amount of a compound, material, or pharmaceutical composition, as described herein that is effective to achieve a particular biological result. Effectiveness for treatment of the aforementioned conditions may be assessed by improved results from at least one neuropsychological test. These neuropsychological tests are known in the art and include Clinical Global Impression of Change (CGIC), Rey Auditory Verbal Learning Test (RAVLT), First-Last Names Association Test (FLN), Telephone Dialing Test (TDT), Memory Assessment Clinics Self-Rating Scale (MAC-S), Symbol Digit Coding (SDC), SDC Delayed Recall Task (DRT), Divided Attention Test (DAT), Visual Sequence Comparison

(VSC), DAT Dual Task (DAT Dual), Mini-Mental State Examination (MMSE), and Geriatric Depression Scale (GDS), among others.

**[0087]** The term "cognitive function" refers to the special, normal, or proper physiologic activity of the brain, including, without limitation, at least one of the following: mental stability, memory/recall abilities, problem solving abilities, reasoning abilities, thinking abilities, judging abilities, capacity for learning, perception, intuition, attention, and awareness.

**[0088]** "Enhanced cognitive function" or "improved cognitive function" refers to any improvement in the special, normal, or proper physiologic activity of the brain, including, without limitation, .. at least one of the following: mental stability, memory/recall abilities, problem solving abilities, reasoning abilities, thinking abilities, judging abilities, capacity for learning, perception, intuition, attention, and awareness, as measured by any means suitable in the art. "Reduced cognitive function" or "impaired cognitive function" refers to any decline in the special, normal, or proper physiologic activity of the brain.

**[0089]** In another aspect, the methods of the present invention further comprise determination of the patients' genotype or particular alleles. In one aspect, the patient's alleles of the apolipoprotein E gene are determined. It has been found that non-E4 carriers performed better than those with the E4 allele when elevated ketone body levels were induced with MCT. Also, those with the E4 allele had higher fasting ketone body levels and the levels .. continued to rise at the two hour time interval. Therefore, E4 carriers may require higher ketone levels or agents that increase the ability to use the ketone bodies that are present.

**[0090]** In one aspect, the pharmaceutical compositions of the disclosure are administered orally. Therapeutically effective amounts of the therapeutic agents can be any amount or dose sufficient to bring about the desired effect and depend, in part, on the severity and stage of the condition, the size and condition of the patient, as well as other factors readily known to those skilled in the art. The dosages can be given as a single dose, or as several doses, for example, divided over the course of several weeks, as discussed elsewhere herein.

**[0091]** The pharmaceutical compositions of the disclosure, in one aspect, are administered in a dosage required to increase blood ketone bodies to a level required to treat and/or prevent the occurrence of any disease- or age-associated cognitive decline, such as AD, AAMI, and the like. Appropriate dosages may be determined by one of skill in the art.

**[0092]** In one aspect, oral administration of a pharmaceutical composition of the disclosure results in hyperketonemia. Hyperketonemia, in one aspect, results in ketone bodies being utilized for energy in the brain even in the presence of glucose.

**[0093]** Additionally, hyperketonemia results in a substantial (39%) increase in cerebral blood flow (Hasselbalch, S.G., et al., Changes in cerebral blood flow and carbohydrate metabolism during acute hyperketonemia, *Am J Physiol*, 1996, 270:E746-51). Hyperketonemia has been reported to reduce cognitive dysfunction associated with systemic hypoglycemia in normal humans (Veneman, T., et al., Effect of hyperketonemia and hyperlacticacidemia on symptoms, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia in normal humans, *Diabetes*, 1994, 43:1311-7). Please note that systemic hypoglycemia is distinct from the local defects in glucose metabolism that occur in any disease- or age-associated cognitive decline, such as AD, AAMI, and the like.

**[0094]** Administration can be on an as-needed or as-desired basis, for example, once-monthly, once-weekly, daily, or more than once daily. Similarly, administration can be every other day, week, or month, every third day, week, or month, every fourth day, week, or month, and the like. Administration can be multiple times per day. When utilized as a supplement to ordinary dietetic requirements, the composition may be administered directly to the patient or otherwise contacted with or admixed with daily feed or food.

**[0095]** The pharmaceutical compositions provided herein are, in one aspect, intended for "long term" consumption, sometimes referred to herein as for 'extended' periods.

**[0096]** "Long term" administration as used herein generally refers to periods in excess of one month.

**[0097]** Periods of longer than two, three, or four months comprise one aspect of the instant .. invention. Also included are aspects comprising more extended periods that include longer than 5, 6, 7, 8, 9, or 10 months. Periods in excess of 11 months or 1 year are also included. Longer terms use extending over 1, 2, 3 or more years are also contemplated herein.

**[0098]** "Regular basis" as used herein refers to at least weekly, dosing with or consumption of the compositions. More frequent dosing or consumption, such as twice or thrice weekly are included. Also included are regimens that comprise at least once daily consumption. The skilled artisan will appreciate that the blood level of ketone bodies, or a specific ketone body, achieved may be a valuable measure of dosing frequency. Any frequency, regardless of whether expressly exemplified herein, that allows maintenance of a blood level of the measured compound within acceptable ranges can be considered useful herein. The skilled artisan will appreciate that dosing frequency will be a function of the composition that is being consumed or administered, and some compositions may require more or less frequent administration to maintain a desired blood level of the measured compound (e.g., a ketone body).

**[0099]** Administration can be carried out on a regular basis, for example, as part of a treatment regimen in the patient. A treatment regimen may comprise causing the regular ingestion by the patient of a pharmaceutical composition of the disclosure in an amount effective to enhance cognitive function, memory, and behavior in the patient.

**[0100]** Regular ingestion can be once a day, or two, three, four, or more times per day, on a daily or weekly basis.

**[0101]** Similarly, regular administration can be every other day or week, every third day or week, every fourth day or week, every fifth day or week, or every sixth day or week, and in such a regimen, administration can be multiple times per day. The goal of regular administration is to provide the patient with optimal dose of a pharmaceutical composition of the disclosure, as exemplified herein.

**[0102]** Dosages of the disclosed compositions, such as, for example, those comprising MCT, may be administered in an effective amount to increase the cognitive ability of patients afflicted with diseases of reduced neuronal metabolism, such as in patients with any disease- or age-associated cognitive decline, such as, AD, AAMI, and the like.

**[0103]** In one aspect, the disclosed compositions result in elevating ketone concentrations in the body, and in this aspect, the compositions are administered in an amount that is effective to induce hyperketonemia. In one aspect, hyperketonemia results in ketone bodies being utilized for energy in the brain.

**[0104]** In one aspect, the composition increases the circulating concentration of at least one type of ketone body in the mammal or patient. In one aspect, the circulating ketone body is D-beta-hydroxybutyrate. The amount of circulating ketone body can be measured at a number of times post administration, and in one aspect, is measured at a time predicted to be near the peak concentration in the blood, but can also be measured before or after the predicted peak blood concentration level. Measured amounts at these off-peak times are then optionally adjusted to reflect the predicted level at the predicted peak time. In one aspect, the predicted peak time is at about two hours. Peak circulating blood level and timing can vary depending on factors known to those of skill in the art, including individual digestive rates, co-ingestion or pre- or post-ingestion of foods, drinks, etc., as known to one of skill in the art. In one aspect, the peak blood level reached of D-beta-hydroxybutyrate is between about 0.05 millimolar (mM) to about 50 mM. Another way to determine whether blood levels of D-beta-hydroxybutyrate are raised to about 0.05 to about 50 mM is by measurement of D-beta-hydroxybutyrate urinary excretion a range in the range of about 5 mg/dL to about 160 mg/dL. In other aspects, the peak blood level is raised to about 0.1 to about 50 mM, from about 0.1 to about 20 mM, from about 0.1 to about 10 mM, to about 0.1 to about 5 mM, more preferably raised to about 0.15

to about 2 mM, from about 0.15 to about 0.3 mM, and from about 0.2 to about 5 mM, although variations will necessarily occur depending on the formulation and host, for example, as discussed above. In other aspects, the peak blood level reached of D-beta-hydroxybutyrate will be at least about 0.05 mM, at least about 0.1 mM, at least about 0.15 mM, at least about 0.2 mM, at least about 0.5 mM, at least about 1 mM, at least about 1.5 mM, at least about 2 mM, at least about 2.5 mM, at least about 3 mM, at least about 4 mM, at least about 5 mM, at least about 10 mM, at least about 15 mM, at least about 20 mM, at least about 30 mM, at least about 40 mM, and at least about 50 mM.

**[0105]** Effective amount of dosages of compounds for the disclosed compositions, i.e., compounds capable of elevating ketone body concentrations in an amount effective for the treatment of or prevention of loss of cognitive function caused by reduced neuronal metabolism will be apparent to those skilled in the art. As discussed herein above, such effective amounts can be determined in light of disclosed blood ketone levels. Where the compound capable of elevating ketone body concentrations is MCT, the MCT dose, in one aspect, is in the range of about 0.05 g/kg/day to about 10 g/kg/day of MCT. In other aspects, the dose will be in the range of about 0.25 g/kg/day to about 5 g/kg/day of MCT. In other aspects, the dose will be in the range of about 0.5 g/kg/day to about 2 g/kg/day of MCT. In other aspects, the dose will be in the range of about 0.1 g/kg/day to about 2 g/kg/day. In other aspects, the dose of MCT is at least about 0.05 g/kg/day, at least about 0.1 g/kg/day, at least about 0.15 g/kg/day, at least about 0.2 g/kg/day, at least about 0.5 g/kg/day, at least about 1 g/kg/day, at least about 1.5 g/kg/day, at least about 2 g/kg/day, at least about 2.5 g/kg/day, at least about 3 g/kg/day, at least about 4 g/kg/day, at least about 5 g/kg/day, at least about 10 g/kg/day, at least about 15 g/kg/day, at least about 20 g/kg/day, at least about 30 g/kg/day, at least about 40 g/kg/day, and at least about 50 g/kg/day.

**[0106]** As described herein, the present compositions are provided as a liquid formulation for administration to a subject in need thereof. The compositions may be advantageously combined and/or used in combination with other therapeutic or prophylactic agents, different from the disclosed MCT compounds. In many instances, administration in conjunction with the subject compositions enhances the efficacy of such agents. For example, the compounds may be advantageously used in conjunction with antioxidants, compounds that enhance the efficiency of glucose utilization, and mixtures thereof. **[0080]** The daily dose of MCT can also be measured in terms of grams of MCT per kg of body weight (BW) of the mammal. The daily dose of MCT can range from about 0.01 g/kg to about 10.0 g/kg BW of the mammal. Preferably, the daily dose of MCT is from about 0.1 g/kg to about 5 g/kg BW of the mammal. More preferably, the daily dose of MCT is from about 0.2 g/kg to about 3 g/kg of the mammal.

Still more preferably, the daily dose of MCT is from about 0.5 g/kg to about 2 g/kg of the mammal.

#### IV. Terminology

**[0107]** The phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. For example, the use of a singular term, such as, “a” is not intended as limiting of the number of items. Also, the use of relational terms such as, but not limited to, “top,” “bottom,” “left,” “right,” “upper,” “lower,” “down,” “up,” and “side,” are used in the description for clarity in specific reference to the figures and are not intended to limit the scope of the present disclosure or the appended claims.

**[0108]** Any term of degree such as, but not limited to, “substantially” as used in the description and the appended claims, should be understood to include an exact, or a similar, but not exact configuration. For example, “a substantially planar surface” means having an exact planar surface or a similar, but not exact planar surface. Similarly, the terms “about” or “approximately,” as used in the description and the appended claims, should be understood to include the recited values or a value that is three times greater or one third of the recited values. For example, about 3 mm includes all values from 1 mm to 9 mm, and approximately 50 degrees includes all values from 16.6 degrees to 150 degrees. For example, they can refer to less than or equal to  $\pm 5\%$ , such as less than or equal to  $\pm 2\%$ , such as less than or equal to  $\pm 1\%$ , such as less than or equal to  $\pm 0.5\%$ , such as less than or equal to  $\pm 0.2\%$ , such as less than or equal to  $\pm 0.1\%$ , such as less than or equal to  $\pm 0.05\%$ .

**[0109]** The terms “comprising,” “including,” and “having” are used interchangeably in this disclosure. The terms “comprising,” “including,” and “having” mean to include, but not necessarily be limited to the things so described.

