

Review

Improving Product Quality with Entrapped Stable Emulsions: From Theory to Industrial Application

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Abstract: Entrapment of sub-micron scale emulsions containing active ingredients into macro-scale matrices has exhibited great potential as a delivery vehicle with controlled release capabilities, however optimization remains unrealized. Reported here are methods used to improve product quality by optimizing the emulsion formation steps. These methods are in conjunction with the precepts of Process Intensification (PI). Success with pharmaceuticals and chemical reacting systems provides a strategy for a wide range of applications; the emphasis here being nutraceuticals. Use of a nano-technology platform assists in: (a) product quality improvements through better nutrient dispersion, and thus bio-efficacy; and (b) production efficiencies through implementation of PI concepts. A continuous methodology, utilizing these PI concepts, that approximates a bottom-up approach to the creation of sub-micron and nano-emulsions is the basis of the technology presented here. Note that solid particles may result during post-processing. The metrics of successful processing include obtainment of nano-scale species with minimal input energy, reduced processing steps at higher throughput rates, and improved quality without over-usage of key ingredients. In addition to flavor and wellness characteristics, product stability for extended shelf life along with an appreciable cargo load in the entrapped emulsion is a major concern. Experimental protocols and path forward recommendations to overcome challenges and meet expectations in these emerging opportunities are also presented.

Keywords: emulsion; nutraceuticals; bio-efficacy; nanoemulsion; emulsion stability

1. Introduction

It is well documented that the majority of Americans suffer from vitamin and essential fatty acid (such as Omega-3) deficiencies [1,2]. The ever-quickening pace of modern life-styles and correlate high stress and stress-related behaviors (such as smoking [3]) increase the likelihood that an individual will consume less than the required daily intake of these vital substances. Consequently, a push in the nutraceuticals industry to place these substances in higher, more-efficiently dispersed quantities in our food has resulted.

Despite success with advanced delivery and transport technologies in the areas of pharmaceuticals and chemical reacting systems, barriers to success in the area of nutraceuticals exist. These include quality, taste, efficiency of delivery (which is directly tied to stability), and subsequent shelf-life of the products, as well as requirements by regulatory bodies such as the US FDA, and other consumer acceptance concerns [4–12]. The use of nano-emulsions directly, for example in fluids, such as beverages, is an obvious application already commercialized [7–9,13]. A not so obvious use is the entrapment of nano-emulsions in solid matrix materials that are further incorporated into final products. The demand for emulsion entrapment technologies is estimated to be rising at an extremely high rate—about 10 percent every year, with new markets and opportunities emerging on a consistent basis. [4–9]. A major driving force underlying entrapment innovation is the growing functional foods market.

This work is concerned with submicron emulsions, which are typically kinetically stable and substantially more stable than micron size emulsions [14]. Such emulsions are not necessarily nano-emulsions, which are typically at the very end of the size spectrum, below 100 nm in size, and are thermodynamically stable. Currently, there are no regulations regarding the use of either submicron or nano-emulsions, but recently, the US FDA communicated its intent for regulations on nano- materials used in food and cosmetics in the future. In this work, the terms “nanoemulsions” and “sub-micron” emulsions are used interchangeably, mainly due to the lack of widely accepted terminology in the literature.

This work is an effort to provide comprehensive insights into the fundamental mechanisms involved in the production steps and the protocol for a rational optimization strategy to expand this nano-emulsion/entrapment process to a wide range of products. Further, this work illustrates how to select suitable process parameters to optimize the manufacturing process and quality of products that contain entrapped nano-scale emulsions, specifically in the area of nutraceuticals. The underlying motivation is to improve nutritional value while maintaining and/or enhancing shelf-life and consumer acceptance.

The processing technology presented here is a continuous methodology that utilizes PI concepts to approximate a bottom-up approach to the creation of nano-emulsions for incorporation into the entrapping matrix. Post-processing, the emulsified droplet may be transformed to a solid phase. Representative model compounds used here for illustration are algal oil, vitamins A and E, and bee’s wax. Algal oil and vitamin E are representative of compounds that remain in the liquid phase when the nano-emulsion is entrapped into a solid matrix, while vitamin A and bee’s wax are representative of those that solidify at room temperature, *i.e.*, post emulsion formation. Furthermore, bee’s wax represents compounds that can be used effectively in a controlled/extended release scenario, post

consumption of the final product. An example is the slow release of caffeine from its cargo-loaded vesicle, which was initially entrapped into a solid food substance.

2. Current Achievements and Objectives

2.1. Materials of Interest

The nutritional value of omega-3 fatty acids is widely accepted; included are the healthy benefits for the heart, blood circulation, and the immune and nervous systems [1,2,15,16]. For supplementation, omega-3 is commonly added in several types of food. However, due to its high degree of unsaturation, autoxidation is a particularly undesirable event. This is especially true for the long chain fatty acids, DHA and EPA. During the oxidation process, volatile secondary lipid oxidation compounds are formed. Many of these compounds have a very low odor threshold, leading to development of off-flavors, and thus a decreased shelf-life.

The selection of algal oil as one of the key ingredients to be studied here is also based upon a general consensus in the food industry to add omega-3 (*i.e.*, polysaturated fatty acid docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) derived from algal oil) into long-shelf-life foods, such as cereal. In response to the sensitivity and easily oxidizable property of omega-3, entrapment is required to protect these fatty acids from autoxidation and thus prevent off-flavor. Use of flavor-masking compounds, in addition to the benefits obtained with entrapment technologies, is also viable in improving consumer acceptance. With the aims of achieving more effective entrapment and driving down DHA/EPA cost per serving, optimized entrapment technology is currently undergoing substantial research efforts throughout multiple industries [17–23].

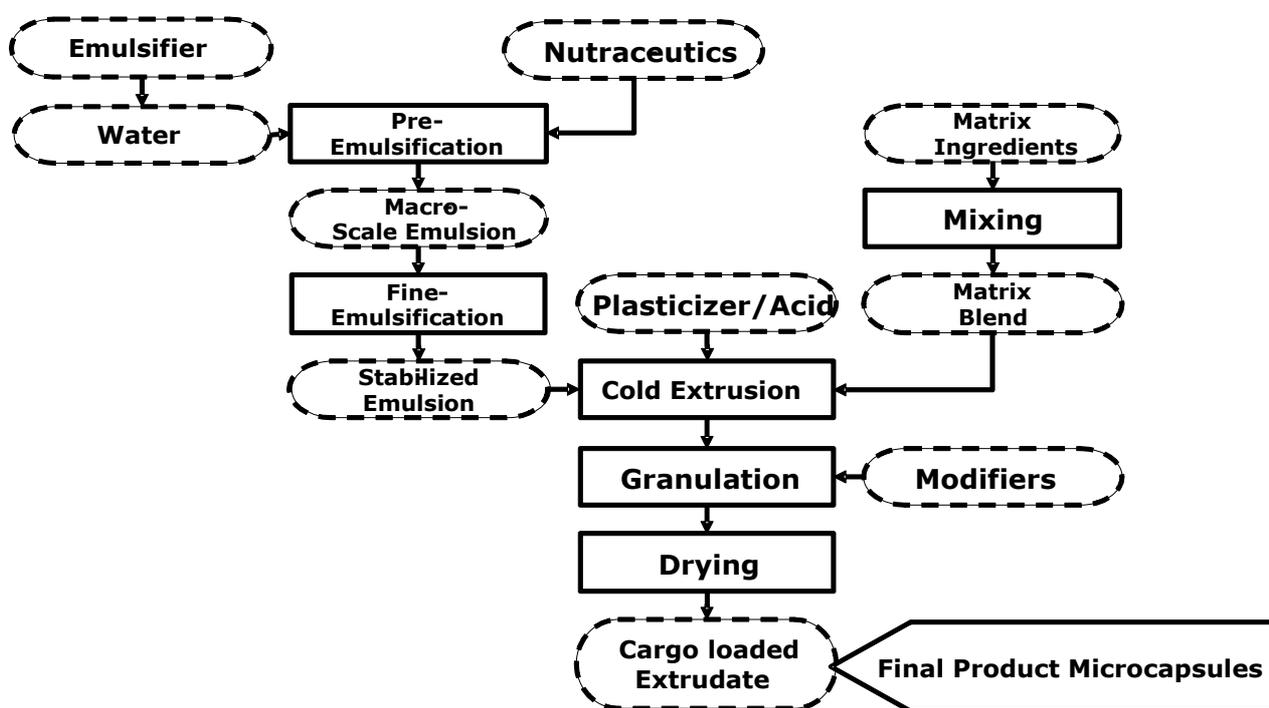
Vitamin E (α -tocopherol) is also a lipid soluble substance that has similar characteristics to that of algal oil. It is mentioned here primarily since it can be used as an antioxidant in the algal emulsion, in addition to its direct use as a nutraceutical.

