Nanoemulsion Workflow & General Information

using the #Q700CA Sonicator

for Cannabinoids

Definitions:

Aqueous: Water-based.

Hydrophile: A molecule that readily dissolves in water.

Lipophile: A molecule that readily dissolves in fat.

Surfactant: A surface active molecule-often amphipathic.

Solubility: The affinity for one substance to dissolve into another.

Suspension: An often temporary dispersion of insoluble particles in a solvent.

Solution: A mixture of a solute into a solvent.

Solvent: The material in which a solute dissolves into.

Solute: The material in which a solvent contains.

Second Law of Thermodynamics: Entropy of the universe is constantly increasing.

Conglomeration: The huddling of lipid molecules-often small droplets becoming one large droplet.

Complex (bond): A bond that is not covalent, but rather through intermolecular forces such as hydrogen bonding.

Colloid: The dispersed phase of an emulsion.

Emulsion: A system containing two parts: a dispersed phase and a continuous phase. This system is mediated by a surfactant.

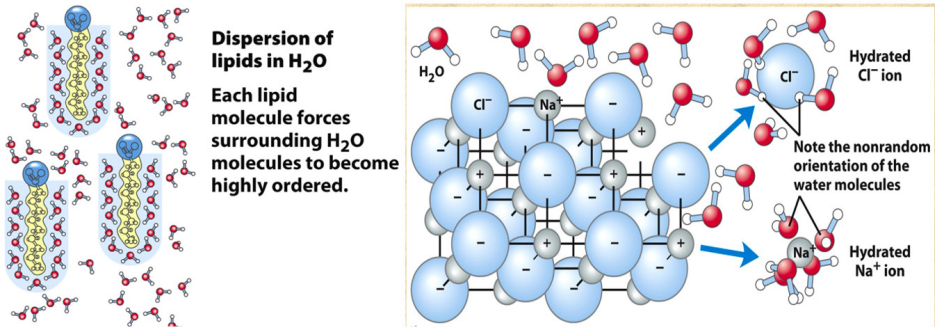
Amphipathic: Containing both polar and non-polar groups

Surface active: Mediate's surface tension between water and fat by the reduction of interface tension

Background:

Although a long-established and well-documented concept in the pharmaceutical space, nanotechnology has received increased attention in cannabis applications as a promising tool to enhance lipophile compatibility in aqueous solutions. An aqueous solution is considered to be preferred in many drug delivery applications due to water's compatibility with the human body, and it is known that fat-soluble drugs are limited by reduced bioavailability due to poor water solubility. The fundamental insolubility of lipophilic molecules in water is due to the entropic disruption of these hydrogen bonds, and the amount of disruption has a positive relationship with particle size all else held equal. On a molecular level, water must rearrange itself around nonpolar moieties if they are introduced into a solution. The electrostatic forces imposed by the water on fat droplets causes conglomeration in order to maximize hydrogen bonding complexes, therefore, increasing entropy in a manner consistent with the second law of thermodynamics. The mechanism by which particle size reduction mediates entropic unfavorability is through increasing the surface area to volume ratio of a fat droplet. Figure 1.1 shows the activity of water before and after the introduction of a lipid.

Figure 1.1: Entropic Arrangement of Water Due to the Hydrophobic Effect



Hydrophobic Effect Ionic Effect

A conventional colloid suspension such as milk is an example of a kinetically stable emulsion, which simply means separation occurs at a much slower rate relative to a less stable system. If a small enough particle size is achieved, it may be possible to attain a highly kinetically stable suspension, which is often referred to as a nanoemulsion. Ultrasonic processors can reduce particle size to sub-micron

sizes (<100nm) and are used to create nanoemulsions. However, simply reducing particle size is seldom powerful enough to overcome the hydrophobic effect, and the use of a surfactant is often necessary. However, simply reducing particle size is seldom powerful enough to overcome the hydrophobic effect, and the use of a surfactant is often necessary. A broad interpretation of a surfactant is any molecule that exhibits surface activity, and the most commonly known one is soap. The surfactant is surface active due to containing both polar and nonpolar groups, which is defined as being amphipathic. The overall goal of employing a surfactant is to reduce intermolecular tension caused by the hydrophobic effect and mainly occurs at the interface between oil and water. The mechanism by which a surfactant reduces tension at interfaces is by complexing it's polar and non-polar groups accordingly. For example, the carbon chain of surfactant would face the lipophile, while the remaining polar groups would face the water molecules in solution, and a classic example of this is the cell membrane. There are a plethora of nuances in the chemical composition of surface-active molecules, and selectivity is necessary to achieve proper emulsification. If one were to add a random surfactant into a water and oil mixture, it is unlikely the resulting emulsion would be viable. In short, the proper surfactant in the correct amount added to an oil and water mixture is the basis of a successful emulsion, but these emulsions can be massively augmented with particle size reduction techniques. This document can be used to aid in the formulation of conventional emulsions as well as nanoemulsions given the key difference is physical processing.