**[0110]** The terms “or” and “and/or,” as used herein, are to be interpreted as inclusive or meaning any one or any combination. Therefore, “A, B, or C” or “A, B, and/or C” mean any of the following: “A,” “B,” or “C”; “A and B”; “A and C”; “B and C”; “A, B, and C.” An exception to this definition will occur only when a *combination* of elements, functions, steps, or acts are in some way inherently mutually exclusive.

**[0111]** Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this disclosure belongs. The following references provide one of skill with a general definition of many of the terms used in this disclosure: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991), all of which are incorporated by

reference herein. As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

**[0112]** The phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. When introducing elements of the present disclosure or the preferred aspects(s) thereof, the articles “a,” “an,” “the,” and “said” are intended to mean that there are one or more of the elements. The terms “comprising,” “including,” and “having” are intended to be inclusive and mean that there may be additional elements other than the listed elements. Wherever the terms “comprising” or “including” are used, it should be understood the disclosure also expressly contemplates and encompasses additional aspects “consisting of” the disclosed elements, in which additional elements other than the listed elements are not included.

**[0113]** The term “about” or “approximately,” as used herein, can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term “about” can mean an acceptable error range for the particular value, such as 10% of the value modified by the term “about.” As used herein, the term “about,” can mean relative to the recited value, e.g., amount, dose, temperature, time, percentage, etc.,  $\pm 10\%$ ,  $\pm 9\%$ ,  $\pm 8\%$ ,  $\pm 7\%$ ,  $\pm 6\%$ ,  $\pm 5\%$ ,  $\pm 4\%$ ,  $\pm 3\%$ ,  $\pm 2\%$ , or  $\pm 1\%$ .

**[0114]** Further, as the present disclosure is susceptible to aspects of many different forms, it is intended that the present disclosure be considered as an example of the principles of the present disclosure and not intended to limit the present disclosure to the specific aspects shown and described. Any one of the features of the present disclosure may be used separately or in combination with any other feature. References to the terms “aspect,” “aspects,” and/or the like in the description mean that the feature and/or features being referred to are included in, at least, one aspect of the description. Separate references to the terms “aspect,” “aspects,” and/or the like in the description do not necessarily refer to the same aspect and are also not mutually exclusive unless so stated and/or except as will be readily apparent to those skilled in the art from the description. For example, a feature, structure, process, step, action, or the like described in one aspect may also be included in other aspects but is not necessarily included. Thus, the present disclosure may include a variety of combinations and/or integrations of the aspects described herein. Additionally, all aspects of the present disclosure, as described herein, are not essential for its practice. Likewise, other systems, methods, features, and advantages of the present disclosure will be, or become,

apparent to one with skill in the art upon examination of the figures and the description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present disclosure, and be encompassed by the claims.

[0115] As a person skilled in the art will recognize from the detailed description and from the figures and claims, modifications and changes may be made to the aspects of the disclosure without departing from the scope of this disclosure as defined in the following claims.

### EXAMPLES

[0116] The following example illustrates various non-limiting aspects of the present disclosure.

#### Example 1: Development of base emulsion formulations of Tricaprilin

[0117] A list of base formulations developed for clinical studies are provided in **Table 4**. These formulations were manufactured by the general method outlined in **FIG. 1**, and did not contain any flavoring agents. A gamma irradiation protocol was used for bioburden reduction. With reference to **Table 4**: Phospholipon® 90G is a lecithin supplied by Lipoid and contains a minimum of 94% phosphatidylcholine as the main component; Kolliphor® RH40 is the trade name for Polyoxyl 40 Hydrogenated Castor Oil (USP), also referred to as macrogol glycerol hydroxystearate; glycerol is also referred to as glycerine; citrem is formally known as citric acid esters of mono- and diglycerides; 50 mM phosphate buffer at pH 6.8 was prepared by mixing 7.8 mg/mL sodium dihydrogen orthophosphate dihydrate USP/Ph.Eur. with low pyrogen water (Baxter Water for Irrigation); the pH was then adjusted with 2 M sodium hydroxide NF/BP

**Table 4: Unflavored Emulsion Formulations**

| Product Name | Amount per g <sup>1</sup>                                                                                                           | Product Name | Amount per g <sup>1</sup>                                                                                                          |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------|--------------|------------------------------------------------------------------------------------------------------------------------------------|
| AC-OLE-01    | 500 mg Tricaprilin<br>40 mg Phospholipon® 90G<br>20 mg Kolliphor® RH40<br>25 mg Glycerol<br>415 mg Phosphate Buffer (50 mM, pH 6.8) | AC-OLE-06    | 400 mg Tricaprilin<br>32 mg Phospholipon® 90G<br>16 mg Citrem<br>25 mg Glycerol<br>527 mg Phosphate Buffer (50 mM, pH 7.0)         |
| AC-OLE-02    | 500 mg Tricaprilin<br>40 mg Phospholipon® 90G<br>20 mg Kolliphor® RH40<br>440 mg Phosphate Buffer (50 mM, pH 6.8)                   | AC-OLE-07    | 200 mg Tricaprilin<br>16 mg Phospholipon® 90G<br>8 mg Kolliphor® RH40<br>25 mg Glycerol<br>751 mg Phosphate Buffer (50 mM, pH 6.8) |
| AC-OLE-03    | 400 mg Tricaprilin<br>21.3 mg Phospholipon® 90G<br>10.7 mg Kolliphor® RH40                                                          | AC-OLE-08    | 200 mg Tricaprilin<br>24 mg Phospholipon® 90G<br>5 mg Sodium Oleate<br>25 mg Glycerol                                              |

| Product Name     | Amount per g <sup>1</sup>                                                                                             | Product Name     | Amount per g <sup>1</sup>                                                                                                           |
|------------------|-----------------------------------------------------------------------------------------------------------------------|------------------|-------------------------------------------------------------------------------------------------------------------------------------|
|                  | 25 mg Glycerol<br>543 mg Phosphate Buffer (50 mM, pH 6.8)                                                             |                  | 2 mg Kolliphor® RH40<br>744 mg Phosphate Buffer (50 mM, pH 8.0)                                                                     |
| <b>AC-OLE-04</b> | 400 mg Tricaprilin<br>32 mg Phospholipon® 90G<br>16 mg Kolliphor® RH40<br>552 mg Phosphate Buffer (50 mM, pH 6.8)     | <b>AC-OLE-09</b> | 400 mg Tricaprilin<br>32 mg Phospholipon® 90G<br>16 mg Kolliphor® RH40<br>25 mg Glycerol<br>527 mg Phosphate Buffer (50 mM, pH 6.8) |
| <b>AC-OLE-05</b> | 400 mg Tricaprilin<br>21.3 mg Phospholipon® 90G<br>10.7 mg Kolliphor® RH40<br>568 mg Phosphate Buffer (50 mM, pH 6.8) | <b>AC-OLE-10</b> | 200 mg Tricaprilin<br>16 mg Phospholipon® 90G<br>8 mg Kolliphor® RH40<br>776 mg Phosphate Buffer (50 mM, pH 6.8)                    |

[0118] These formulations were used in clinical trials. However, comments from clinical trial participants made it necessary to improve the taste of the emulsions. Taste assessment showed that Gamma irradiation may have a negative impact on the taste of the formulations. To this end studies to develop a flavored versions of some lead formulations was undertaken. AC-OLE-1 and AC-OLE-03 were used in subsequent trials.

#### Example 2: Sweetness and flavor optimization

[0119] A few flavoring agents were short listed based on initial inputs. These included vanilla, mango and/or berry. In addition to flavoring agents various sweeteners were also tested. Two sweeteners were considered for further testing: sucralose and stevia (see Table 6). The compositions of the base formulations used for addition of flavors and sweeteners are outlined in Table 5 below.

**Table 5: Compositions of base formulations used for flavor optimization studies**

| Ingredient                      | AC-OLE-01 Active (% by weight) | AC-OLE-03 Active (% by weight) |
|---------------------------------|--------------------------------|--------------------------------|
| Tricaprilin                     | 50%                            | 40%                            |
| Safflower oil                   | N/A                            | N/A                            |
| Phospholipon® 90G               | 4%                             | 2.13%                          |
| Kolliphor® RH40                 | 2%                             | 1.07%                          |
| Glycerol                        | 2.5%                           | 2.5%                           |
| Phosphate Buffer, 50 mM, pH 6.8 | q.s. to 100%                   | q.s. to 100%                   |

[0120] Sweetener concentrations were assessed over 3 separate trials as listed in Table 6:

**Table 6: Sweeteners and the concentrations used for selecting sweetener level and type**

| Sweetener       | Supplier   | Sweetness                           | Concentrations Assessed |
|-----------------|------------|-------------------------------------|-------------------------|
| Sucralose       | Kanbosweet | ~600 times sweeter than sucrose     | 0.025, 0.05, 0.1%       |
| Stevia (NSF-12) | PureCircle | ~200-350 times sweeter than sucrose | 0.1, 0.2, 0.3%          |

**[0121]** Formulations were prepared using the concentrations of sweetener detailed in **Table 6** with 0.3% Givaudan Vanilla. No clear difference was identified between sucralose and stevia. Sucralose was selected as the sweetener for further trials as it could be used at a lower concentration with 0.05% selected as the concentration for additional studies. In addition, the Givaudan Vanilla concentration was halved for additional studies as it was found to be too intense in this trial.

**[0122]** Five vanilla flavors from 3 different companies were used for screening. A study was performed on AC-OLE-03 samples to compare the flavors and identify the preferred options. Concentrations were selected based on manufacturer recommendations and initial trials performed with sucralose set at 0.05%, as recommended from the previous sweetener selection study. The tested flavoring agents are detailed in **Table 7**.

**Table 7: Details of flavor screening experiments**

| Flavoring agent    | Solubility | Concentration |
|--------------------|------------|---------------|
| Givaudan Vanilla 1 | Water      | 0.15%         |
| Sensient Vanilla 1 | Water      | 0.3%          |
| Sensient Vanilla 2 | Oil        | 0.2%          |
| Scentral Vanilla 1 | Water      | 0.2%          |
| Scentral Vanilla 2 | Oil        | 0.2%          |
| Sensient Mango 1   | Water      | 0.2%          |
| Sensient Mango 2   | Oil        | 0.2%          |
| Sensient Berry     | Water      | 0.3%          |
| Scentral Mango 1   | Water      | 0.2%          |
| Scentral Mango 2   | Oil        | 0.2%          |

**[0123]** It was determined that the oil-soluble flavors had a more balanced taste with a reduced after taste. Sensient® Vanilla 2 (also known as Intense Vanilla Flavor and referred to henceforth as vanilla flavor or vanilla) was selected, at 0.2% concentration. The identification of alternative flavor options, including a mango flavor and a berry flavor, was also performed. Four mango flavors were used. A study was performed on AC-OLE-03 samples to (i) compare the flavors, along with a berry flavor supplied, and (ii) identify the preferred option. In general, mango flavors did not have a nice taste. Scentral Mango 1 was considered the best. However,

the berry flavor was preferred to all mango flavors and was recommended as back-up flavor at 0.3% concentration.

[0124] During the flavor selection studies, AC-OLE-01, a formulation with greater concentration of both oil and emulsifiers was also tested. AC-OLE-01 active and placebo samples were flavored at the optimized concentrations detailed in **Table 8** and tasted. In all cases, the taste of the flavored samples was acceptable and comparable to the AC-OLE-03 equivalents. However, the AC-OLE-01 samples had a creamier/thicker mouthfeel.

**Table 8: Summary of taste-matched active**

| Sample    | Active Lead | Active Back-up |
|-----------|-------------|----------------|
| Sucralose | 0.05%       | 0.05%          |
| Vanilla   | 0.2%        | N/A            |
| Berry     | N/A         | 0.3%           |

[0125] Additionally, it was determined that the formulations so far tested were too sweet and that the vanilla flavor was too strong. As such, formulations with reduced sweetness were tested. Formulations with reduced sweetness that were tested are provided in **Table 9** below.

**Table 9: Formulations with reduced vanilla and sweetness**

| Formulation | Vanilla Conc. | Sucralose Conc. | Observations                            |
|-------------|---------------|-----------------|-----------------------------------------|
| 1           | 0.1%          | 0%              | No sweetness, minimal vanilla, not nice |
| 2           | 0.2%          | 0%              | No sweetness, minimal vanilla, not nice |
| 3           | 0.1%          | 0.01%           | Weak vanilla, ok sweetness, not nice    |
| 4           | 0.2%          | 0.01%           | Ok vanilla, weak sweetness, not nice    |
| 5           | 0.1%          | 0.015%          | Mild vanilla, slightly sweet, nice      |
| 6           | 0.2%          | 0.015%          | Sweet, too strong vanilla, ok overall   |

[0126] From these studies, formulation 5 was selected as the best option as a third formulation with lower vanilla and sucralose concentrations. In general, it was observed that a minimum of 0.015% sucralose was needed and that a balance of flavor and sweetener was required to bring out the best characteristics of each component.