Another entrapment target, Vitamin A, is a fat-soluble and easily oxidized nutraceutical that plays a critical role in promoting eye and skin health [1,24]. Therefore, entrapment is employed to protect these sensitive ingredients against adverse environmental conditions and to control their release. Since there are potential toxicity issues with over-dosage, controlled release becomes an important feature. Eye health is related to Retinal (a metabolite that is a hormone-like growth factor), and epithelial and other cells are affected by Retinol, also available after in-situ biochemical reactions. Despite its over-dosage toxicity, Vitamin A is an essential nutraceutical. It is estimated that one-third of children under the age of 5 worldwide are deficient in Vitamin A, and that in developing countries 500,000 each year become blind because of this deficiency [24].

The approach proposed here, via an emerging technology platform, is to incorporate Vitamin A into a solid matrix (such as starch) from its nano-emulsion state after it is processed as a liquid. Since it is a solid at room temperature it presents an interesting model compound for studies directed at the entrapment of nano-particulates. Prior commercial successes such as plant sterols in orange juice provide additional incentives and confidence that the probability of a successful wide-scale implementation into human-quality foods is high, and will thus lead to an expanding market [11,17–23].

Bee's wax, being a solid at room temperature, provides the ability for a controlled, sustained delivery protocol for its cargo. Caffeine was mentioned previously as an example. This strategy is, however, applicable to both water and lipid soluble compounds, and thus a wide spectrum of nutraceuticals, especially volatile hydrophobic ones. The current, widely accepted emulsion/entrapment process consists of three major unit operations: emulsification, entrapment, and drying. In general, emulsification is used to create oil-in-water nano-emulsions for subsequent stabilization; entrapment is to embed the oil droplets into matrix material to create final pellets; subsequent drying is used to remove superfluous water within this matrix product. The schematic process is shown in Figure 1. The dashed-line portions represent ingredients and the solid boxes indicate processing steps.

Figure 1. Overview of the current nano-emulsion/entrapment process.



The microcapsule product has a core-shell-matrix configuration. The inner sphere is the nutraceutical agent (as a solid or cargo loaded liquid droplet); the surrounding shell/film is composed of emulsifiers, with these emulsified vesicles dispersed throughout the entrapping matrix material.

2.2. Processing Methods

Preparation of the nano-emulsion using a pre-existing nano-technology platform [4–13,25,26] will assist in (a) product quality improvements through better nutrient dispersion (and thus bio-efficacy); and (b) production efficiencies through compatibility with the precepts of Process Intensification (PI). The measures of successful processing include obtainment of nano-scale species with minimal input energy, reduced processing steps at higher through-put rates, and improved quality while using less emulsifier.

Optimization of the emulsification process and surface active agent formulation are major considerations since a quality emulsion, with nano-sized droplets in a narrow size distribution, is a key component for enhanced stability. Furthermore, higher entrapment efficiency can be expected.

Reduction in emulsifier requirements, while maintaining this quality emulsion, can result in significant cost savings. Additional wellness benefits can be obtained since allergen (and possible toxin) levels are thereby lowered. Potential optimization strategies for production of a nano-emulsion prior to the stabilization step are discussed in detail in later sections (along with pertinent references).

The key parameters affecting the production of a nano-emulsion are identified; including energy input and efficiency, apparatus type, emulsifier type and concentration, and operating conditions such as temperature and pressure. The main focus lies in discussing the potential benefits of process innovations such as system configurations and operational maps for this emulsification step. Optimization strategies are proposed for implementation at the commercial production scale that involves screening for the most efficient emulsification conditions.

With respect to the entrapment step, the cold extrusion process serves as the current method. However, this method involves numerous aspects subject to improvement, such as matrix type, matrix ingredients and concentrations, use of antioxidant, *etc.* Characteristics of the entrapping matrix, such as its glassy state, porosity and viscosity, determine its functionality in the extrusion process. Furthermore, water content and pH play a critical role in preventing oxidation of the emulsified cargo. Parameters for this evaluation include observation of excess surface oil, oxidative stability, and accelerated shelf-life testing of the final pellets. These data also provide an indication of the efficacy of the nano-emulsion production process itself.

2.3. Objectives of This Work

Priority R&D project objectives for emulsification/entrapment of nutraceuticals, particularly with respect to oxidation susceptible substances such as omega-3, must include: (i) extension of shelf-life stability to at least 12 months (for example in cereal products); (ii) removal of allergen ingredients used currently, such as dairy allergen from sodium caseinate and wheat allergen from the matrix; (iii) identification of label-friendly antioxidants that maintain or slow the current rate of product oxidation; (iv) increasing the cargo load in final matrix pellet without loss in stability, thereby lowering total ingredient costs and reducing the mass of pellets that need to be applied to the final consumer product; and (v) overcoming several operational challenges in the extrusion process, such as die plugging due to non-hydrated ingredients.

Suppliers of other nutrient rich supplements (*i.e.*, vitamins, minerals, *etc.*) anticipate that similar nano-emulsion/entrapment processes will extend beyond the preliminary stages with innovative food formulators, thus mirroring the expansions experienced in the pharmaceutical industry with respect to nano-medicines [8,9,27].

3. Evaluation of Technology and Process Changes

Presented in the next few sections are the most important aspects of the technology, from preparation of the emulsions to their entrapment inside solid matrices. Specifically, included are the processing parameters, such as the energy input and formulation parameters that may affect the stability and functionality of emulsions and the final products. Table 1 below contains the summary of the parameters to be discussed.

Table 1. Factors influencing emulsion preparation and entrapment.

	Emulsion Preparation	Emulsion Entrapment
Processing Parameters	<ul style="list-style-type: none"> • Mechanical energy • Energy intensity • Equipment type • Bottom up vs. top down • Time scales • Energy input and transfer • Temperature 	
Formulation parameters	<ul style="list-style-type: none"> • Surface Active agent • Emulsifier concentration • Oil Content • Physicochemical properties • Other 	<ul style="list-style-type: none"> • Matrix type • Glass transition temp • Porosity and O2 permeability • Viscosity • Water content and humidity • Antioxidants • pH

The use of the term bottom-up to describe the method used here for emulsion preparation is not strictly correct. It is the term adopted here since the technology platform used for illustration is that which is applicable in the production of nano-materials through bottom-up, molecular processing [4,7,10–12]. When used for the formation of solid particles through crystallization or chemical reactions, the method is purely bottom-up in which particles are growing within turbulent eddies. However, when used for the formation of emulsions the process may not be strictly bottom-up at the molecular level. Nevertheless, it is single step and continuous, while the top down method has additional steps and is usually a batch process. Additional comments and comparisons are given in later sections. For purposes of clarity and continuity in presenting these comparisons, *etc.*, we will no longer refer to the single step approach as approximating a bottom-up method, but simply as one.

3.1. Emulsification

To meet the objective of increased shelf-life of products, the generally accepted hypothesis is that a stable nano-emulsion with a narrow size distribution is required. An optimum quantity of emulsifier is also important. There must be enough to provide sufficient surface coverage and to yield adsorption/interaction kinetics greater than droplet-droplet collision frequencies, without an excess that would contribute to reduced performance, poor quality, and increased costs. The micelles formed by the excess emulsifier can alter the physicochemical properties of the media that affect processing, in addition to flavor and possible allergen issues.