Determine Desired Emulsion:

In a simplified model, there are two types of emulsions: oil in water (o/w), and water in oil (w/o). **The most popular emulsion in pharmaceuticals, as well as the cannabis space, is an oil in water emulsion**. This type of emulsion is useful when the majority of the solvent is water, but the chemist wishes to add oil in a smaller amount and will be the main topic of this document. The type of emulsion desired greatly influences the resulting process, and will also dictate which surfactant to be used.

Determine Surfactant Composition and Mixture:

In order to determine the best surfactant for the desired emulsion, the hydrophilic-lipophilic difference (HLB) method remains the most popular method of navigating available choices. In short, the HLB system assigns a unitless, arbitrary number to a molecule as a means of describing its chemical

composition. The values begin at 0 and end at 18. Per figure 1.2, the lower value indicates fat solubility, while the higher numbers indicate water solubility. The overall HLB value of surfactant(s) should generally correspond with the dominant solvent, therefore, the (o/w) emulsion is most receptive to higher HLB molecules. Table 1.1 shows a list of popular surfactants with their corresponding HLB value. While a convenient method, there are additional considerations when formulating such as:

1. The general safety of surfactant(s): The FDA has identified a database of chemicals considered to be generally regarded as safe (GRAS), and is a common guide when considering a reagent.
2. Consumer appeal: The cannabis and cannabinoid market is intrinsically based off of the idea that natural products are better for overall health. Choosing a reliable, yet artificial surfactant such as polysorbate 80 may be problematic to your customer base.
3. Blending surfactants: Many companies currently market proprietary surfactants and these products are often blends that have been experimentally determined by the company to be versatile and effective. Blending surfactants are often more effective than using just 1 molecule for reasons beyond the scope of this document. **It is common practice in the formulation space to combine high HLB molecules with low HLB molecules**.

Table 1.1: Commonly Used Surfactants and Their HLB Values

Trade Name	INCI Name	HLB Value
Glyceryl monostearate	Glyceryl monostearate	3.8
PEG 400 Monoleate	Polyoxyethylene monooleate	11.4
PEG 400 Monostearate	Polyoxyethylene monostearate	11.6
PEG 400 Monolaurate	Polyoxyethylene monolaurate	13.1
Potassium oleate	Potassium oleate	20.0
Sodium lauryl sulfate	Sodium lauryl sulfate	40
Sodium oleate	Sodium oleate	18
Span 20	Sorbitan monolaurate	8.6
Span 40	Sorbitan monopalmitate	6.7
Span 60	Sorbitan monostearate	4.7
Span 65	Sorbitan tristearate	2.1

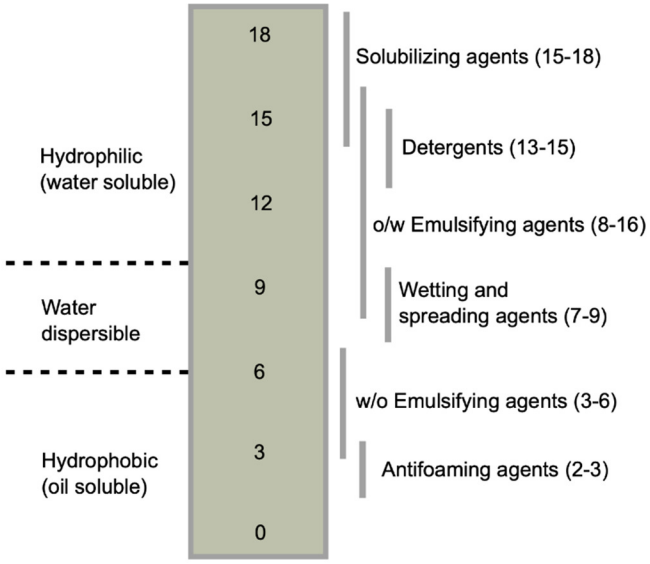
Span 80	Sorbitan monooleate	4.3
Span 85	Sorbitan trioleate	1.8
Triethanolamine oleate	Triethanolamine oleate	12
Tween 20	Polyoxyethylene sorbitan monolaurate	16.7
Tween 21	Polyoxyethylene sorbitan monolaurate	13.3
Tween 40	Polyoxyethylene sorbitan monopalmitate	15.6
Tween 60	Polyoxyethylene sorbitan monostearate	14.9
Tween 61	Polyoxyethylene sorbitan monostearate	9.6
Tween 65	Polyoxyethylene sorbitan tristearate	10.5
Tween 80	Polyoxyethylene sorbitan monooleate	15.0
Tween 81	Polyoxyethylene sorbitan monooleate	10.0
Tween 85	Polyoxyethylene sorbitan trioleate	11.0

For your convenience, table 1.2 is a list of popular, “natural” surfactants in the industry.