[0127] In addition to these studies several other samples were tested for flavor optimization. The observations and conclusions from these studies are summarized below.

### **Example 3: Impact of Gamma and Xray irradiation on flavoring**

[0128] The initial protocols for manufacturing the tricaprilin formulations used gamma irradiation for bioburden reduction. Therefore, the impact of this process on flavor was assessed. The first study involved flavoring AC-OLE-03 samples, which had been previously irradiated, with the lead vanilla flavor. Concentrations up to 0.5% of vanilla and 0.2% of

sucralose were used. In all cases, the flavored and unflavored AC-OLE-03 samples tasted bad due to a 'cooked'/ off flavor imparted by irradiation.

[0129] The next study looked at irradiating flavored AC-OLE-03 samples to identify whether the presence of flavoring during the process could inhibit/mask the development of the unpleasant off flavors. Both stevia and sucralose along with vanilla, berry and lemon-lime flavors were used. In all cases, the flavors after irradiation were poor with minimal hints of the flavoring agents retained in some cases. It was therefore recommended to look at alternative methods of bioburden reduction.

[0130] One of the alternative bioburden reduction methods assessed was X-ray irradiation. This provided the capability to irradiate with lower overall irradiation doses. Doses of 2 kGy and 5 kGy X-ray irradiation were tested in comparison to gamma irradiation doses of 10 kGy. A range of vanilla and berry flavors were tested for both AC-OLE-01 and AC-OLE-03 formulations. The cooked flavor persisted in these formulations, albeit slightly attenuated, in formulations treated at both X-ray irradiation doses. Methods for bioburden reduction other than irradiation-based methods were tested in subsequent formulations. In some cases, formulations without any bioburden reduction treatment were also tested for shelf life and flavor.

#### Example 4: Conclusions from flavor optimization studies

[0131] A wide range of studies were completed to identify a suitable flavor and sweetener combination for oral emulsion products. In summary: The recommended lead and back-up formulations based on these studies were:

[0132] Lead active formulation: 0.2% Vanilla, 0.05% Sucralose (refer to **Table 10** for full formulation details)

[0133] Back-up active formulation: 0.3% Berry, 0.05% Sucralose

[0134] The use of reduced vanilla (0.1%) and sucralose (0.015% and 0.025%) concentrations also produce acceptable tasting formulations. Addition of the same concentration of sweeteners and flavors to both AC-OLE-01 and AC-OLE-03 formulations produces comparable taste results Gamma and x-ray irradiation create flavors that could not be adequately masked by added flavors.

**Table 10:** Lead vanilla flavored active formulation (AC-OLE-01-VA)

| Ingredient        | Amount per g |
|-------------------|--------------|
| Tricaprilin       | 500 mg       |
| Phospholipon® 90G | 40 mg        |

|                                                                                                                        |          |
|------------------------------------------------------------------------------------------------------------------------|----------|
| Kolliphor® RH40                                                                                                        | 20 mg    |
| Glycerol                                                                                                               | 25 mg    |
| Sucralose                                                                                                              | 0.5 mg   |
| Vanilla Flavor<br>(Supplier: Sensient®; Commercial Product Code: AA000851015;<br>Product Name: Intense Vanilla Flavor) | 2 mg     |
| Phosphate Buffer, 50 mM, pH 6.8                                                                                        | 412.5 mg |

#### Example 5: Flavor and sweetener addition process and effect of nitrogen headspace

[0135] After optimization of the flavor and sweetener types, suppliers, and concentrations, the flavor addition process was considered. Two different points of addition were considered as illustrated in FIG. 2. The first point of addition was mixing the oil soluble vanilla flavor with the tricaprilin oil at the start of the process before the oil is added to the buffer and emulsifier mixture. This process was considered preferable since the flavor would be well mixed with the oil. It would also be easier to control the bioburden compared to adding the flavor later in the process. However, it was a concern if the flavor would be affected if added at the start and thereby going through the whole manufacturing process. The second point of addition was towards the end of the process after high pressure homogenization and just before filling. An advantage of this point of addition was that the flavor would not go through the preceding manufacturing process whereby the flavor would be expected to be unchanged. A potential disadvantage would be that it would be challenging to mix the oil soluble flavor with the emulsion oil droplets in a homogeneous way, which could potentially affect the emulsion stability. In addition, it may be challenging to add the flavor at the end of the process without potentially affecting the bioburden.

[0136] To study the best point of addition, laboratory studies were undertaken where:

- a) The vanilla flavor was mixed with tricaprilin at the start of the process until visibly homogenous. In this process, the sucralose sweetener was mixed with the buffer and emulsifiers at the start of the process.
- b) The vanilla flavor and the sucralose sweetener were added at the end of the process. This was done by adding flavor and sucralose to 450 g of high-pressure homogenized emulsion in a 1 Liter beaker and mixing at 750 rpm with an overhead stirrer fitted with a 60 mm diameter propeller type impeller.

[0137] It was observed that the oil soluble vanilla flavor dispersed easily in tricaprilin with minimal stirring. When sucralose and Vanilla flavor were added at the end of the process (Process b) they dispersed very rapidly forming a homogenous emulsion. The vanilla flavor

(Intense Vanilla, Sensient® product code: QAA0016060002/AA000851015) was incorporated at 0.2% (w/w) and Sucralose sweetener (KANBOSWEET) at 0.05% (w/w) in all cases.

[0138] To study the effect of nitrogen headspace, some of the samples where vanilla and sucralose were added at the start of the process were filled into glass bottles and uncapped bottles and caps were transferred to a glove bag attached to a nitrogen cylinder. The bag was purged three times with nitrogen and the bottles then capped inside the bag. Once removed from the bag, Teflon tape was wrapped around the rim of the bottles to seal and prevent air penetration into the samples.

[0139] Formulations prepared by the different processes, were filled into 30 mL amber glass bottles and placed on stability.

[0140] No clear difference between adding the flavor at the beginning (pre-addition) or at the end (post-addition) of the process was evident when comparing data of color, pH, particle size, assay and related substances up until the 2 month time point. This indicates that it makes no difference to these key physico-chemical parameters whether the flavor is added at the start or at the end of the process.

[0141] There was one minor difference in related result for the active formulation filled under nitrogen (formulation – pre-addition process, N<sub>2</sub> headspace) compared to samples not filled under nitrogen in that some impurity is present at a slightly higher level in the N<sub>2</sub> headspace sample at the 1 month time point at 25 °C and at 2 months' time point at 2-8 °C. However, the difference is minor and it seems unlikely that a nitrogen headspace would generate more impurities so it is unlikely to be of significance.

#### **Example 6: Taste testing of samples prepared with pre- or post-addition process and with nitrogen headspace**

[0142] To examine if the point of addition and filling under nitrogen influenced taste, taste testing was conducted at both the initial (T<sub>0</sub>) and at the 1-month timepoints. A triangle taste testing method was used. The taste panel each tasted 3 samples, composed of two of one sample and one of the other and attempted to identify the odd sample. The results from initial time point show that the point of flavor addition does not significantly affect the taste samples.

[0143] Similar triangle taste testing was also done at the 1-month time point and the results are shown in **Table 8**. At this time point it was tested if panelists were able to tell the difference between the following samples:

- a) Active pre-addition samples vs active post-addition samples, both stored at 25 °C

- b) Active pre-addition samples stored at 25 °C vs active post-addition samples stored at 2-8 °C
- c) Active pre-addition samples vs active pre-addition samples filled under nitrogen, both stored at 25 °C
- d) Active pre-addition samples stored at 25 °C vs active freshly prepared samples

**[0144]** No significant differences between pre-and post-addition, between 2-8 °C storage temperature, between filling under nitrogen or ambient air, or between 1-month old sample or freshly prepared samples were identified indicating that none of these factors have a significant impact on taste at the 1-month time point. It is also possible that differences may show up at later time points, however the insignificant differences after 1 month indicates that any differences that could show up at later times are likely to be minor.

**[0145]** Overall, it was concluded that adding the flavor at the beginning or at the end of the process or filling under nitrogen has no significant impact on the physico-chemical properties of the active or the placebo formulations, nor does it have a significant impact on the taste up until the 1 month time point.

**[0146]** Based on these results it is therefore recommended to mix the flavor with the oil at the beginning of the process, as shown schematically in Fig. 3, since it is more certain that the flavor will be more equally mixed between different oil droplets in the final emulsion and because it is easier to control the bioburden compared to adding the flavor at the end of the process.

#### **Example 7: Detailed manufacturing process**

##### Preparation of 2 M NaOH

**[0147]** Low pyrogen water was transferred into an appropriate beaker. An appropriate amount of sodium hydroxide was weighed into an appropriate container. Sodium hydroxide was transferred into the beaker of low pyrogen water and stirred until all sodium hydroxide had dissolved using an overhead stirrer equipped with a stainless steel stirrer.

##### Preparation of 50 mM phosphate buffer pH 6.8

**[0148]** Sodium Dihydrogen Phosphate Dihydrate was weighted into an appropriate container. Low pyrogen water was weighed into a stainless-steel mixing tank. Sodium Dihydrogen Phosphate Dihydrate was added to the low pyrogen water and stirred until all the Sodium Dihydrogen Phosphate Dihydrate dissolved. The pH of the solution was adjusted to 6.8 (target  $\pm 0.1$ ) with the 2 M NaOH prepared above. The solution was stirred for less than 1 minute after each addition of sodium hydroxide solution.

Emulsion preparation

[0149] Kolliphor® RH40 was warmed to a temperature of 60 °C or less in an oven. When the Kolliphor® melted, the material was stirred manually for at least 2 minutes before weighing into the phosphate buffer solution and the mixing was started using a stainless steel stirrer. Glycerol was weighed into an appropriate container and transferred to the Kolliphor® RH40 solution in phosphate buffer. Sucralose was weighed into an appropriate container and then transfer to Kolliphor® RH40/ glycerol solution in phosphate buffer. Phospholipon® 90G was weighed and added to the sucralose/ Kolliphor® RH 40/ glycerol solution. The solution was stirred for 15 minutes.

[0150] An oil and flavor mixture was prepared. tricaprilin (active) or safflower oil (control) were weighed in a different container. Intense vanilla flavor was weighed and added to a container. The vanilla flavor was added to the container with either the tricaprilin or safflower oil. The mixture was mixed for 5 minutes.

[0151] The oil and flavor mixture was added to the Phospholipon® 90G/ sucralose/ Kolliphor® RH 40/ glycerol solution in phosphate buffer to form a bulk solution.

[0152] An in-line high shear emulsifier was set up and connected to the stainless steel mixing tank containing the bulk solution. The bulk solution was passed through the emulsifier into a 30 L receiver stainless steel tank. The bulk solution was manually stirred in the receiver tank and the temperature was measured. The bulk solution was fed into the in-line high shear emulsifier two times. The temperature of the bulk solution was maintained at 40 °C. In process samples were removed and particle size measured until the particle size ( $d(0.5)$ ) was under 5  $\mu\text{m}$ . The actual particle size was 2.853  $\mu\text{m}$  resulting in the formation of a course emulsion.

[0153] An EmulsiFLEX-C55® (Avestin) high pressure homogenizer was set up to homogenize the contents of tank. The course emulsion was high pressure homogenized in the 50 L tank at 10,000 PSI and transferred to another tank. The high pressure homogenizing between the tanks was repeated multiple times and was conducted for a total of 2-5 times depending on the batch. The setting of the tank is listed below in **Table 11**. In process samples were measured to check for the particle size during the high-pressure homogenization. The process was continued until a particle size ( $d(50)$ ) was less than 0.4  $\mu\text{m}$  was achieved. The measured particle size  $d(0.5)$  was 0.133  $\mu\text{m}$  resulting in a fine emulsion.