Inspired by the broad application of nano-technology for dispersions, crystallization, and emulsions/entrapments in the pharmaceuticals industry, several studies have confirmed these nano-technology based concepts are also able to deliver benefits for food manufacturers and customers [17]. The production and control of submicron emulsions with a narrow size distribution has received considerable attention. The emphasis is toward use of a platform technology that was designed, developed and validated in both the pharmaceuticals and advanced materials industries by producing nano-scale entities [7]. Applying this approach here yields a high probability of increased

emulsion stability and thus the efficiency of the subsequent microentrapment process, as well as final product bio-efficacy.

3.1.1. Advantages of Nano-Emulsions

Benefits obtained by using nutraceutical-enriched nano-emulsions for entrapment within a food matrix include:

- (a) Nano-emulsions can persist as kinetically stable systems: Although they are thermodynamically unstable, emulsions containing nano-scale droplets appear to be kinetically stable systems able to resist several instability phenomena (Appendix) [14]; that is, (i) the small droplet sizes significantly reduce gravitational effect; (ii) collision frequency is lower (Brownian diffusion) helping to prevent creaming and sedimentation; (iii) steric limitations associated with smaller droplet sizes reduces the probability for coalescence; and (iv) narrow droplet size distributions lead to a reduction in Ostwald ripening.
- (b) Stable nano-emulsions are a prerequisite for an efficient entrapment process: Characteristics such as stability and droplet size play a key role in optimizing the entrapment efficiency [28–32]. Smaller droplet sizes lead to higher dispersability in downstream processing and contribute to less deformation under shear forces encountered during the entrapment process (see Section 3.2). An increase in droplet size has been reported to correlate with a decrease in microentrapment efficiency [33]. A stable nano-emulsion can also increase the retention of volatiles via vapor-pressure suppression and improve shelf-life of entrapped oil products through reduction of the amount of free oil present from leakage and/or being initially unbound [18,34–36].
- (c) Nano-emulsions contribute to enhanced product performance: Release rates can be controlled more effectively using small droplet sizes and associated barrier properties of the surface film formed by the emulsifying agent. Timely release of controlled amounts of the active agent can prevent undesired systemic clearance rates and improve bioefficacy. Further, small droplet sizes facilitate translocation of nutrients across bio-barriers for better absorption (uptake) rates [17]. The transport parameters of the barrier can also help protect the cargo from degradation mechanisms such as oxidation. Nano-emulsions are also found to increase the synergetic influence of introduced antioxidants [17].

3.1.2. Preparation of Nano-Emulsions-Theoretical Considerations

To produce nano-emulsions with high stability, several key factors must be considered, as follows [7]:

- (a) Surface active agent: The type and concentration of emulsifiers must be carefully selected based on their transport properties, their adsorption interactions with both coated and uncoated droplets (*i.e.*, collision frequency, effectiveness, and energetics), and stabilization requirements [14].
- (b) Time scales: The above mentioned interaction considerations help establish critical time scales. Droplet size will be minimized by ensuring that the following ratio of characteristic times is much less than one [37].

$$\frac{\tau_{adsorption}}{\tau_{collision}} = \frac{6\pi\Gamma\phi}{dC_s} \ll 1 \quad (1)$$

Where $\tau_{adsorption}$ denotes emulsifier adsorption time on the surface of emulsion droplets and $\tau_{collision}$ denotes collision time between droplets; ϕ is the dispersed phase volume fraction; Γ denotes excess surface concentration of the emulsifier; d is droplet diameter; and C_s is the concentration of emulsifier in the system. Thus, the faster the diffusion and interfacial adsorption processes, the smaller the droplet size that can be maintained. The concept is that once the smallest droplets obtained through the turbulent energy dissipation mechanism are formed, they need to be coated with emulsifier molecules to stabilize their size before they grow (via Ostwald ripening and/or collisions with coalescence and re-aggregation).

- (c) Physicochemical properties: In addition to the transport properties of the fluids, the interfacial tension and the physicochemical properties contribute significantly to the critical mixing process and associated time scales involved. For example, turbulent intensity is governed by the system's Reynolds numbers and thus the Kolmogorov parameters associated with diffusion length and time scales.
- (d) Process design: Fewer processing units, lower energy input, and amount of emulsifier can contribute to cost saving. Under specific processing conditions the pre-emulsion step can be eliminated, cycle times reduced (by minimizing the number of passes through the emulsification device) and the amount of emulsifier required can be minimized. Both up- and down-stream processing techniques must be efficient; avoiding detrimental over-processing. PI concepts employed here can prove highly effective on product quality and reduction of costs.
- (e) Selection of the emulsification device: The method and equipment used to create the emulsion is crucial to its quality. It is essential to provide a high energy density transfer rate, ensuring that a large fraction of the energy is being used to create oil-water interfaces rather than viscous dissipation and other loss mechanisms.

Additional comments with respect to the above mentioned factors that influence the droplet size and stability of the emulsion produced during the emulsification step are given in the following sections. Relative optimization strategies to create these nano-emulsions are also discussed.

3.1.3. Energy Intensity

It is generally accepted that increasing the energy density transport rate and/or interaction times results in reduced droplet sizes. The emulsification method, device, and operating pressure all contribute to the required energy intensity.

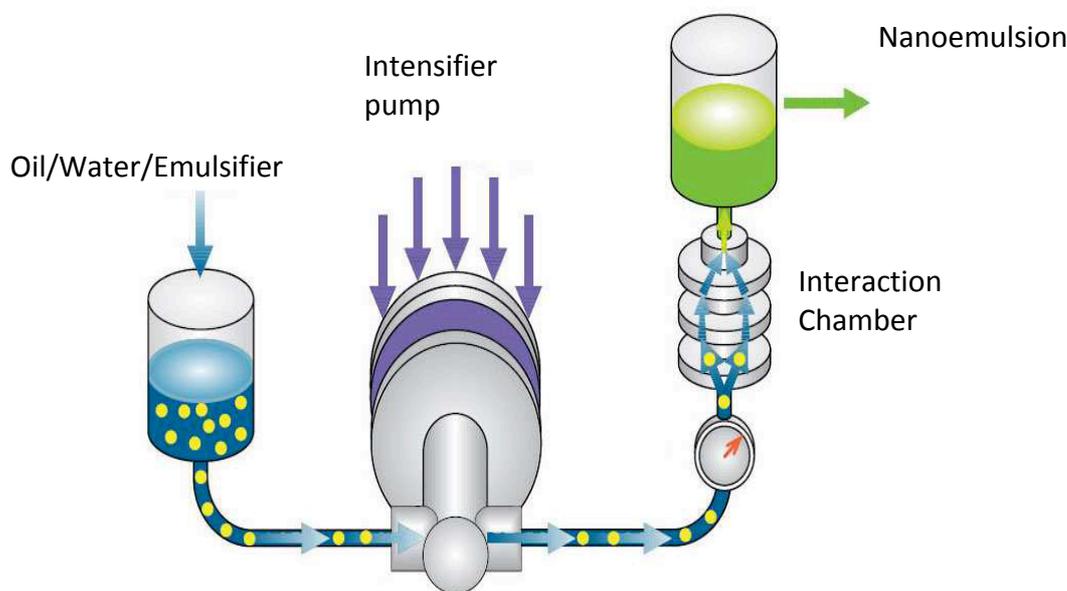
3.1.3.1. Creating Nano-Emulsions

Production of a nano-emulsion can be accomplished by several methods, including use of high-speed mixers, high-pressure homogenizers or devices that utilize confined impinging jets (CIJ) as in the microfluidic platform technology to be discussed subsequently. With these devices, smaller droplet sizes and narrower distributions are obtained compared to traditional emulsifying devices, such as high speed blenders [38]. Typically, high speed mixing systems convert an appreciable amount of

mechanical energy to heat due to viscous dissipation, consequently less energy is devoted to generating surfaces. These high speed mixers are generally used for producing coarse emulsions (in a top down process) while a CIJ system (for example, a Microfluidizer™ processor) is used to create fine emulsions via either a top-down or bottom-up technique. The high efficiency of a CIJ system is not always evident when compared with high pressure homogenizers (HPH), especially those with an orifice valve [39]. Recent studies on nano-emulsification by high pressure systems further substantiate that fact [40–43]. The use of the Microfluidizer™ platform technology in this work as illustrative hardware is based upon our familiarity with this equipment and the successful applications as reported throughout this document. Potential users of either the CIJ or HPH technologies need to carefully evaluate their needs as related to their specific product specifications and performance requirements.