Table 1.2: Clean Label Surfactants/Emulsifiers:

Trade Name	INCI Name	HLB Value
Q Naturale	Quillaja extract	~14
PC90	Phosphatidylcholines *Known as Lecithin(s) If purity <60%	~8-9
Yucca Schidigera Extract	Saponin Rich Yucca Extract	>10
Cyclobetadextrins	Cyclobetadextrins	>8

Figure 1.2: Emulsifier Scale



Select Carrier Oil:

Many formulas choose to dissolve their cannabis compounds into a carrier oil, but it is a misconception that they are essential every time. The overall purpose of carrier oils is to modify the properties of the dispersed (oil) phase in oil in water emulsions. The chief property of interest for the purpose of this document is the density of the dispersed phase. Figure 1.3 shows Stoke's equation, and is the mathematical framework by which many drug formulators design their emulsion. For example, if one were to observe the density of a full spectrum oil versus water, it is possible to find it is denser than water. In this case, it would be wise to use a carrier oil with a density lower than water in order to achieve an average density close to water. There is also speculation that using a carrier oil that is better received by the body in a physiological environment may increase the bioavailability of cannabinoids, and that longer chain oils promote long term stability. Table 1.3 is a list of popular carrier oils used in the industry.

Figure 1.3: Stoke's Equation

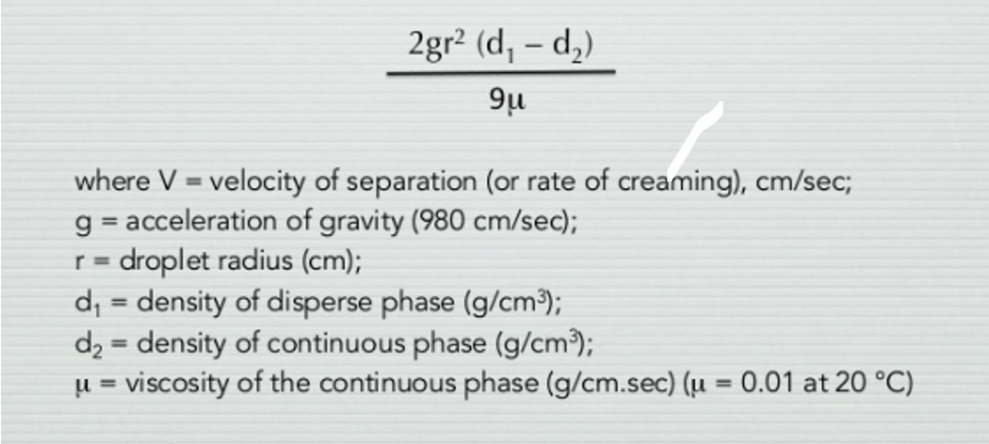


Table 1.3: Common Carrier Oils:

Trade Name	Chain Length
Olive Oil	~14-24
Castor Oil	~17
Medium Chain Triglyceride (MCT) Oil	~6-12
Mineral Oil	~17

*Oil composition varies by manufacturer

Additional Formulation Considerations:

While the selection of the proper emulsion type, surfactant blend, and carrier oil are the fundamentals of creating a stable emulsion, there are many more factors that determine the efficacy of an emulsion. These factors include but are not limited to pH, amount of ions in solution, temperature, and viscosity of the continuous phase. While there are effective nonionic surfactants, many surfactants are ionc, which means their charge can be modulated through changes in pH. Depending on the particular sample matrix, it is essential for one to experimentally target the best pH for them. The most common agents used to alter pH in the industry are **citric acid, maleic acid, phosphoric acid and sodium bicarbonate**.

While altering pH is directly related to the number of ions in solution, it at times necessary to neutralize ions without severely affecting pH for formulation reasons. Therefore, **chelating agents such**

as EDTA are used for this application. For the scientific basis of this concept, please see DLVO theory, and a link will be included at the bottom.

Temperature and viscosity are two components of an emulsion that must be considered. While it is almost always necessary to heat components to get them in solution, knowing the individual melting and boiling points of reagents is crucial to ensuring long term stability. For example, the surfactant quillaja will begin to boil around the temperature phosphatidylcholine melts at an appreciable rate. Allowing this to occur will result in an adultered reagent, as the composition is no longer truly known.