**Table 11.**

|                                                                                                                                                |
|------------------------------------------------------------------------------------------------------------------------------------------------|
| Settings:<br>-C55 pump head chiller: 5°C<br>-C55 heat exchange chiller: 10°C<br>Homogenisation pressure: 10000 PSI<br>High Pressure: 12000 PSI |
|------------------------------------------------------------------------------------------------------------------------------------------------|

High Pressure cutoff: 15000 PSI  
 Low pressure: 0 PSI  
 Low pressure cutoff: 0 PSI  
 Feed pump pressure: 31 PSI  
 Ensure emulsion is no more than (NMT) 45 °C  
 Ensure contents of Tank 4 are NMT 40 °C  
 Temperature measurements for each pass:  
 Pass 1: Initial: 40.6°C Final: 29.8°C  
 Pass 2: Initial: 29.6°C Final: 26.6°C

**[0154]** The fine emulsion was filled into 100 mL amber glass containers with screw-cap closures. The amber bottles had been presterilized by gamma irradiation.

**[0155]** Examples of particle size distributions measured during lab scale formulation development for the lead active formulation AC-OLE-01-VA (0.2% Intense Vanilla Flavor, 0.05% Sucralose) are shown in **FIG. 4**, respectively. The top particle size distribution represents the measurement done after high shear mixing and the bottom particle size distribution represents the measurement done after seven passes of high-pressure homogenization (Step 4.12) in **FIG. 4**.

**Example 8: Bioburden of formulations processed by high pressure homogenization alone and without gamma irradiation**

**[0156]** High pressure homogenisation has previously been reported to have a bioburden reduction effect, although the effect has mainly been reported at pressures around 100-300 MPA, which is greater than the pressures used in the current manufacturing method (10,000 PSI; 69 MPA). To investigate if high pressure homogenisation alone without gamma irradiation was an efficient bioburden reduction method the microbial load of samples was followed. Samples used for these studies are provided in **Table 12** and **Table 14**. The results for these sample types are provided in **Table 13** and **Table 15A and 15B** respectively.

**Table 12: Sample set 1 for bioburden analysis**

|                                     |                | Batch No       |                |
|-------------------------------------|----------------|----------------|----------------|
| Pre - high-pressure homogenisation  | CER22005-F3a-a | CER22005-P1b-a | CER22005-F2b-a |
| Post - high pressure homogenization | CER22005-F3a-b | CER22005-P1b-b | CER22005-F2b-b |
| Ingredient                          | % (w/w)        | % (w/w)        | % (w/w)        |
| Tricaprilin                         | 50%            | N/A            | 50%            |
| Safflower Oil                       | N/A            | 50%            | N/A            |
| Phospholipon® 90G                   | 4%             | 4%             | 4%             |
| Kolliphor® RH40                     | 2%             | 2%             | 2%             |
| Glycerol                            | 2.5%           | 2.5%           | 2.5%           |

|                                     |                                                                                                               |       |       |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------|-------|-------|
| Sucralose                           | 0.05%                                                                                                         | N/A   | N/A   |
| Vanilla Flavor<br>(Intense Vanilla) | 0.2%                                                                                                          | N/A   | N/A   |
| Phosphate Buffer,<br>50 mM, pH 6.8  | 41.25%                                                                                                        | 41.5% | 41.5% |
| Container/Closure                   | 30 mL amber glass bottle.<br>GL18 Child Resistant Cap with PE Liner.<br>4 ml GC bottles (used for appearance) |       |       |
| Storage Conditions<br>/Orientation  | <ul style="list-style-type: none"> <li>25°C/60%RH, Upright</li> <li>40°C/75% RH, Upright</li> </ul>           |       |       |

**Table 13:** Microbial loads of samples processed by high pressure homogenization alone and collected before and after the high-pressure homogenization step.

| Batch 1: CER22005-F2b              |               |           |           |           |                            |           |
|------------------------------------|---------------|-----------|-----------|-----------|----------------------------|-----------|
| Test                               | Specification | 0 months  | 2 weeks   | 1 month   | 2 months                   |           |
|                                    |               | 40°C      | 40°C      | 40°C      | 25°C                       | 40°C      |
| Pre-high pressure homogenization   |               |           |           |           |                            |           |
| TAMC                               | <1000 cfu/g   | <10 cfu/g | <10 cfu/g | <10 cfu/g |                            |           |
| TYMC                               | <100 cfu/g    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g                  | <10 cfu/g |
| E. coli                            | Absent in 1 g | Absent    | Absent    | Absent    | Absent                     | Absent    |
| Post-high pressure homogenization  |               |           |           |           |                            |           |
| TAMC                               | <1000 cfu/g   | <10 cfu/g | <10 cfu/g | <10 cfu/g |                            |           |
| TYMC                               | <100 cfu/g    | <10 cfu/g | <10 cfu/g | <10 cfu/g | ~1.8x10 <sup>3</sup> cfu/g | <10 cfu/g |
| E. coli                            | Absent in 1 g | Absent    | Absent    | Absent    | Absent                     | Absent    |
| Batch 2: CER22005-F3a              |               |           |           |           |                            |           |
| Test                               | Specification | 0 months  | 2 weeks   | 1 month   | 2 months                   |           |
|                                    |               | 40°C      | 40°C      | 40°C      | 25°C                       | 40°C      |
| Pre-high pressure homogenization   |               |           |           |           |                            |           |
| TAMC                               | <1000 cfu/g   | <10 cfu/g | <10 cfu/g | <10 cfu/g |                            |           |
| TYMC                               | <100 cfu/g    | <10 cfu/g | <10 cfu/g | <10 cfu/g | 60 cfu/g                   | <10 cfu/g |
| E. coli                            | Absent in 1 g | Absent    | Absent    | Absent    | Absent                     | Absent    |
| Post-high pressure homogenization  |               |           |           |           |                            |           |
| TAMC                               | <1000 cfu/g   | <10 cfu/g | <10 cfu/g | <10 cfu/g |                            |           |
| TYMC                               | <100 cfu/g    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g                  | <10 cfu/g |
| E. coli                            | Absent in 1 g | Absent    | Absent    | Absent    | Absent                     | Absent    |
| Batch 3: CER22005-P1b              |               |           |           |           |                            |           |
| Test                               | Specification | 0 months  | 2 weeks   | 1 month   | 2 months                   |           |
|                                    |               | 40°C      | 40°C      | 40°C      | 25°C                       | 40°C      |
| Pre-high pressure homogenization   |               |           |           |           |                            |           |
| TAMC                               | <1000 cfu/g   | <10 cfu/g | <10 cfu/g | <10 cfu/g |                            |           |
| TYMC                               | <100 cfu/g    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g                  | <10 cfu/g |
| E. coli                            | Absent in 1 g | Absent    | Absent    | Absent    | Absent                     | Absent    |
| Post- high pressure homogenization |               |           |           |           |                            |           |
| TAMC                               | <1000 cfu/g   | <10 cfu/g | <10 cfu/g | <10 cfu/g |                            |           |

|         |               |           |           |           |           |           |
|---------|---------------|-----------|-----------|-----------|-----------|-----------|
| TYMC    | <100 cfu/g    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| E. coli | Absent in 1 g | Absent    | Absent    | Absent    | Absent    | Absent    |

[0157] The outlet hose was removed from the high-pressure homogeniser for the final pass and samples were collected directly from the steel outlet. This was also wiped with ethanol prior to collection.

[0158] TAMC, TYMC and the presence of E. coli were measured by Eurofins. TAMC and TYMC were validated with a limit of detection of 10 cfu/g. Results below this are reported as <10 cfu/g. This limit is well below the specifications of <1000 cfu/g and <100 cfu/g for TAMC and TYMC, respectively.

[0159] Bioburden results for samples treated by high pressure homogenization alone and collected before and after the high-pressure homogenization step are shown in **Table 13**. It is seen that no microbial load is detected in any of the samples after storage at 40 °C for 1 month.

[0160] At the 2 months' time point, aerobic microbes as well as yeasts and molds were detected at levels above specification at 25 °C for batch 1 (CER22005-F2b) for the post homogenization sample while no microbes were detected for the pre-homogenization sample. For batch 2 (CER22005-F3a), low levels of aerobic microbes and yeasts and molds below the specification were detected for the pre-homogenization sample while no microbes were detected in the post-homogenization sample. For both batch 1 and 2, no microbials were detected at 40 °C for either of the pre-homogenization or post-homogenization samples. For the third batch (batch 3, CER22005-P1b) no microbials were detected at 25 °C or 40 °C for any samples.

**Table 14:** Sample set 2 for bioburden analysis

|                                                 |                                                                                                     |
|-------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| Irradiated – filled under laminar hood:         | CER2201 8-F1a                                                                                       |
| Homogenized only – filled under laminar hood:   | CER22018-F1b                                                                                        |
| Homogenized only – filled outside laminar hood: | CER22018-F1c                                                                                        |
| Ingredient                                      | % (w/w)                                                                                             |
| Tricaprilin                                     | 50%                                                                                                 |
| Phospholipon® 90G                               | 4%                                                                                                  |
| Kolliphor® RH40                                 | 2%                                                                                                  |
| Glycerol                                        | 2.5%                                                                                                |
| Phosphate Buffer, 50 mM, pH 6.8                 | 41.25%                                                                                              |
| Container/Closure                               | 30 mL amber glass bottle.<br>GL18 Child Resistant Cap with PE Liner.                                |
| Storage Conditions /Orientation                 | <ul style="list-style-type: none"> <li>25°C/60%RH, Upright</li> <li>40°C/75% RH, Upright</li> </ul> |

[0161] Part of the batch was sent for gamma irradiation as a control. This part was filled into glass bottles in a laminar flow hood after gamma irradiation (irradiated group). The remaining part of the batch was retained and not subjected to gamma irradiation (homogenization only group). Part of the homogenization only group was filled in a laminar flow hood and the other part was filled outside a laminar flow hood.

[0162] TAMC, TYMC and the presence of *E. coli* were measured by Eurofins. TAMC and TYMC were validated with a limit of detection of 10 cfu/g. Results below this are reported as <10 cfu/g. This limit is well below the specifications of <1000 cfu/g and <100 cfu/g for TAMC and TYMC, respectively.

[0163] Bioburden results for the 2<sup>nd</sup> set of formulations are shown in **Table 15A** and **15B**. The results show that no microbial load was detected in any of the samples after storage at 25 °C or 40 °C for 12 weeks regardless of whether the samples were filled inside or outside of the laminar flow hood.

**Table 15A:** Microbial load of formulations for set 2.

| Test           | Specification                                                 | 0 months  |           |           |           |           |
|----------------|---------------------------------------------------------------|-----------|-----------|-----------|-----------|-----------|
|                |                                                               | 1 week    |           | 4 weeks   |           |           |
|                |                                                               |           | 25°C      | 40°C      | 25°C      | 40°C      |
| <b>Batch:</b>  | <b>CER22018-F1a – Irradiated, filled in laminar flow hood</b> |           |           |           |           |           |
| TAMC           | <1000 cfu/g                                                   | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| TYMC           | <100 cfu/g                                                    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| <i>E. coli</i> | Absent in 1 g                                                 | Absent    | Absent    | Absent    | Absent    | Absent    |
| <b>Batch:</b>  | <b>CER22018-F1b – Non-Irradiated, filled under hood</b>       |           |           |           |           |           |
| TAMC           | <1000 cfu/g                                                   | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| TYMC           | <100 cfu/g                                                    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| <i>E. coli</i> | Absent in 1 g                                                 | Absent    | Absent    | Absent    | Absent    | Absent    |
| <b>Batch:</b>  | <b>CER22018-F1c – Non-Irradiated, filled outside hood</b>     |           |           |           |           |           |
| TAMC           | <1000 cfu/g                                                   | <10 cfu/g | N/A       | <10 cfu/g | N/A       | <10 cfu/g |
| TYMC           | <100 cfu/g                                                    | <10 cfu/g | N/A       | <10 cfu/g | N/A       | <10 cfu/g |
| <i>E. coli</i> | Absent in 1 g                                                 | Absent    | N/A       | Absent    | N/A       | Absent    |

**Table 15B:** 8-12 week data for set 2

| Test           | Specification                                                 | 8 weeks   |           | 12 weeks  |           |
|----------------|---------------------------------------------------------------|-----------|-----------|-----------|-----------|
|                |                                                               | 25°C      | 40°C      | 25°C      | 40°C      |
| <b>Batch:</b>  | <b>CER22018-F1a – Irradiated, filled in laminar flow hood</b> |           |           |           |           |
| TAMC           | <1000 cfu/g                                                   |           |           |           |           |
| TYMC           | <100 cfu/g                                                    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| <i>E. coli</i> | Absent in 1 g                                                 | Absent    | Absent    | Absent    | Absent    |
| <b>Batch:</b>  | <b>CER22018-F1b – Non-Irradiated, filled under hood</b>       |           |           |           |           |
| TAMC           | <1000 cfu/g                                                   |           |           |           |           |
| TYMC           | <100 cfu/g                                                    | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| <i>E. coli</i> | Absent in 1 g                                                 | Absent    | Absent    | Absent    | Absent    |
| <b>Batch:</b>  | <b>CER22018-F1c – Non-Irradiated, filled outside hood</b>     |           |           |           |           |

|         |               |     |           |     |           |
|---------|---------------|-----|-----------|-----|-----------|
| TAMC    | <1000 cfu/g   | N/A | <10 cfu/g | N/A | <10 cfu/g |
| TYMC    | <100 cfu/g    | N/A | <10 cfu/g | N/A | <10 cfu/g |
| E. coli | Absent in 1 g | N/A | Absent    | N/A | Absent    |

**[0164]** To conclude, the fact that none of the samples prepared contained any microbial load above the detection limit even when filled outside a laminar flow hood indicate that the manufacturing process itself, without gamma irradiation, appears to be sufficient to maintain the bioburden below the detection limit. However, one pre-homogenization sample and one post-homogenization sample of set 1 did show microbial counts at 25 °C after 2 months storage, which makes it impossible to determine the extent of bioburden reduction effect provided by the high-pressure homogenization. Rather, the inconsistent presence of microbial contamination is most likely due to contamination of individual vials during filling as a result of the non-aseptic lab environments. Overall, the results of set 2 indicate that it is possible to prepare formulations without any detectable microbial load without gamma irradiation or other bioburden reduction steps. It is nonetheless recommended to fill under aseptic conditions. It is furthermore recommended that the final commercial manufacturing process includes ultra-high temperature (UHT) treatment as a bioburden reduction step to remove any potential microbial contaminants in the raw materials or during manufacturing steps upstream of high pressure homogenization and filling.