A top down process is illustrated in Figure 2. This approach utilizes a pre-emulsion with coarse droplet sizes that are to be reduced in the interaction chamber. Multiple passes through the system are typically required to obtain the desired fine emulsion with narrow droplet size distribution. High pressure generated in the intensifying pump forces the stream through micro-channels at high velocities into an impingement zone, creating an intense turbulent mixing action. Compared to a high speed mixer, the emulsification is more uniform due to the fixed geometry and localized high-energy dissipation; thus the necessary high-energy density transfer rates. In addition, the operation can be conducted in a continuous fashion and/or with a recycle configuration to provide for multi-pass capabilities [26].

Figure 2. Microfluidizer™ processor used for top-down process.



Selection of the interaction chamber type: Two different jet impingement arrangements are currently available; jet to wall (Z-chamber) and jet to jet (Y-chamber). Various channel and chamber dimensions are also available, determined by user specifications on rates and emulsion quality. Both arrangements are shown in Figures 3,4 [26].

Within a Z-chamber, enhanced turbulent mixing is created when the high-velocity jet impacts the wall. In the Y-chamber, the turbulent mixing is created by the impingement of two identical jets with each other. Compared to the Z-chamber, this impinging jet system minimizes wall shear, reducing the

amount of energy dissipated as heat and/or other losses; thus maximizing the utilization of energy for interface generation. A large amount of energy is transferred into a small volume of fluid in a short period of time, accomplishing a higher energy density transfer rate. Furthermore, the mixing produced with the Y-chamber is more homogeneous, producing a more uniform droplet size distribution.

Figure 3. Z-type interaction chamber.

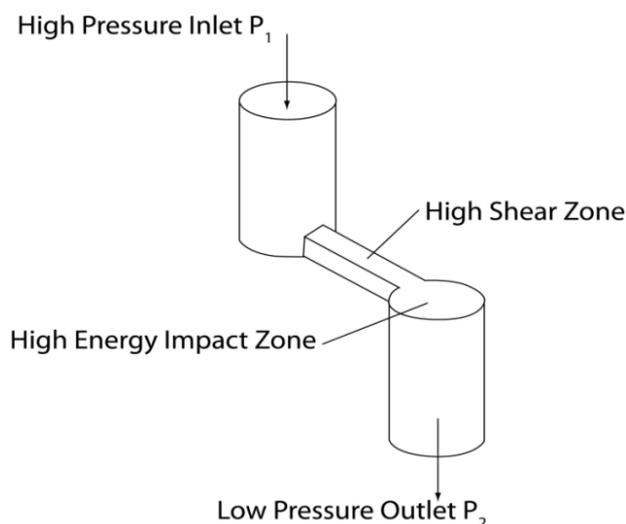
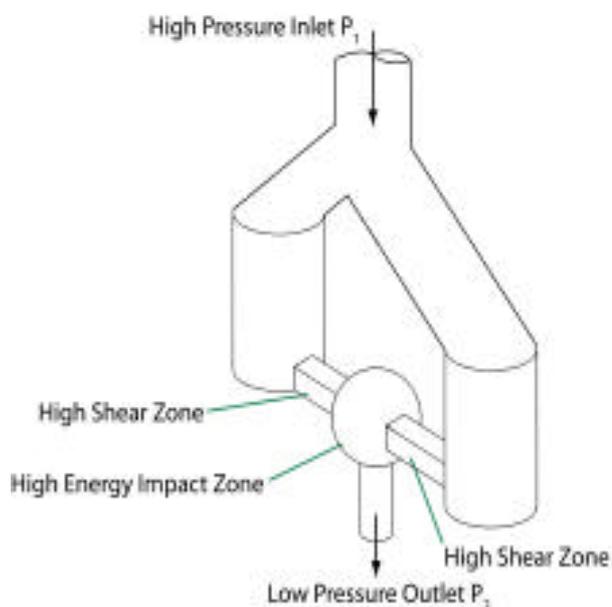


Figure 4. Y-type interaction chamber.

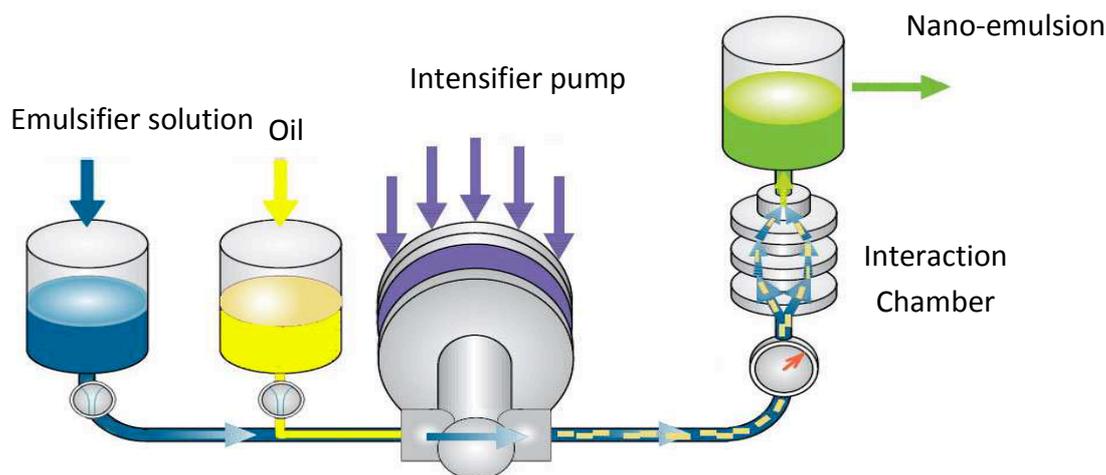


3.1.3.2. Bottom Up vs. Top Down Processing Protocols

For discussion purposes here, top down processing refers to emulsions prepared in two stages; a pre-emulsion is formed before the interaction chamber that must be “broken down” into a nano-emulsion by the impingement mechanism. Conversely, bottom-up processing refers to generation of emulsions that are “directly produced” in the chamber without the premix step, *i.e.*, essentially a one-step process.

In the top-down process, the pre-mixed oil/water/emulsifier stream is fed into the system through one inlet line. In the bottom-up process, oil and emulsifier solution are fed via two separate inlets. Through use of co-axial feed scheme there is minimal interfacial disruption between the co-flowing fluids (*i.e.*, insufficient residence time for centerline jet break-up) prior to the intense mixing in the impingement zone. The system configuration for bottom-up processing is illustrated in Figure 5 [26,44].

Figure 5. Microfluidics' processor configuration for bottom-up processing.



During any emulsification process, the faster the emulsifier molecules adsorb on the surface of the freshly produced droplet, the faster the decrease of the interfacial tension; thus rapid stabilization of these droplets against re-growth mechanisms. However, the collisions among the naked droplets themselves are also rapid due to intense mixing. If the timescale of emulsifier adsorption is longer than the timescale of collision, the fresh interface will not be completely covered and will lead to re-coalescence, and possibly growth via Ostwald ripening itself, independent of collisions [45–47]. Rapid stabilization of new interfaces by sufficient emulsifier molecules (the threshold amount) is an effective way to prevent re-coalescence [39,48–51]. However, this stabilization at the pre-emulsion stage can be problematic, requiring more extensive second stage processing. Furthermore, note that higher oil content, lower emulsion (continuous phase) viscosity and higher energy input might increase collision frequency and lead to an increase in droplet size. This is particularly true if there is insufficient emulsifier concentration and transport rates are low; a major concern with bottom-up processing (discussed in detail later).

Often, with top-down processing, one pass through the Microfluidizer™ is not sufficient to break up large droplets formed during the pre-emulsion step. In addition to the increase in operating costs due to energy consumption, multiple passes may not always be beneficial. Specific circumstances such as higher pressures and longer emulsification times might lead to “over-processing,” which is manifested as a re-coalescence of droplets that can occur via various mechanisms. Once a minimum size is obtained, with regard to the turbulent intensity of the flow field (*i.e.*, eddy size), any excess energy will be dissipated in a detrimental manner. For example, viscous heating will alter emulsifier performance. This phenomenon of re-coalescence has been reported in several emulsion systems and is always a possibility when multiple passes are necessary for a given application [52–55].