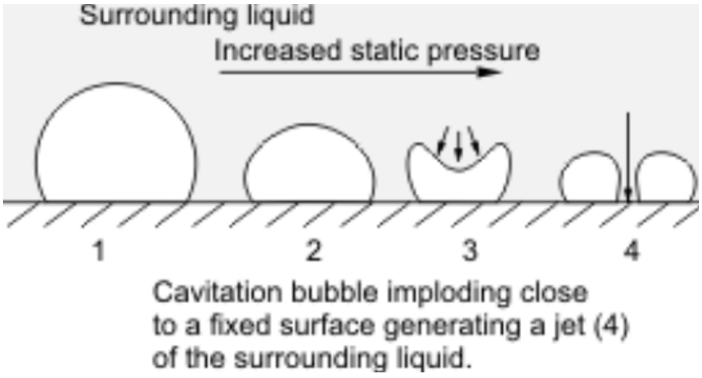
An additional component of Stoke's law is the viscosity of the continuous phase. Nearly all emulsions in dietary supplements contain thickening agents as a means of slowing oil and water separation. At a molecular level, these thickeners help to physically inhibit the conglomeration of oil by making it more difficult for atoms to move about in solution, and therefore stifling Brownian motion. The two most popular thickening agents for the application of this document are **xanthan gum and gum Arabic**.

Determine Hardware Processing Parameters:

This document is applicable to any conventional emulsion as well as nanoemulsions. If one wishes to create a simple emulsion, then the only further step is to use a conventional overhead stirrer/homogenizer to mix ingredients. A stir plate is not sufficient outside of a pre-emulsion, which is an optional step used to prime reagents in solution and will be included in a separate protocol document.

To create a true nanoemulsion a Sonicator is required. The process of Sonication utilizes the sinusoidal nature of sound waves to create strong pressure differentials, therefore, resulting in a phenomenon known as cavitation. In short, cavitation reduces particle size in a way quite similar to a molecular explosion. Figure 1.4 shows a basic diagram of the process of cavitation. A byproduct of cavitation is heat so temperature control is essential. Samples must be chilled during Sonication. A simple ice bath is sufficient for most applications but larger volumes may benefit from the use of a chiller. In addition, the Sonicator has a pulse function and can be programmed to cycle on/off to help maintain an acceptable sample temperature.

Figure 1.4: Simplified Diagram of Cavitation



Different sample formulations for an emulsion will affect the processing method, and higher oil loads will generally require longer run times and/or higher intensity/amplitude settings. The Q700CA Sonicator system includes 2 probes for a range of processing volumes up to approximately 1,000ml. The Q2000 system can be used for production scale volumes.

For batch sizes of roughly 200ml or less, the ½" diameter probe is sufficient for processing and should be done in a beaker that has enough overhead to contain any possible foaming. If your formulation has a high concentration of surfactant, foaming will likely occur. Foaming should be minimized by adjusting probe depth. For instance, if the liquid is excessively foaming at modest surfactant concentrations, the probe is likely too shallow and the particle size reduction will not occur homogenously. Conversely, observing no activity such as rippling or bubbling at the top of the solution indicates a probe that is overly submerged. Using a properly sized beaker the probe should be inserted approximately halfway into the liquid volume. A 250ml beaker is acceptable for 100-200ml samples. Use of a larger beaker for this volume will result in poor outcomes.

For batch sizes of approximately 400-1,000ml, it is recommended to use the 1" diameter probe. Beaker size should correlate with sample volume. For example, a 2,000ml beaker is recommended for 1,000ml samples.

In addition to cooling the sample, the Converter (silver cylindrical component above the titanium probe) must also be prevented from overheating. The ½" probe does not require external cooling but the 1" probe must be run on an air cooled converter. See the instruction manual for guidance on how to use an air compressor to cool the converter.

It is strongly advised to closely monitor sample conditions during processing, and the apparatus should not be left unattended while running.

Verify Results:

In order to remain compliant with CFR 201/502 of the Food Drug and Cosmetic Act, one must provide verified test results in order to make any label claims related to nanosizing. The best method of analyzing particle size is dynamic light scattering (DLS) and is a common 3rd party laboratory service. However, if the sole purpose of verification is for R&D, homogeneity and particle size can be estimated with qualitative methods. For example, a strong laser pointer will display the distribution of the dispersed phase for an estimate. Additionally, the particle size of the dispersed phase shares an inverse relationship with solution clarity. If the emulsion began as milky white but is now relatively clear, micro/nanoparticles are likely to present in the sample.

Additional Resources:

Link to DLVO Theory Lecture: https://www.youtube.com/watch?v=nrymj8NOG-8

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