#### **Example 9: Studying small variations in formulations using design of experiments (DOE)**

**[0165]** Formulations were screened for variations in pH, buffer strength, flavor type, flavor concentration, and sweetener concentration. The ranges shown in **Table 16** below were used for each factor:

**Table 16:**

| <b>Factor</b>           | <b>Range</b>                                                                     |
|-------------------------|----------------------------------------------------------------------------------|
| pH                      | 6.5 – 7.8                                                                        |
| Buffer Strength         | 50 mM – 150 mM                                                                   |
| Flavor                  | Vanilla or Berry                                                                 |
| Flavor Concentration    | 0.10 – 0.20% (w/w) for Vanilla Active<br>0.15 - 0.30% (w/w) for Berry Active     |
| Sweetener Concentration | 0.015 - 0.050% (w/w) for Vanilla & Berry active<br>0.10% (w/w) for Berry Placebo |

**[0166]** The stability of the formulations was followed for 3 months at 2-8 °C, 25 °C and 40 °C. From the data, the following observations are evident:

**[0167] Appearance:** The appearance all formulations is unchanged and within specifications as “White to off white emulsion with no phase separation” at all storage conditions throughout the 3 months stability study.

**[0168] Assay:** The assay is well within specification at all storage conditions throughout the 3 months for all active formulations and there is no evidence of a decline in assay.

**[0169] Related substances assay:** There is no evidence of an increase in total related substances and no concerning rise in any individual impurities either for any of the active formulations tested at any storage condition.

**[0170] pH:** There is a clear decrease in pH during the 3-month stability study for all formulations tested. This will be discussed in more detail in later Examples.

**[0171] Particle size:** The particle size is stable for all active and placebo formulations at all storage conditions.

**[0172] Viscosity:** Viscosity was acceptable for all formulations with no major changes of concern throughout the 3-month study. Viscosity data was collected for information only.

**[0173] Colour:** Colour was acceptable for all formulations with no major changes of concern throughout the 3-month study. Colour data was collected for information only.

**[0174] Taste:** Taste changed for all formulations when stored at 25 °C or 40 °C for 3 months, whereas there was minimal or no change at 2-8 °C storage.

**[0175]** An overall conclusion was that the liquid emulsions are robust to changes in factors varied in the design (pH, buffer strength, flavor type, flavor concentration, and sweetener concentration) with respect to appearance, assay, related substances, particle size, viscosity and colour. Taste is difficult to evaluate as it is very subjective and difficult to quantitate without engaging large sensory panels, which was beyond the scope of this formulation study. It could be concluded, however, that the type of flavor (Vanilla or Berry) was not of major importance for flavor stability on storage and they both behaved similarly on storage.

**[0176]** Apart from taste, the only other attribute that was not stable during storage was pH, which decreased during the 3-month stability study. The decrease was greater at higher storage temperatures and depended on the formulation since some formulations decreased more in pH than others.

**[0177]** To evaluate the most important factors responsible for the decrease in pH, the active formulations were ranked in order from greatest to smallest pH decrease at 25 °C as shown in FIG. 5A-5I. The change at 25 °C was found to be most useful for comparing pH stability of the formulations since room temperature is the anticipated storage condition. By comparing

the pH stability ranking the following conclusions can be made. **Starting pH:** The formulations with starting pH = 6.5 were the most stable and the formulations with starting pH = 7.8 were the least stable. However, at 40 °C the formulations with starting pH of 6.5 started to drop off at 3 months, most likely as a result of the pH moving away from the pKa of the phosphate buffer (pKa 6.8) where the buffer effect is most effective. Based on these results it was decided to keep the pH slightly higher at 6.8 for formulations to optimize pH stability and avoid the drop off observed at later time points at 40 °C for formulations with low starting pH

**[0178] Flavor level:** The most stable formulations have a low flavor level and the least stable formulations have a high flavor level.

**[0179] Flavor type:** The vanilla and berry flavored formulations behave similarly and there is no clear difference in pH stability with respect to flavor type.

**[0180] Buffer strength:** The most stable formulations have a high buffer strength, and the least stable formulations have a low buffer strength. However, it was fairly certain that both flavor level and buffer strength were important to pH stability since previous experiments had indicated that flavored formulations were less stable than unflavored formulations and it appears logical that higher buffer strength would increase pH stability.

**[0181] Sucralose level:** Sucralose level does not appear to be a major factor for pH stability.

**[0182]** The overall conclusion is that formulations with higher buffer strength, lower flavor levels and a starting pH of 6.8 improve pH stability.

#### **Example 10: Incorporation of Ultra High Temperature (UHT) treatment as Bioburden control step**

**[0183]** AC-OLE-01-VA batches manufactured at 85 Kg scale were stable and did not show any microbial growth during 12 month storage at 25 °C even in the absence of a specific bioburden reduction step in the process. In spite of this, it is considered necessary to include a bioburden reduction step in the final commercial manufacturing process. Gamma irradiation was employed for previously manufactured unflavored formulations, but this was found to have a negative impact on the taste and was therefore not suitable for a commercial product. Sterile filtration was attempted but the formulations were found to block sterilizing grade filters. High pressure thermal processing (HPTP) was also considered but this process had limitations regarding access to manufacturing scale equipment.

**[0184]** Instead, Ultra Heat Treatment (UHT) was examined and found to be a suitable process, which is also scalable to large batch sizes. The following section describes the development of an UHT process suitable for inclusion in the manufacturing process. .

[0185] A study was designed to study the impact of UHT parameters on the AC-OLE-01-VA oral liquid emulsion. The active AC-OLE-01-VA formulation was assessed in the DOE study, which had the composition shown in **Table 17** below.

**Table 17:** Composition of AC-OLE-01-VA formulation used for UHT study

| Ingredient                       | Concentration (% w/w) |
|----------------------------------|-----------------------|
| Tricaprilin                      | 50%                   |
| Phospholipon® 90G                | 4%                    |
| Kolliphor® RH40                  | 2%                    |
| Glycerol                         | 2.5%                  |
| Sucralose                        | 0.05%                 |
| Vanilla Flavor (Intense Vanilla) | 0.2%                  |
| Phosphate Buffer, 50 mM, pH 6.8  | 41.25%                |

[0186] The emulsion preparation procedure involved three main process steps in the following order:

- a) High Shear Mixing
- b) Ultra-heat treatment (UHT)
- c) High pressure homogenization (HPH)

[0187] Batch sizes of 10 L were prepared for UHT processing. The initial 7 L and final 1 L of the UHT processed batches were discarded and only the middle 2 L was collected for high pressure homogenization. This was to ensure dilution did not impact the results as previous studies had shown that the first few liters were diluted. The dilution effect is due to the UHT first being run with pure water to stabilize the flow rate and temperature and the formulation then added when the UHT system has heated up and parameters are stable.

[0188] Five UHT process parameters, which can be controlled in the UHT process were identified. One of these factors, the processing volume, was not included in the initial DOE design but was intended to be assessed once the final process had been identified. The other four process parameters were assessed in a fractional factorial DOE as outlined in **Table 18** below. Three center points were included to assess system repeatability.

**Table 18:** UHT Process development DOE design

| Run Order | Flow Rate (L/hr)* | UHT Temperature (°C) | Chiller On or Off | Holding Tube (s) | Hold Time (s)* |
|-----------|-------------------|----------------------|-------------------|------------------|----------------|
| 2         | 40                | 130                  | Off               | 3                | 7.5            |
| 6         | 100               | 130                  | On                | 3                | 3              |
| 9         | 40                | 145                  | On                | 3                | 7.5            |
| 5         | 100               | 145                  | Off               | 3                | 3              |
| 10        | 40                | 130                  | Off               | 18               | 45             |

| Run Order | Flow Rate (L/hr)* | UHT Temperature (°C) | Chiller On or Off | Holding Tube (s) | Hold Time (s)* |
|-----------|-------------------|----------------------|-------------------|------------------|----------------|
| 3         | 100               | 130                  | On                | 18               | 18             |
| 4         | 40                | 145                  | On                | 18               | 45             |
| 1         | 100               | 145                  | Off               | 18               | 18             |
| 11        | 70                | 137.5                | On                | 8                | 11.4           |
| 8         | 70                | 137.5                | On                | 8                | 11.4           |
| 7         | 70                | 137.5                | On                | 8                | 11.4           |

\*Controlled by flow rate and holding tube

[0189] The study was primarily focused on assessing how UHT impacts the product quality attributes rather than following the long-term stability. Samples were tested after preparation for taste, description, particle size, viscosity, assay and relateds. Samples were also placed at 25 °C and tested for particle size and description after two weeks and one month storage to identify any effects of UHT on stability. Apart from this, the study was not intended to assess the long-term stability of UHT processed formulations. Later studies using an optimized UHT method were used to prepare samples for long-term stability.

[0190] Table 19 and Table 20 present the assay and total relateds presented as a function of hold time and UHT temperature. Without being bound by theory, if UHT treatment resulted in chemical degradation of the AC-OLE-01-VA formulation one would expect the assay to decrease and relateds to increase for UHT runs with a high temperature and long hold times. In other words, one would expect assay values to decrease and total relateds values to increase when moving from the top left-hand corner of the tables (130 °C & 3s) towards the bottom right-hand corner (145 °C & 45s). However unexpectedly, this is not the case and there is no indication whatsoever that higher temperatures and longer hold times lead to chemical degradation of tricaprilin. There is no indication of individual impurities increasing for higher temperatures and longer hold times either. It is therefore concluded that the formulation is highly robust to UHT treatment and the entire range of hold times and temperatures and hold times studied in the DOE can be selected without impacting assay and relateds of the formulation.

**Table 19:** Assay results for UHT process development DOE batches.

| Hold time (s) | Assay(%)          |                                       |                   |
|---------------|-------------------|---------------------------------------|-------------------|
|               | UHT Temperature   |                                       |                   |
|               | 130 °C            | 137.5 °C                              | 145 °C            |
| 3 s           | 97.2 %<br>(Run#6) | N/A                                   | 97.9 %<br>(Run#5) |
| 7.5 s         | 98.0 %<br>(Run#2) | N/A                                   | 97.2 %<br>(Run#9) |
| 11.4 s        | N/A               | 95.6%; 96.2%; 96.4%<br>(Run#7;#8;#11) | N/A               |

|      |                    |     |                   |
|------|--------------------|-----|-------------------|
| 18 s | 95.4 %*<br>(Run#3) | N/A | 95.9 %<br>(Run#1) |
| 45 s | 98.4 %<br>(Run#10) | N/A | 97.0 %<br>(Run#4) |

**Table 20:** Total relateds results for UHT process development DOE batches.

|               | Total Relateds (%) |                                       |                   |
|---------------|--------------------|---------------------------------------|-------------------|
|               | UHT Temperature    |                                       |                   |
| Hold time (s) | 130 °C             | 137.5 °C                              | 145 °C            |
| 3 s           | 1.72 %<br>(Run#6)  | N/A                                   | 1.66 %<br>(Run#5) |
| 7.5 s         | 1.68 %<br>(Run#2)  | N/A                                   | 2.16 %<br>(Run#9) |
| 11.4 s        | N/A                | 1.78%; 1.80%; 1.74%<br>(Run#7;#8;#11) | N/A               |
| 18 s          | 1.65 %*<br>(Run#3) | N/A                                   | 1.83 %<br>(Run#1) |
| 45 s          | 1.72 %<br>(Run#10) | N/A                                   | 1.67<br>(Run#4)   |

\*The UHT process failed for Run#3 (batch D22081702,1802) as the UHT pump stopped during the run due to pressure build-up in the UHT system. This data is therefore shown in grey font as it is not representative of a true UHT run of the selected UHT parameters.