Employing a bottom-up process can provide several advantages over top-down processing: (1) no pre-mixing is needed, which will decrease operating costs and possibly eliminate some process vessels (capital costs); (2) multiple pass requirements are minimized due to higher energy efficiency potential in the emulsification process itself with no need to break down pre-stabilized larger droplets from the pre-mix step; (3) emulsions produced with smaller droplet sizes due to more efficient energy utilization and minimization of re-coalescence; (4) the phase boundary produced has higher electro-chemical stability since a pre-mix step leads to unfavorably large contact points between immiscible streams; (5) a more narrow droplet size distribution due to the absence of uneven droplet break-up; and (6) greater flexibility in the control of ingredient feed conditions can be achieved *i.e.*, oil and surfactant can be added in a fed-batch manner and the feeding ratios can be tuned as needed.

3.1.3.3. Time Scale Considerations

Residence time in the interaction zone and overall processing time will significantly affect emulsion quality. These process variables, along with system configuration, chamber geometry, and operating pressure and temperature determine performance metrics, and are essential in establishing the time scales relevant for the mechanisms associated with the transport phenomena occurring; *i.e.*, heat, mass and turbulent momentum transfer.

The residence times for one pass through the chambers are in the range of 1–5 milliseconds in currently available CIJ systems [26]. For the bottom-up process, this typically is enough time for the emulsifier to cover an interface and create the stable droplet. For the top-down process, which may require multiple passes through the system, there will be an optimal processing time due to the consideration of “over-processing” as mentioned earlier. Different flow rates, numbers of passes, and length of transport lines can be used to vary residence and processing times [26].

3.1.3.4. Input Energy Density and Transfer Rate Requirements

The total energy input to the system can be varied by changing the operational pressure and/or the number of passes. However, it is the energy density transfer rate that is the significant factor in emulsion formation [5044]. With a CIJ system, the higher operating pressure drops obtained in the system produces large turbulent energy dissipation rates in the small volume chambers. The subsequent higher energy density transfer rates (compared to other mixing devices) produce smaller eddies and hence smaller droplet sizes. The use of HH systems, as mentioned earlier, may also give comparable results [39–43]. Consequently, the user application, product specifications, and performance requirements dictate which technology is selected.

Typically, under turbulent conditions, increasing the power input will decrease the ratio $\tau_{adsorption}/\tau_{collision}$, resulting in a decrease in adsorption time, which, in turn, leads to a decrease in the tendency for coalescence. However, total energy input must be viewed with caution. This is especially true for the top-down process since excessive energy input may lead to increases in droplet size due to poor stabilization of the newly formed droplets (over-processing) [56]. A reasonable explanation is that force-induced phenomena such as cavitation, shear, turbulence, and temperature-rise emerge simultaneously, each altering the structure and properties of emulsifiers; in particular for modified starch and protein emulsifiers [57–59].

In summary, evaluation of energy input efficiency can be used to evaluate emulsion quality. Fluids subjected to extremely high wall shear rates, experience large energy dissipation rates as heat due to frictional losses, and thus decreased energy efficiency with respect to forming larger interfacial areas. The CIJ platform technology can ensure that input energy is used for enhanced surface formation rather than squandered via viscous dissipation. This is crucial in terms of producing stable emulsions with smaller droplet sizes.

3.1.4. Selection of Surface Active Agent

Another important factor in producing a quality emulsion is the type of emulsifier used. This requires a careful selection process [7,14]. Proteins and polysaccharides are the most commonly used biopolymer-based emulsifiers, especially in the food and drug industries. Both can serve as stabilizers and thickeners and are highly compatible with the vast majority of biological systems. At equal concentration levels, different types of emulsifiers produce different droplet sizes; their stability depending on their surface load, the rate at which they reach the interface, the ability of the resultant film/membrane to prevent droplet coalescence and the degree to which they reduce interfacial tension.

Molecular weight is one of many criteria useful in the decision process. For the most part, molecular weight is an important factor in determining the diffusivity of the emulsifier. Results from a number of research groups support the premise that the rate of surface coverage is instrumental in maintaining size integrity. In one study [33] three different molecular weight emulsifiers, (Gum Arabic, Sodium Caseinate, and a Starch) were evaluated with respect to final droplet size obtained. The starch used had a lower molecular weight than Gum Arabic, thus it had more mobility and more rapidly stabilized the newly developed oil-water interface. Among these three emulsifiers, the droplet size followed the order: Gum Arabic > Sodium Caseinate > Starch. In another study [32,60], the resultant lowering of droplet size with lower molecular weight emulsifiers, nOSA-starch *versus* Gum Arabic, was also related to the superior mobility of the starch. The low molecular weight of nOSA-starch also allows a denser packaging of the molecules at the oil-water droplet interface and thus provides a better protective layer for the oil. However, seemingly contradictory results with respect to molecular weight considerations alone were observed by others [61]. Upon further analysis it was concluded that when the emulsion is being created, larger emulsifier molecules adsorb more rapidly than smaller ones depending upon the length of their hydrophobic chains. That is, the hydrophobicity of these molecules is a factor that must also be considered. Furthermore, it was noted that large casein micelles adsorb faster than individual casein molecules; this most likely is simply due to the fact that a large number of molecules are delivered to the surface via micelle collisions.

The Hydrophilic-lipophilic balance (HLB) value serves as a useful metric to characterize different emulsifier types. Low HLB value corresponds to high hydrophobicity, and vice versa. According to Bancroft's rule [62,63], the nature of an emulsion (*i.e.*, oil-in-water or water-in-oil) is determined by the emulsifier type and whether the emulsifier is soluble in the continuous phase. For example, if an oil-in-water emulsion is desired, an emulsifier with a relatively high HLB value (*i.e.*, low hydrophobicity) should be selected [7,14,62,63].

When a mixture of more than two types of emulsifiers is used to prepare an emulsion, their interaction and the competitive adsorption mechanism at the oil-water interface should also be

investigated [7,40–44,64–68]. Low molecular weight surfactants are much smaller in size than biopolymers; thus they can reduce interfacial tension more efficiently and quickly by adsorbing a large number of molecules within the same surface area. If both are present at high enough bulk concentrations they are likely to dominate at the interface after equilibration [69–72].

Emulsifiers can also influence the oxidative stability of the final product. It was reported that Sodium Caseinate contains traces of transition metal ions, especially iron. Thus, excessive addition of caseinate could result in maximum addition of ferrous ions, which, even at small levels, can promote oxidation by facilitating degradation of lipid hydroperoxides [73].

3.1.4.1. Emulsifier Concentration

To ensure emulsion quality and avoid adverse solution properties, an optimum quantity of emulsifier needs to be determined. Insufficient droplet surface coverage has already been shown to be problematic with respect to stability. An excess beyond the coverage requirement can lead to consumer acceptance complications in addition to increased operating costs. The two major factors to be considered here are the equilibrium droplet size and micelle formation. Droplet size is related to the size of the smallest eddies as estimated by the degree of turbulence (Kolmogorov scale [74]), setting a minimum concentration level. Micelle formation is determined by emulsifier solubility and its critical micelle concentration (CMC); setting an upper limit target.

The size of the smallest eddies, which form in the interaction chamber, can be estimated using the Kolmogorov length scale λ_K defined as [74–76]

$$\lambda_K = \left(\frac{\nu^3}{\varepsilon} \right)^{1/4} \quad (2)$$

where ν is the kinematic viscosity of water, ε denotes turbulent energy dissipation rate per unit mass (or volume, if convenient) which is affected by the operating pressure establishing the flow rate in the system and thus momentum exchange. This Kolmogorov length scale is therefore useful in setting a lower limit on droplet size estimates.