**[0191]** The physical stability and particle size of the emulsions was also assessed. Comparing the particle size before high pressure homogenization and at T0 it is clear that the particle size distribution varies widely before high pressure homogenization but the final particle sizes after high pressure homogenization are very similar. This means that the particle size is affected by the UHT process but the high pressure homogenization step eliminates this difference and results in practically identical particle size distribution of the final products irrespective of the UHT process. It is also noted that the particle size distribution is practically unchanged after 1 month storage at 25 °C for all batches except for the one batch (run#3) where the UHT pump stopped during the UHT process due to pressure build up in the system. This caused some of the product to stay in the system for a prolonged period of time until the pump was restarted and is therefore not representative of a true UHT run and should be disregarded. It is therefore concluded that the AC-OLE-01-VA is also highly robust to UHT treatment with respect to particle size and the entire range of hold times and temperatures studied in the DOE can be selected without impacting the particle size distribution of the finished product or the physical stability.

**[0192]** Taste of the UHT treated batches was also assessed and found to be acceptable for all combinations of UHT temperature and hold time.

[0193] Overall, it can be concluded that the formulation is robust to UHT treatment with respect to appearance, chemical stability, physical stability and taste within a UHT temperature range from 130 °C to 145 °C and hold times from 3 seconds to 45 seconds.

[0194] From a manufacturing process point, it was noted that one UHT run failed due to the UHT pump shutting off during the run as the pressure in the system approached a cutoff point of 25 bar. This occurred for run#3 with a flow rate of 100 L/hr; UHT temperature of 130 °C and chiller temperature of: 20 °C. It was found that higher flow rates and lower temperatures resulted in greater pressure build-up and it is therefore possible to avoid this happening for manufacturing batches by controlling these parameters.

[0195] To assess the bioburden reduction efficiency of UHT treatment of the AC-OLE-01-VA liquid emulsion, microbial challenge testing was performed. An AC-OLE-01-VA liquid emulsion (50 mM phosphate buffer pH 6.8, 0.2% Vanilla, 0.05% Sucralose) was prepared and a coarse emulsion prepared by high shear mixing.

[0196] The formulation was microbiologically challenged with *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* bacteria as well *Geobacillus Stearothermophilus* spores and subsequently UHT treated at 135 °C for 2.35 seconds.

[0197] Log reduction was measured by comparing colony forming units per gram (cfu/g) before and after UHT treatment, which gave the results in **Table 21** below.

**Table 21:** Log reduction for different challenge organisms after UHT treatment at 135 °C for 2.35 seconds

| Microorganism                                   | Replicate | N <sub>0</sub><br>Log <sub>10</sub><br>cfu/g | N <sub>F</sub><br>Log <sub>10</sub><br>cfu/g | Reduction<br>Log <sub>10</sub><br>cfu/g |
|-------------------------------------------------|-----------|----------------------------------------------|----------------------------------------------|-----------------------------------------|
| <i>E. coli</i>                                  | 1         | 6.41                                         | 0                                            | 6.41                                    |
|                                                 | 2         |                                              | 0                                            | 6.41                                    |
|                                                 | 3         |                                              | 0                                            | 6.41                                    |
| <i>Salmonella</i>                               | 1         | 6.51                                         | 0                                            | 6.51                                    |
|                                                 | 2         |                                              | 0                                            | 6.51                                    |
|                                                 | 3         |                                              | 0                                            | 6.51                                    |
| <i>Listeria monocytogenes</i>                   | 1         | 6.52                                         | 0                                            | 6.52                                    |
|                                                 | 2         |                                              | 0                                            | 6.52                                    |
|                                                 | 3         |                                              | 0                                            | 6.52                                    |
| <i>Staphylococcus Aureus</i>                    | 1         | 6.54                                         | 0                                            | 6.54                                    |
|                                                 | 2         |                                              | 0                                            | 6.54                                    |
|                                                 | 3         |                                              | 0                                            | 6.54                                    |
| <i>Bacillus Cereus</i>                          | 1         | 5.91                                         | 0                                            | 5.91                                    |
|                                                 | 2         |                                              | 0                                            | 5.91                                    |
|                                                 | 3         |                                              | 0                                            | 5.91                                    |
| <i>Geobacillus stearothermophilus</i><br>spores | 1         | 5.94                                         | 2.86                                         | 3.08                                    |
|                                                 | 2         |                                              | 3.18                                         | 2.76                                    |
|                                                 | 3         |                                              | 3.51                                         | 2.43                                    |

[0198] **Commercial sterility test:** As a further evaluation of the efficiency of the UHT treatment, a commercial sterility test was also performed. Formulations that were microbiologically challenged with separate UHT runs of 135 °C for 2.35 seconds with *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* were incubated after UHT treatment at 30 °C for 14 days and 55 °C for 7 days and microbial growth was then assessed. The results are shown in **Table 22** below.

**Table 22:** Results of commercial sterility tests of microbiologically challenged and UHT treated formulation

| Microorganism used in the microbiological challenge testing of UHT at 135 °C for 2.35 seconds | Test                                            | Microbial Growth Test results | Commercial Sterility Result |
|-----------------------------------------------------------------------------------------------|-------------------------------------------------|-------------------------------|-----------------------------|
| <i>E. coli</i>                                                                                | Commercial sterility test/Microbial growth test | No microbial growth           | Commercially sterile        |
| <i>Salmonella</i>                                                                             | Commercial sterility test/Microbial growth test | No microbial growth           | Commercially sterile        |
| <i>Listeria monocytogenes</i>                                                                 | Commercial sterility test/Microbial growth test | No microbial growth           | Commercially sterile        |
| <i>Staphylococcus Aureus</i>                                                                  | Commercial sterility test/Microbial growth test | No microbial growth           | Commercially sterile        |
| <i>Bacillus Cereus</i>                                                                        | Commercial sterility test/Microbial growth test | No microbial growth           | Commercially sterile        |

[0199] No microbial growth was observed demonstrating that UHT of 135°C for 2.35 seconds was able to achieve a commercially sterile formulation after UHT treatment for the pathogens studied.

**Example 11: Stability of formulations prepared with UHT treatment included in manufacturing process**

[0200] A number of batches were prepared with UHT treatment included in the manufacturing process. These batches were made with varying buffer strength and vanilla flavor concentrations to optimize the long term stability since the formulation screening DOE showed that lower flavor concentration and higher buffer strength increased pH stability of the formulations. The formulations prepared are shown in **Table 23** and the compositions are shown in **Table 24**.

**Table 23:** Active formulations with varying flavor concentrations and buffer strength prepared with UHT treatment at 135 °C and 5 second hold time.

| Product               | Batch/Lot Number                                 | Buffer Strength | Flavor       | Container Type | UHT process                                        |
|-----------------------|--------------------------------------------------|-----------------|--------------|----------------|----------------------------------------------------|
| Active formulations   |                                                  |                 |              |                |                                                    |
| AC-OLE-01-VA (Active) | 070622-2                                         | 50mM            | 0.2% Vanilla | Glass/HDPE     | UHT temperature: 135 °C<br>Holding time: 5 seconds |
| AC-OLE-01-VA (Active) | D22102601<br>D22102602<br>D22102603<br>D22102604 | 100mM           | 0.2% Vanilla | Glass/PET      | UHT temperature: 135 °C<br>Holding time: 5 seconds |
| AC-OLE-01-VA (Active) | D22111016                                        | 100mM           | 0.1% Vanilla | Glass          | UHT temperature: 135 °C<br>Holding time: 5 seconds |
| AC-OLE-01 (Active)    | D23042701                                        | 100mM           | Unflavored   | Glass          | UHT temperature: 135 °C<br>Holding time: 5 seconds |

Table 24: Composition of UHT treated active formulations

| Active Formulation varieties of AC-OLE-01 & AC-OLE-01-VA |                          |                           |                            |                            |
|----------------------------------------------------------|--------------------------|---------------------------|----------------------------|----------------------------|
| Ingredient                                               | Amount per g             |                           |                            |                            |
|                                                          | 100 mM Buffer Unflavored | 50 mM Buffer 0.2% Vanilla | 100 mM Buffer 0.2% Vanilla | 100 mM Buffer 0.1% Vanilla |
| Tricaprilin                                              | 500 mg (50%)             | 500 mg (50%)              | 500 mg (50%)               | 500 mg (50%)               |
| Phospholipon® 90G                                        | 40 mg (4%)               | 40 mg (4%)                | 40 mg (4%)                 | 40 mg (4%)                 |
| Kolliphor® RH40                                          | 20 mg (2%)               | 20 mg (2%)                | 20 mg (2%)                 | 20 mg (2%)                 |
| Glycerol                                                 | 25 mg (2.5%)             | 25 mg (2.5%)              | 25 mg (2.5%)               | 25 mg (2.5%)               |
| Vanilla Flavor                                           | N/A                      | 2 mg (0.2%)               | 2 mg (0.2%)                | 1 mg (0.1%)                |
| Sucralose                                                | N/A                      | 0.5 mg (0.05%)            | 0.5 mg (0.05%)             | 0.5 mg (0.05%)             |
| Phosphate Buffer, pH 6.8 (50 mM or 100 mM)               | 415 mg (50 mM)           | 412.5 mg (50 mM)          | 412.5 mg (100 mM)          | 413.5 mg (100 mM)          |

[0201] From the data it was evident that all formulations are stable with respect to appearance, assay, related substances, particle size and color. The only attributes that display significant change during storage is pH, which decreases during storage, and taste, which changes somewhat when stored at elevated temperatures.

[0202] FIG. 6A-6D shows graphs of the pH evolution of active formulations with varying levels of vanilla flavor and buffer concentration. From these graphs it is evident that the active

formulations can be ranked in order from most stable to least stable with respect to pH as follows:

*100 mM buffer, unflavored > 100 mM buffer, 0.1% Vanilla > 100 mM buffer, 0.2% Vanilla > 50 mM buffer, 0.2% Vanilla*

**[0203]** These results demonstrate that both higher buffer strength (100 mM vs 50 mM) and lower flavor concentration results in more stable formulations. This is in agreement with the results from the formulation screening DOE.

**[0204]** It is noted that the unflavored formulations have only reached the 3-month time point and the data on the unflavored formulations is therefore limited. Nevertheless, the 3-month data already shows an indication that pH is more stable than flavored formulations since the pH of even the 40 °C samples has barely changed at all.

**[0205]** The overall conclusion that was drawn from the batches prepared with UHT treatment in the manufacturing process is that UHT was successfully incorporated into the process and batches were prepared at higher buffer strength of 100 mM instead of 50 mM. The batches with 100 mM buffer strength displayed better long term stability than batches with 50 mM buffer strength but otherwise equivalent composition. Lowering the flavor concentration (e.g. from 0.2% to 0.1% vanilla) improved the stability even further. There is therefore an option for improving storage stability by adjusting the buffer strength to 100 mM phosphate buffer and/or lowering the vanilla flavor concentration.

**[0206]** Based on the successful incorporation of UHT treatment, the recommended final manufacturing process is as shown in the **FIG. 7**, where UHT is included between the High shear mixing and High pressure homogenization.

#### **Example 12: Large scale manufacturing process for unflavored emulsions**

**[0207]** Large scale batches of unflavored AC-OLE-01 to AC-OLE-10 and vanilla flavored AC-OLE-01-VA formulations have been manufactured for use in clinical trials for unflavored emulsions and vanilla flavored emulsions. A brief overview of the manufacturing process of these large-scale batches and the storage stability are provided. The unflavoured liquid emulsions were manufactured at 20 Kg scale. Formulation AC-OLE-06 as indicated in **Table 1**, containing citrem and AC-OLE-08, also included pH adjustment steps and slightly different excipients but were otherwise identical. The manufacturing process may use an overhead high shear mixer instead of an in-line high shear mixer. It is noted that gamma irradiation of the product after filling was used as bioburden reduction method here.

**[0208]** Ten unflavored formulations named AC-OLE-01 to AC-OLE-10 were manufactured for clinical trials. A stepwise summary of the manufacturing steps is provided below and illustrated in FIG. 1:

STEP 1: Preparation of NaOH

- 1.1 Low pyrogen water was transferred into an appropriate beaker.
- 1.2 Weigh sodium hydroxide into an appropriate container.
- 1.3 Transfer the Sodium hydroxide to the beaker of low pyrogen water and stir until all sodium hydroxide has dissolved using an overhead stirrer equipped with a stainless steel stirrer.

STEP 2: Preparation of 50 mM phosphate buffer pH 6.8

- 2.1 Weigh Sodium Dihydrogen Phosphate Dihydrate into an appropriate container.
- 2.2 Weigh low pyrogen water into a 30L steel Tank1.
- 2.3 Add the Sodium Dihydrogen Phosphate Dihydrate weighed in step 2.1 to the low pyrogen water weighed in step 2.2 and stir the mixture until all the Sodium Dihydrogen Phosphate Dihydrate in the stainless steel Tank1 dissolves using an overhead stirrer.
- 2.4 Adjust the pH of the solution to 6.8 (Target  $\pm 0.1$ ) with the 2M NaOH prepared in step 1.3. Stir the solution for NLT 1 minute after each addition of NaOH solution.

STEP 3: Emulsion preparation

| Step | Action                                                                                                                                                                                                                                                                 | Comments |
|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| 3.1  | Warm Kolliphor® RH40 in an oven with an oven temperature NMT 60 °C. When the Kolliphor® has melted, stir the material manually for NLT 5 minutes before weighing into the solution prepared in step 2.4 and start stirring the mixture using a stainless steel stirrer |          |
| 3.2  | Weigh glycerol into an appropriate container and then transfer to the solution prepared in step 3.1 and continue stirring                                                                                                                                              |          |
| 3.3  | Weigh Phospholipon® 90G into an appropriate container and then transfer to the solution                                                                                                                                                                                |          |

|            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                         |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            | prepared in step 3.2 and continue stirring for NLT 15 minutes                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                         |
| <b>3.4</b> | Weigh tricaprilin into an appropriate container and then transfer to the solution prepared in step 3.3 and continue stirring for NLT 5 minutes                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                         |
| <b>3.5</b> | <p>Set up an IKA in-line high shear emulsifier and connect Stainless Steel Tank1 containing the bulk solution to the inlet of the IKA emulsifier and connect an empty 30L stainless steel Tank2 to the outlet of the IKA emulsifier.</p> <p>Pass the bulk through the IKA emulsifier into 30L steel Tank2. Manually stir the bulk in Tank2 and measure temperature. Gravity transfer the contents of Tank 2 to Tank 1.</p> <p>Pass the bulk through the IKA emulsifier two more times</p> | <p>Chiller system setting: 5 °C</p> <p>Pressure of pressure container: NMT 2 bar.</p> <p>System speed: 13046 rpm</p> <p>Ensure the bulk temperature is NMT 40 °C</p> <p>Temperature measurements for each pass:</p> <p>Pass 1:Initial: 20.5°C Final: 28.1°C</p> <p>Pass 2:Initial: 28.1°C Final: 34.0°C</p> <p>Pass 3:Initial: 34.0°C Final: 38.2°C</p> |
| <b>3.6</b> | In process control sample for particle size measurement                                                                                                                                                                                                                                                                                                                                                                                                                                   | <p>Acceptance criteria: A single peak observed with a d(0.5) particle size NMT 5.0 µm</p> <p>Actual Measurement: d(0.5)=3.118 µm, single peak</p>                                                                                                                                                                                                       |
| <b>3.7</b> | <p>Set up Emulsiflex C55 high pressure homogenizer.</p> <p>Transfer the high shear mixed coarse emulsion from 30L Tank2 to 50L Tank3.</p> <p>High Pressure Homogenize the contents of 50 L Tank3 to 50L Tank4 at 10000 PSI.</p>                                                                                                                                                                                                                                                           | <p>Settings:</p> <p>-C55 pump head chiller: 5°C</p> <p>-C55 heat exchange chiller: 5°C</p> <p>Homogenisation pressure: 10000 PSI</p>                                                                                                                                                                                                                    |

|     |                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|     | <p>Then High Pressure Homogenize the contents of 50 L Tank4 to 50L Tank3 at 10000 PSI.</p> <p>Repeat high pressure homogenizing between Tanks 3 and 4 for a further 3 passes equal to a total of 5 passes.</p> | <p>High Pressure: 12000 PSI</p> <p>High Pressure cutoff: 15000 PSI</p> <p>Low pressure: 0 PSI</p> <p>Low pressure cutoff: 0 PSI</p> <p>Feed pump pressure: 31 PSI</p> <p>Ensure contents of Tank 3 are NMT 45 °C</p> <p>Ensure contents of Tank 4 are NMT 40 °C</p> <p>Temperature measurements for each pass:</p> <p>Pass 1: Initial: 33.7°C Final: 23.9°C</p> <p>Pass 2: Initial: 23.9°C Final: 23.8°C</p> <p>Pass 3: Initial: 23.8°C Final: 33.1°C</p> <p>Pass 4: Initial: 23.1°C Final: 22.2°C</p> <p>Pass 5: Initial: 22.2°C Final: 19.3°C</p> |
| 3.8 | In process control sample for particle size measurement                                                                                                                                                        | <p>Acceptance criteria: A single peak observed with a d(0.5) particle size NMT 0.4 µm</p> <p>Actual Measurement: d(0.5)=0.143 µm, single peak</p>                                                                                                                                                                                                                                                                                                                                                                                                   |

**STEP 4: Filling**

| Step | Action | Comments |
|------|--------|----------|
|------|--------|----------|

|     |                                                                                  |                                                            |
|-----|----------------------------------------------------------------------------------|------------------------------------------------------------|
| 4.1 | The emulsion is filled into 30 mL amber glass containers with screw-cap closures | Fill limits:<br>Lower limit: 23.0 g<br>Upper limit: 25.0 g |
|-----|----------------------------------------------------------------------------------|------------------------------------------------------------|

**STEP 5: Gamma Irradiation**

| Step | Action                                                                    | Comments              |
|------|---------------------------------------------------------------------------|-----------------------|
| 5.1  | The bottles filled in step 4.1 were packed and sent for gamma irradiation |                       |
| 5.2  | Bottles were gamma irradiated at 10 kGy target dose                       | Actual dose: 14.1 kGy |

**[0209]** In process particle size measurements after high sheer mixing (Top) and after pass 5 of high pressure homogenization are provided in **FIG. 8A** and **FIG 8B**.

**Example 13: Large scale manufacturing process for flavored emulsions**

**[0210]** Three vanilla flavored active batches (AC-OLE-01) were manufactured at 85 Kg scale, using a manufacturing process as outlined in **FIG. 9**. The manufacturing process for the vanilla flavored formulations contains six main differences compared to the method used for the unflavored formulations described in **Example 12**:

1. Vanilla flavor and sucralose is added to the buffer-emulsifier mixture. Sucralose is added directly to the buffer-emulsifier mixture, and vanilla flavor is added by first mixing with the tricaprilin.
2. Bottles and caps were gamma irradiated before filling. This was done because no gamma irradiation was done after filling.
3. Formulations were filled in 100 mL amber glass bottles instead of 30 mL bottles.
4. Bottles were filled in a bio-cabinet to avoid microbial contamination. This was also done because no gamma irradiation was done after filling.
5. Gamma irradiation was not done after filling because gamma irradiation had a negative effect on taste.
6. Only 2 passes of high-pressure homogenization were done since particle size measurements showed that this was sufficient.

**[0211]** In process particle size measurements after high sheer mixing (**FIG. 10A**) and after pass 2 of high-pressure homogenization for one of the active formulations (**FIG. 10B**) are shown.

**[0212]** The stability of the emulsions with vanilla flavor at 2-8 °C, 25 °C and 60% relative humidity (RH), and 40 °C and 75% RH at 3 months, 6 months, 9 months, and 12 months is shown in **Table 25**.

Table 25: Characteristics of the manufactured emulsion

| Test                                                              | Acceptance Criteria                                                       | AC-OLE-01-VA lot DE472201 Stability Time Point    |                                                                        |                                                                        |                                                                        |                                                                         |                                                                         |                                                                                            |                                         |                                                                                            |            |
|-------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|-----------------------------------------|--------------------------------------------------------------------------------------------|------------|
|                                                                   |                                                                           | T0                                                | 3 Months                                                               |                                                                        |                                                                        | 40°C/75%RH                                                              | 2-8°C                                                                   | 6 Months                                                                                   |                                         | 9 Months                                                                                   | 12 Months  |
|                                                                   |                                                                           |                                                   | 2-8°C                                                                  | 25°C/60%RH                                                             | White emulsion with no phase separation                                |                                                                         |                                                                         | 25°C/60%RH                                                                                 | White emulsion with no phase separation |                                                                                            |            |
| Appearance                                                        | White to off white emulsion with no or easily reversible phase separation | White emulsion with no phase separation           | White emulsion with no phase separation                                | White emulsion with no phase separation                                | White emulsion with no phase separation                                | White emulsion with no phase separation                                 | White emulsion with no phase separation                                 | White emulsion with no phase separation                                                    | NS                                      | White emulsion with no phase separation                                                    | 25°C/60%RH |
| Particle size d <sub>10</sub> , d <sub>50</sub> , d <sub>90</sub> | Report Results                                                            | 0.070<br>0.137<br>0.259                           | 0.074<br>0.134<br>0.237                                                | 0.075<br>0.138<br>0.247                                                | 0.074<br>0.136<br>0.242                                                | 0.074<br>0.135<br>0.238                                                 | 0.075<br>0.138<br>0.249                                                 | 0.067<br>0.132<br>0.254                                                                    | NS                                      |                                                                                            |            |
| Assay                                                             | 90% to 110% of label claim                                                | 97%                                               | 98%                                                                    | 99%                                                                    | 99%                                                                    | 99%                                                                     | 99%                                                                     | 97%                                                                                        | NS                                      | 97%                                                                                        |            |
| Impurities and Related Substances (% area)                        |                                                                           | Specified                                         | Specified                                                              | Specified                                                              | Specified                                                              | Specified                                                               | Specified                                                               | Specified                                                                                  | NS                                      | Specified                                                                                  |            |
| Glyceryl-diactanoate (Imp 1)                                      | NMT 1.5%                                                                  | Imp 1: 0.51%                                      | Imp 1: 0.68%                                                           | Imp 1: 0.65%                                                           | Imp 1: 0.73%                                                           | Imp 1: 0.57%                                                            | Imp 1: 0.56%                                                            | Imp 1: 0.53%                                                                               |                                         |                                                                                            |            |
| Sum of imp 2-7                                                    | NMT 3.0%                                                                  | Sum of imp 2-7: 0.82%                             | Sum of imp 2-7: 0.82%                                                  | Sum of imp 2-7: 0.82%                                                  | Sum of imp 2-7: 0.82%                                                  | Sum of imp 2-7: 0.82%                                                   | Sum of imp 2-7: 0.83%                                                   | Sum of imp 2-7: 0.83%                                                                      |                                         |                                                                                            |            |
| Imp 8 – (Sum of)                                                  | NMT 0.4%                                                                  | Sum of Imp 8: <0.05%                              | Sum of Imp 8: <0.05%                                                   | Sum of Imp 8: <0.05%                                                   | Sum of Imp 8: <0.05%                                                   | Sum of Imp 8: <0.05%                                                    | Sum of Imp 8: <0.05%                                                    | Sum of Imp 8: <0.05%                                                                       |                                         |                                                                                            |            |
| Any unspecified impurities                                        | NMT 0.2%                                                                  | Unspecified: RRT 0.819: 0.05%<br>RRT 0.821: 0.07% | Unspecified: RRT 0.20: 0.07%<br>RRT 0.819: <0.05%<br>RRT 0.821: <0.05% | Unspecified: RRT 0.20: 0.05%<br>RRT 0.819: <0.05%<br>RRT 0.821: <0.05% | Unspecified: RRT 0.20: 0.10%<br>RRT 0.819: <0.05%<br>RRT 0.821: <0.05% | Unspecified: RRT 0.20: <0.05%<br>RRT 0.819: <0.05%<br>RRT 0.821: <0.05% | Unspecified: RRT 0.20: <0.05%<br>RRT 0.819: <0.05%<br>RRT 0.821: <0.05% | Unspecified: RRT 0.20: <0.05%<br>RRT 0.819: <0.05%<br>RRT 0.821: <0.05%<br>RRT 1.01: 0.07% | NS                                      | Unspecified: RRT 0.20: <0.05%<br>RRT 0.819: <0.05%<br>RRT 0.821: <0.05%<br>RRT 1.01: 0.07% |            |
| Total impurities                                                  | NMT 3.5%                                                                  | 1.5%                                              | 1.6%                                                                   | 1.6%                                                                   | 1.7%                                                                   | 1.5%                                                                    | 1.5%                                                                    | 1.4%                                                                                       | NS                                      | 1.4%                                                                                       |            |
| pH                                                                | 5.5-7.5                                                                   | 6.6                                               | 6.7                                                                    | 6.5                                                                    | 5.8                                                                    | 6.7                                                                     | 6.3                                                                     | 5.9                                                                                        | NS                                      | 5.9                                                                                        |            |
| Microbial Limits: Total Aerobic Microbial Count                   | ≤ 1000 CFU/ g                                                             | < 10 CFU/g                                        | < 10 CFU/g                                                             | < 10 CFU/g                                                             | < 10 CFU/g                                                             | < 10 CFU/g                                                              | < 10 CFU/g                                                              | < 10 CFU/g                                                                                 | < 10 CFU/g                              | < 10 CFU/g                                                                                 |            |
| Total Combined Yeasts and Molds Count                             | ≤ 100 CFU/ g                                                              | < 10 CFU/g                                        | < 10 CFU/g                                                             | < 10 CFU/g                                                             | < 10 CFU/g                                                             | < 10 CFU/g                                                              | < 10 CFU/g                                                              | < 10 CFU/g                                                                                 | < 10 CFU/g                              | < 10 CFU/g                                                                                 |            |
| Specified Microorganisms <i>E. coli</i>                           | Absent in 1 g                                                             | Absent in 1g                                      | Absent in 1g                                                           | Absent in 1g                                                           | Absent in 1g                                                           | Absent in 1g                                                            | Absent in 1g                                                            | Absent in 1g                                                                               | Absent in 1g                            | Absent in 1g                                                                               |            |