The critical micelle concentration (CMC) is defined as the maximum concentration of emulsifier dissolved in the continuous phase without micelle formation. Due to the limited solubility of surfactants (or emulsifiers), when the concentration is beyond CMC, the surfactant/emulsifier molecules tend to form micelles. These entities are an aggregation form in which, for an oil- water system, the hydrophobic tails are shielded from water by the hydrophilic head groups. Therefore, when a fraction of surfactant is not used to coat the oil droplets these micelles exist in the aqueous phase. The presence of micelles inhibits the formation of small eddies which will adversely increase droplet size [7]. Furthermore, these surfactant micelles and unadsorbed biopolymers located in the aqueous phase can induce destabilization of emulsions by mechanism of “depletion flocculation” [53], resulting in increased droplet aggregation and subsequent enhanced sedimentation and phase separation. Furthermore, on the premise that the interfaces have been completely covered by emulsifiers, minimizing emulsifier usage can, as mentioned earlier, reduce operating costs and help prevent potential toxicity and/or an allergic response incurred by excessive usage [36]. More in-depth

discussions pertaining to micelle formation being detrimental to formation of a stable interface are available elsewhere [77–79].

Micelle formation can be detected by monitoring the osmotic pressure or surface tension of water as a function of surfactant concentration. Osmotic pressure measurements can be used to determine both the critical micelle concentration and the aggregation number for various emulsifiers in solution [64,65]. The aggregation number not only is an indication of the size of the self-assembled micelles in water but it also aids in interpretation of the size distribution data. Further, micelle formation is considered to trigger an abrupt change in the physicochemical properties, thereby influencing the formation and stability of the emulsion system [7]. There doubtless will be some difference in the critical value of emulsifier concentration in an emulsion system compared to that measured in the continuous phase alone. However, the CMC value can still be an evaluation factor for relative comparison of emulsions prepared using different emulsifiers and different amounts.

The minimum amount of emulsifier used to create an emulsion with a given size distribution can be estimated by a surface load measurement technique. For example, using sodium caseinate as the emulsifier, inherent empirical relationships describe minimum surface concentration for monolayer coverage. The value is typically between 1 and 3 mg/m². On the basis of this information, the amount of emulsifier for a given droplet size distribution can be estimated, which should be regarded as a lower bound. However, in practice, some excess is needed. The practical accumulation of emulsifier molecules at an interface is characterized by a surface excess concentration (surface load), which is equal to the excess emulsifier concentration divided by the surface area of a single droplet [19,77]. Another reason why the input amount of emulsifiers needs to surpass the theoretical values is that excessive emulsifier might act as wall matrix material during a subsequent entrapment process.

3.1.5. Temperature

Since key solution properties are temperature dependent, proper temperature control will lead to better emulsions. Interfacial tension, Laplace pressure, and the viscosities of the components of an emulsion are all reduced with a temperature increase. These result in reducing the energy necessary for processes that facilitate production of smaller droplets [7,80]. However, for certain emulsifiers, there is a critical temperature beyond which biopolymers can lose their emulsifying properties. Studies indicate that temperature rise can be detrimental during emulsification because of the complex mechanisms initiated [47]. The major concern is allocation of the dissipated energy; toward interface formation *versus* loss mechanisms such as heat, cavitation and sound.

3.1.6. Oil Content in the Emulsion

The oil-water ratio influences emulsion quality for both types of continuous phase systems. For example, in an oil-water emulsion, increasing the oil content leads to increases in droplet size [56]. The possible explanations given are that: (1) there might be insufficient emulsifier molecules to completely cover the new droplet; (2) the greater fraction of the higher viscosity dispersed phase will create more difficulties with droplet size disruption; and (3) higher collision frequency of droplets due to the greater volume fraction enhances the probability of coalescence.

3.1.6.1. Physicochemical Properties of the Emulsion System

The emulsification efficiency also depends on other physicochemical properties such as viscosity, interfacial tension, and density. The roles that interfacial tension and density play have been mentioned earlier with respect to energy requirements, droplet stability, mixing/separation (downstream processing), and storage. Additional comments are given later with respect to matrix entrapment steps.

The viscosity of each phase impacts all aspects, but particularly the emulsion preparation stage. Increasing the viscosity of the dispersed phase might increase the droplet size [56]. The correlation function between mean droplet diameter (d) and viscosity of dispersed phase (η_d) is [81,82]:

$$d = C\eta_d^b \quad (3)$$

with the exponent b ranging from 0.25–0.4 for rotor-stator systems and 0.4–0.9 for CIJ systems. It is commonly accepted that low viscosity continuous phases enhance the tendency of droplet collision during processing, especially in the top-down approach. A higher viscosity of either phase can reduce the rate of sedimentation and/or droplet creaming as well as lowering the collision frequency.

Reducing the droplet size can be achieved by ensuring the viscosity ratio falls within the optimum range for droplet breakup (preferentially within 0.1–5) [7]. This can be achieved by varying temperature or by adding thickening agents such as proteins and polysaccharides [83]. The preference is for compounds of higher molecular weight and more extended structures, since these lead to a lower concentration demand.

Although no validated mechanism is identified, one research group [59] found that for O/W emulsions stabilized with a slow adsorption rate protein, at a sufficiently high continuous phase viscosity (by adding some stabilizers), the same droplet sizes were obtained as those obtained when using a fast stabilizing surfactant. This further illustrates that viscosity plays a critical role in the emulsion preparation stage.

3.1.7. Other Components

Impurities in the oil phase include free fatty acids, monoacylglycerols, and diacylglycerols. These surface active lipid components tend to accumulate at the oil-water interface to affect the emulsion formation [47]. The presence of short-chain alcohols reduce interfacial tension and thereby droplet size. Other minor components such as piperonal (heliotropin) in vanilla essential oil will affect interface and/or antioxidant properties.

3.1.8. Emulsion Characterization

The quality of an emulsion can be identified through useful, readily obtained metric measurements. These include:

- (a) Droplet size and distribution measurement: The droplet size measurement is typically done using light scattering techniques. These give the mean and distribution, along with percentile reports. Bi-modal distributions are often observed and recorded using imbedded instrument software.

- (b) Emulsion stability index: An indication of the emulsion's short and long term stability can be obtained through centrifugation studies. A stability index can be measured by accelerating the evolution of unstable behavior of freshly produced emulsions in a centrifuge. Low speed operation can be used for predicting creaming by measuring the volume of that layer when present. High speed centrifugation can be used for predicting coalescence by monitoring the droplet size over time.
- (c) Emulsion viscosity: The viscosity can be measured, for example, by a Brookfield viscometer. A correlation between emulsion viscosity and droplet size or final product quality can be obtained such as that given earlier.
- (d) Oil stability index against oxidation: The standard oil stability index (OSI) is applicable for determining the relative resistance of fat or oil samples to oxidation. This is accomplished using an oxygen uptake technique. However, it may have some limitations when dealing with an emulsion. Further discussions with respect to the entrapment process are given in later sections.

3.2. Entrapment

Now that the key parameters affecting the production of a nano-emulsion are identified; including energy input and efficiency, apparatus type, emulsifier type and concentration, and operating conditions such as temperature and pressure, the focus lies in discussing the entrapment stage. The potential benefits of process innovations such as system configurations and operational maps will be outlined in detail. The method selected for illustration here is the cold extrusion process. Although this serves as the current method, it involves numerous aspects subject to improvement, such as matrix type, matrix ingredients and concentrations, use of antioxidant, *etc.* Characteristics of the encapsulating matrix, such as its glassy state, porosity and viscosity, determine its functionality in the extrusion process. Furthermore, water content and pH play a critical role in preventing oxidation of the emulsified cargo. Parameters for this evaluation include observation of excess surface oil, oxidative stability, and accelerated shelf-life testing of the final pellets. These also provide an indication of the efficacy of the nano-emulsion production process itself.

It is important to keep in mind that preparing a stable nano-emulsion cannot guarantee a superior entrapment efficiency of the active ingredient into a matrix (pellet) intermediary or final product. It therefore becomes a necessary, but not sufficient condition. During the entrapment step the emulsion may be subjected to additional environmental stress. For example in the manufacture of microcapsules (pellets) containing omega-3 in a starch matrix, a twin screw extruder is used inducing high levels of mechanical stress/strain, heat load, *etc.* During this entrapment process, the matrix (starch), the so-called wall material, will have some direct and indirect influences on the entrapped emulsion's properties. With the goal of increasing physical and oxidative stability of the final pellet produced, formulation optimization can be conducted by carefully selecting matrix material, plasticizer and antioxidant. Operational maps and subsequent optimization can be achieved by altering pressure, temperature and applied shear during the extrusion process.