NS = Test Not scheduled

**Example 14: Alternate large-scale manufacturing process.**

[0213] A new manufacturing process was developed with essentially the same steps as shown in FIG. 9, but with some modifications (see FIG. 11). Key differences between the two procedures include the use of di-sodium hydrogen phosphate instead of sodium hydroxide to adjust pH to 6.8. Sodium dihydrogen phosphate and di-sodium hydrogen phosphate can be added in correct proportions to reach the target pH of 6.8. Additional differences include the use of high shear mixing with recirculation and use of 7,500 PSI instead of 10,000 PSI during high pressure homogenization.

**Example 15: Summary and conclusions**

[0214] The formulations provided had very good stability, even at room temperature, however a decrease in pH occurred over time. In order to improve stability even further it was therefore decided to investigate factors that impacted pH stability and it was determined that a lower flavor concentration and higher buffer strength significantly improved pH stability.

[0215] To improve long term stability at room temperature storage, it is therefore recommended that the buffer strength is increased to 100 mM and the vanilla flavor concentration is decreased to 0.1%. This gives the compositions shown in Table 23, and this formulation is called AC-OLE-01-VA-b.

**Table 23:** Vanilla flavored active formulation, AC-OLE-01-VA-b

| <b>AC-OLE-01-VA-b</b>                                                                                                  |                     |
|------------------------------------------------------------------------------------------------------------------------|---------------------|
| <b>Ingredient</b>                                                                                                      | <b>Amount per g</b> |
| Tricaprilin                                                                                                            | 500 mg              |
| Phospholipon® 90G                                                                                                      | 40 mg               |
| Kolliphor® RH40                                                                                                        | 20 mg               |
| Glycerol                                                                                                               | 25 mg               |
| Sucralose                                                                                                              | 0.5 mg              |
| Vanilla Flavor<br>(Supplier: Sensient®; Commercial Product Code:<br>AA000851015; Product Name: Intense Vanilla Flavor) | 1 mg                |
| Phosphate Buffer, 100 mM, pH 6.8                                                                                       | 413.5 mg            |

[0216] Although it was possible to prepare microbially stable formulations without any bioburden reduction steps, it is considered necessary to include a bioburden reduction step in the final commercial manufacturing process.

[0217] A UHT process was developed and it was shown that the AC-OLE-01-VA formulation was very robust to UHT treatment and there was no noticeable difference between UHT

treatment at low temperature (130 °C) and high (145 °C) UHT treatment temperatures or short (3 seconds) and long (45 seconds) hold times with respect to physical and chemical attributes or taste. In other words, the formulation can handle a wide range of UHT process conditions without any impact on product quality.

**CLAIMS**

What is claimed is:

1. An emulsion comprising:
  - about 20%-60% by weight of tricaprilin;
  - about 0.1-10% by weight of emulsifier;
  - about 0.1-10% by weight of glycerol; and
  - about 10 mM - 200 mM phosphate buffer (pH 6.0-8.0).
2. The emulsion of claim 1, wherein the emulsifier comprises phospholipids, macroglycerol hydroxystearate, citric acid ester of mono- and diglycerides, or any combination thereof.
3. The emulsion of claim 1 or claim 2, wherein the emulsifier is Phospholipon 90G, Kolliphor® RH40, or a combination thereof.
4. The emulsion of any one of claims 1-3, comprising:
  - 50% by weight of tricaprilin;
  - 4% by weight of Phospholipon® 90G;
  - 2% by weight of kolliphor RH40;
  - 2.5% by weight of glycerol; and
  - 20 mM - 150 mM phosphate buffer (pH 6.8).
5. The emulsion of claim 1-4, further comprising a sweetener.
6. The emulsion of claim 4, wherein the sweetener comprises acesulfame potassium, advantame, aspartame, saccharin, sucralose, luo han guo, purified stevia leaf extracts, or any combination thereof.
7. The emulsion of any one of claims 5 or 6, comprising about 0.01% to about 1% by weight of sweetener.
8. The emulsion of claim 7, wherein the sweetener is sucralose and is present in a concentration of about 0.05% by weight.
9. The emulsion of any one of claims 1-8, further comprising a flavor.

10. The emulsion of claim 9, wherein the flavor is oil-soluble.
11. The emulsion of any one of claims 9 or 10, wherein the flavor is vanilla, mango, berry, or any combination thereof.
12. The emulsion of claim 11, comprising about 0.1% to about 0.3% by weight vanilla flavor.
13. The emulsion of claim 12, wherein the vanilla is present in a concentration of about 0.1% or about 0.2% by weight.
14. The emulsion of any one of claims 1-13, wherein the phosphate buffer is 100 mM phosphate buffer, and the flavor is vanilla present in a concentration of about 0.1% by weight.
15. The emulsion of any one of claim 1-13, wherein the phosphate buffer is 50 mM phosphate buffer, and the flavor is vanilla present in a concentration of about 0.2% by weight.
16. An emulsion comprising:
  - a medium chain triglyceride (MCT);
  - one or more emulsifiers;
  - a buffer; and
  - a sweetener and/or a flavoring agent;wherein the emulsion is an emulsion for oral administration.
17. The emulsion of claim 16, wherein the MCT is tricaprilin.
18. The emulsion of claim 16, further comprising a triol.
19. The emulsion of claim 16, wherein the one or more emulsifiers comprise phospholipids, macroglycerol hydroxystearate, citric acid ester of mono- and diglycerides, or a combination thereof.
20. The emulsion of claim 18, wherein the triol comprises a glycerol.

21. The emulsion of claim 16, wherein the sweetener comprises acesulfame potassium, advantame, aspartame, saccharin, sucralose, luo han guo, purified stevia leaf extracts, or a combination thereof.

22. The emulsion of claim 21, wherein the sweetener is sucralose, stevia, or any combination thereof.

23. The emulsion of any one of claims 21 or 22, comprising about 0.01% to about 1% by weight of sweetener.

24. The emulsion of claim 23, wherein the sweetener is sucralose and is present in a concentration of about 0.05% by weight.

25. The emulsion of claim 16, wherein the flavor is oil-based.

26. The emulsion of claim 25, wherein the flavor comprises vanilla, mango, berry, or a combination thereof.

27. The emulsion of any one of claims 25 or 26, wherein the flavor is present in an amount of about 0.05% to about 0.5% by weight.

28. The emulsion of any one of claims 25-27, wherein the flavor comprises vanilla.

29. The emulsion of claim 28, wherein the vanilla is present at a concentration of about 0.1% by weight or about 0.2% by weight.

30. The emulsion of any one of claims 25-27, wherein the flavor comprises berry.

31. The emulsion of claim 30, wherein the flavor is present in a concentration of about 0.3% by weight.

32. The emulsion of any one of the preceding claims, wherein the emulsion is stable over a period of at least about 3 months, 6 months, 9 months, 12 months or more at about 2 °C to about 8 °C.

33. The emulsion of any one of the preceding claims, wherein the emulsion is stable over a period of 3 months, 6 months, 9 months, 12 months or more at about 25 °C.
34. The emulsion of any one of the preceding claims, wherein the emulsion is stable over a period of 3 months, 6 months, 9 months, 12 months or more at about 40 °C.
35. A method of making an emulsion comprising tricaprilin, wherein the method comprises
- a) feeding ingredients comprising a buffer, tricaprilin, and one or more emulsifiers to a vessel;
  - b) high shear mixing the buffer, tricaprilin, and one or more emulsifiers in the vessel to form a course emulsion;
  - c) optionally sterilizing the course emulsion to form a sterile emulsion; and
  - d) high pressure homogenizing (HPH) the sterile emulsion to form the emulsion comprising tricaprilin.
36. The method of claim 35, wherein step (c) is a ultra-heat-treatment (UHT) step, an irradiation step, or a combination thereof.
37. The method of claim 35 or claim 36, wherein step (c) is a UHT step.
38. The method of claim 37, wherein the UHT step (step c) is conducted at a temperature of about 130 °C to about 145 °C and for about 3 seconds to about 45 seconds.
39. The method of claim 38, wherein the temperature is about 135 °C and the hold time is about 2-3 seconds.
40. The method of any one of claims 35 - 39, wherein step (c) does not comprise a irradiation step.
41. The method of claim 35, wherein a flavor is mixed with the tricaprilin in step (a).
42. The method of any one of claims 35-41, wherein the one or more emulsifiers comprise Phospholipon® 90G, Kolliphor® RH40, or a combination thereof.
43. The method of any one of claims 35-42, wherein gamma irradiation is not used.

44. The method of any one of claims 35-43, wherein the emulsion is the emulsion of any one of claims 1-34.

45. The method of any one of claims 35-44, wherein the emulsion is stable for at least 3 months, at least 6 months, or at least 12 months.

46. The method of any one of claims 35-45, wherein the emulsion has a particle size of 0.01-10  $\mu\text{m}$  in size.

47. The method of any one of claims 35-45, wherein the high pressure homogenization is done at a pressure of about 5,000 PSI to about 10,000 PSI.

48. The method of claims 47, wherein the high pressure homogenization is done at a pressure of about 7,500 PSI.

49. The method of any one of claims 35-48, wherein the method further comprises a high shear mixing step with recirculation.

50. A method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of the emulsion of any one of claims 1-34.

51. A method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of an emulsion comprising:

- about 20%-60% by weight of tricaprilin;
- about 0.1-10% by weight of emulsifier;
- about 0.1-10% by weight of glycerol; and
- about 10 mM - 200 mM phosphate buffer (pH 6.0-8.0).

52. The method of claim 51, wherein the emulsion comprises:

- 50% by weight of tricaprilin;
- 4% by weight of Phospholipon® 90G;
- 2% by weight of Kolliphor® RH40;
- 2.5% by weight of glycerol; and
- 20 mM - 150 mM phosphate buffer (pH 6.8).

53. The method of any one of claims 47-49, wherein the disease or disorder comprises Age-Associated Memory Impairment (AAMI), Alzheimer's Disease (AD), Parkinson's Disease, Friedreich's Ataxia (FRDA), GLUT1-deficient Epilepsy, Leprechaunism, and Rabson-Mendenhall Syndrome, Coronary Arterial Bypass Graft (CABG) dementia, anesthesia-induced memory loss, Huntington's Disease, infantile Spasms, migraine and related headaches.