3.2.1. Formulation Optimization

3.2.1.1. Matrix Type

The wall matrix material used to encapsulate or embed oil droplets has to be carefully selected. The objective is to maintain durable functional properties and structural integrity, prevent oxidation and/or other forms of degradation, and enable controllable release. When dealing with lipid oxidation in these entrapped forms, the main aspects that must be considered (determined by matrix type) are [84]: (a) the glass transition state of the amorphous matrix system; (b) the presence of metal ions in the wall material that will catalyze reactions; (c) the chemical make-up of the potential reaction media and resultant conversion and/or selectivity; and (d) physical characteristics such as porosity and viscosity that affect transport properties and stability of both the matrix itself and its cargo-loaded emulsion.

Several of the critical factors used to characterize the matrix material in the entrapment process, particularly those associated with oxidation/degradation prevention, are summarized below and also in subsequent sections that include more general aspects of the encapsulate system.

Glass transition temperature: In many situations the rate of oxygen permeation in the matrix is believed to be the rate determining step in oxidative degradation [85]. The glassy state (involving less free volume in a matrix compared with a rubbery state) is more effective in retarding the diffusion of small molecules and thus, the oxidation of lipids [86,87]. Factors that affect the glassy/rubbery state of the capsule wall are therefore parameters that can be used to control the oxidation of entrapped oil [20]. Water and temperature are two such key factors. At the glass transition temperature, the matrix is transformed into the rubbery state whereby the increased diffusive mobility contributes to a greater oxidation potential [85]. Consequently, temperature control during the manufacturing process and subsequent storage is imperative. Since water can plasticize amorphous carbohydrates and lead to the formation of a rubbery state; it also impacts oxidation stability. Therefore, the amount of added water as plasticizer, downstream processing (drying efficiency) and storage conditions play crucial roles.

- (a) **Porosity and oxygen permeability:** The desired pellet structure can be obtained by design through careful selection of matrix components. This structure with its distinguishing bulk and pore characteristics will exhibit distinctive degrees of oxygen permeation in the matrix as well as pore diffusion capabilities. Porosity and surface area measurements, along with imaging techniques, are useful tools to characterize pellets, providing valuable input for matrix selection [32,60].
- (b) **Viscosity of the matrix mixture during processing:** The emulsion being entrapped in the cold extrusion process is subjected to significant shear and temperature impact. The physical stability of oil droplets in a viscoelastic solid (for example, dough) is governed by surface tension, viscosity differences, body forces and shear rate [88]. A complicated analysis includes magnitude of deformation, spacing, and dimensionless parameters such as the Capillary, Weber, Bond (or Eatvos), and Deborah numbers. Of the physical properties involved, viscosity seems to be the most readily manipulative. Consequently, reduction and prevention of droplet deformation and coalescence during the cold extrusion process is possible by means of a decrease of matrix viscosity, either by adding plasticizer or by altering matrix type. The experimental results reported in the literature [60] are consistent with this postulate; that is,

adopting matrix materials with lower viscosities tends to enhance stability. It was observed that the use of high viscosity starch leads to coalescence during the entrapment process.

- (c) Water content and storage humidity: It is well established that water plays a critical role when dealing with oxidation of unsaturated oil, either in terms of water content in the original pellets or via humidity inducing water uptake under storage conditions. Generally, low water content contributes to higher oxidative stability. However, numerous contradictory results have been reported. The rate of lipid oxidation is claimed to be at a minimum under moderate storage humidity. A possible explanation for the low rate at intermediate humidity is that water forms a hydration sphere around metal catalysts, thus reducing their catalytic activity and slowing down the oxidation rate [84]. At both very low and high water activities, lipid oxidation rates are suggested to be appreciably higher than at intermediate water activities [89–92]. In all cases, complicated mechanisms related to metal catalyst activity, mobility to the oil-water interface and availability of active sites that suggest how water plays a critical role in oxidative stability are discussed [85]. Since there appears to be an optimum water content there must exist both anti-oxidative and pro-oxidative effects of water that are dependent on matrix structure, trace components such as metal ions, and interfacial properties [93–96].
- (d) Different antioxidants: Autoxidation is the primary origin of oxidative rancidity derived from lipids. The production of unpalatable odors and flavors results from secondary decomposition products such as aldehydes and ketones. Autoxidation can be inhibited or retarded by the addition of an appropriate antioxidant. Their use in combination with suitable emulsification and entrapment systems are regarded as important protective factors against oxidation of unsaturated lipids. The underlying mechanism might be that antioxidants interact more rapidly with lipid peroxy radicals and compete with unsaturated oils by donating a hydrogen atom to impede the propagation of free radicals.

Both natural and synthetic antioxidants have been broadly used to control oxidation in foods. However, levels of synthetics are limited by the U.S. Food and Drug Administration (FDA) to 0.02% based on lipid content (FDA 1993). Fortunately, the natural antioxidants generally result in greater antioxidation efficacy. For example, treatments at >200 ppm alpha-Tocopherol effectively prolonged the shelf-life of fish oil products. Hydrophobicity can also be a major factor in oxidative stability. In a comparative study, alpha-Tocopherol, a lipophilic antioxidant, showed an enhanced antioxidative effect in both surface and entrapped oil relative to ascorbyl palmitate, an amphiphilic antioxidant [93].

It is worthy of noting that deterioration of omega-3 fatty acids in a pure algal oil system might differ greatly from that in an emulsion or complex encapsulating system due to the barrier properties and/or the presence of trace compounds, as mentioned earlier. The relative efficacy of different antioxidants can vary significantly within a particular matrix system. Thus, the interactions that antioxidants may have with emulsifier or matrix material can lead to either pro- or antioxidant effects on lipids. Various antioxidants need to be tested because no theoretical selection criteria are without limitations.

- (e) pH: In a confidential study conducted by MIT students (as part of a course requirement), it was reported that entrapment efficiency was higher when 15% erythorbic acid (based on matrix weight) was added to a proprietary food matrix/algal oil system as compared to 10%. These

preliminary results suggest that pH is a potentially significant factor determining entrapment efficiency and/or oxidative stability. This is consistent with literature results [21] suggesting that a decrease in pH will lead to a reduction of electrophoretic mobility due to the protonated carboxylic group, driving net surface charge close to zero. Enhanced stability should occur since the surfactant molecules at the interface are closely packed, forming a stable film with high visco-elasticity.

3.2.2. Characterization of the Entrapment Product

The most accurate method of product evaluation is obtained by storing samples of emulsion-entrapped systems under specific environmental conditions and conducting corresponding sensory and physicochemical property tests over time. However, this continuous evaluation protocol spans several months, which restricts process optimization. Therefore, it is common practice to elicit several rapid characterization indexes to predict product quality. Several suggestions regarding current evaluation methods are given here and some new methods are proposed for implementation. For illustrative purposes an oil/water/starch system is selected for discussion.

3.2.2.1. Microencapsulation Efficiency (ME)

The percentage of oil successfully entrapped in the matrix is a commonly used index for determination of entrapment efficiency. The metric used is ME and is defined as $(\text{Total Oil-Surface Free Oil})/\text{Total Oil} \times 100\%$. ME can also be used to predict oxidative stability since the untrapped (surface) oil is directly exposed to oxygen. The concentrations of these quantities, along with the presence of “catalysts” determine oxidation rates.

3.2.2.2. Extent of Oxidation

The induction time obtained from measurement of oxygen uptake rates by dry, oil-containing product in an oxygen bomb serves as a powerful tool to predict oxidative stability.

3.2.2.3. Oxidation Parameters

In order to evaluate the oxidative changes occurring during each step in the manufacturing process, three chemical substances emerging from the oil oxidation reaction are suggested as response variables to be tracked [60]. Conjugated dienes and the peroxide value are from the initial stage of lipid oxidation in the oil fraction. The conjugated dienes can be quantified photometrically (at 234nm after dilution of the oil in 2-propanol) [97], and peroxide value can be determined using the IDF standard method 74A:1991. Volatile aldehydes, resulting from degradation of omega-3 fatty acid, can be analyzed via static headspace chromatography [60].

Tracking the peroxide value seems to be an excellent method to identify the most destructive step in the overall operational procedure affecting oxidative stability. In prior studies [60,93] it was found that the major oxidation process occurred during the entrapment step as compared to the other process steps. This approach is also viable when evaluating shelf-life/storage specifications.

3.2.2.4. Oil Droplet Size in a Reconstituted Emulsion

The reconstitution process is conducted by re-suspending the pellets in water to release the entrapped emulsified oil droplets, whether as the entire vesicle or its oil based cargo. Size and distribution measurements and subsequent comparison with measurements before entrapment indicates whether significant coalescence or other forms of destabilization has occurred [85]. The free oil present would be from (i) leakage from the vesicles; (ii) their disruption from processing; or (iii) barrier breakdown during storage.

3.2.2.5. Water Content and Water Activity Tests

These can be used to evaluate the efficiency of the drying process as well as providing water-related data for the water effect analysis described previously. They also determine shelf-life and packaging design parameters.

3.2.2.6. Accelerated Shelf-Life Test

The current protocol uses high temperature to accelerate shelf-life evaluation. Unfortunately, a study on oxidative stability of entrapped oils [85] did not support use of these accelerated conditions as predictive tools because the behavior differed too much from that at 20 °C. Above T_g of the encapsulate, moisture sorption may release oil from the matrix and hence accelerate lipid oxidation [23]. Furthermore, at elevated temperatures additional chemical reactions can be initiated and/or their rates enhanced such that they produce compounds that would not be of significance at typical storage conditions. A good example is the Maillard reaction network, which is a complex series of reactions between amino acids and reducing sugars. Although studied for nearly a century, they are so complex that many reactions and pathways remain unknown. Many different factors play a role, such as pH, types of amino acids and sugars, temperature, time, presence of oxygen, water, water activity, and other food components. Of the hundreds of reaction products formed, the reducing compounds are antioxidative and thus increase shelf life. Another system of reactions that should be considered is aldol addition and condensation of developing aldehydes that influence the level of lipid oxidation parameters, which were described previously [23].

4. Experimental Program Development

4.1. Bench-scale Screening for the Production of Quality Nano-Emulsions

Due to their capability in screening potential optimal process parameters by varying formulation and operating conditions, platform technologies such as the one developed by Microfluidics International Corporation (MFIC) are systems well suited for use in bench-scale emulsion preparation. The virtual experimental program presented here is one readily implemented by either a single corporate entity or through a strategic partnership arrangement of synergetic collaborators. This discussion is based on use of an oil-based nutrient emulsion for entrapment. Similar strategies can be applied to various other species (as discussed earlier) such as a solid (post-emulsification step) during matrix entrapment.

The parameters to be considered as essential components of the protocol for investigating their effect on droplet size distribution, and emulsion stability index are: (i) the operating pressure; (ii) number of passes; (iii) oil-loading; (iv) emulsifier type; (v) emulsifier concentration; (vi) existence of a pre-mix step; (vii) chamber type and system configuration; and (viii) temperature control. It is worth noting that when dealing with multiple passages in the top-down process with pre-mixing, all ingredients are added into the system initially and the entire mixture is subjected to numerous passes. However, for a bottom-up process, the feed input methodology will be investigated by feeding only a portion of the oil/emulsifier per cycle (batch).

An experimental design to investigate the influence of these eight parameters (or more, since emulsion viscosity is important during entrapment) at three levels would be extremely time consuming and costly, and thus prohibitive. The approach needs to be more innovative and less overwhelming. A series of three variable-two level programs determined using an experimental design software package should prove effective, particularly when utilizing the response surface and sensitivity analysis capabilities. The most useful dependent variables would be the droplet size distribution and a stability index. The outcome of this portion of the overall process scheme is only an indication that the probability of a successful matrix entrapment is sufficiently high to proceed to the commercialization phase.

4.2. Pilot-Plant Scale for the Production of Pellets

Characteristics of the entrapping matrix, such as its glassy state, porosity and viscosity, determine its functionality in the extrusion process. Typically, the operational maps for the processing of the matrix material as a product without the nutrient emulsion are known. Changes must be made when the emulsion is present due to physical property variations and the need to maintain integrity of the cargo-loaded vesicles. Consequently, a series of pilot plant tests must be conducted to determine an effective entrapment process. In addition to emulsion characteristics, all operational variables need to be re-evaluated. This should include new variables such as emulsion injection rate and location. Evaluation of final product quality (e.g., via ME and a stability index) will provide the requisite correlation between quality of emulsion, operational parameters, and final product.

5. Summary and Recommendations

Entrapment of nano-emulsions containing nutraceuticals into a solid matrix presents multiple challenges. Considering the tremendous wellness benefits for consumers, a high demand to overcome these challenges and develop an effective and efficient large-scale production process exists. This manufacturing approach should also be compatible with Process Intensification concepts. Consequently, subsequent R&D programs for entrapment technology should initially focus on the emulsification step, *i.e.*, to produce a stable nano-emulsion with a narrow size distribution, before expanding to the coupled matrix-emulsion system. Optimum processing conditions are then sought through pilot plant studies. The starting point is the adjustment of the operational map obtained by processing the emulsion-free matrix material, followed by tests with the combined materials. Production rate, feed strategy, energy input, physical property modifying ingredients selection and processing units are the most critical factors affecting product quality, process optimization and capital cost.

The priorities for this requisite process development program, consisting of a series of experimental plans to validate corresponding hypotheses, are as follows:

- (1) Verifying whether the bottom-up process leads to a higher quality of emulsion with fewer passes through the system. The emulsion produced is to be compared to that obtained after the pre-mixing step in the top-down protocol.
- (2) Identifying the most suitable emulsifier and its concentration for specific entrapping matrix materials. In addition to the effects that emulsifier types have on emulsion qualities during creation they have differing interactions with the matrix material resulting in modified stability characteristics. Furthermore, by drastically increasing the emulsifier amount, the potentially detrimental impact caused by micelles can be established. If the emulsifier usage can be minimized, while maintaining a stable entrapped emulsion, then Process Intensification concepts are obtained.
- (3) Identifying the optimal operating pressure and processing passes for the various system configurations. Over-processing, which can lead to re-coalescence (excessive energy consumption), must be avoided. The operating parameter selection criteria (considerations) must include: (i) identifying ingredient types that can be processed without detrimental physical property changes within acceptable operational modes; (ii) developing input strategies for the dispersed phase and surface active agents during emulsion formation when multiple passes are required (*i.e.*, bolus or fed batch); and (iii) efficiency of energy dissipation utilization.
- (4) Verifying the benefits of nano-emulsions for the subsequent matrix entrapment process. By correlating the emulsion quality with entrapment efficiency and stability of final product, the benefits of incorporating a nano-emulsion can be demonstrated. Surface oil, oxidative stability, and shelf-life testing of final pellets can be used as responses to evaluate the efficacy of a nano-emulsion.
- (5) Identifying operational map changes from the emulsion free matrix production process. Select ingredient additives that are effective in controlling/modifying physical properties of the materials being processed in the entrapment stage.

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Appendix

Physical instability phenomena: emulsions

Physical instability results in an alteration in the spatial distribution or structural organization of the molecular assemblages. Creaming, flocculation, coalescence, phase inversion and Ostwald ripening are typical examples of physical instability.

Creaming/Sedimentation refer to the migration of droplets resulting from an imbalance of gravitational and buoyancy forces. When droplet density is lower than the surrounding liquid, they rise, leading to creaming. Conversely, the droplets move downward, leading to sedimentation.

Stokes' law describes the migration rate of an isolated spherical particle in a liquid. However, when applied to a liquid droplet, a modification is required. The creaming rate can be described as below:

$$v = v_{stokes} \frac{3(\eta_2 + \eta_1)}{(3\eta_2 + 2\eta_1)} \quad (4)$$

$$v_{stokes} = -\frac{2gr^2(\rho_2 - \rho_1)}{9\eta_1}$$

with η_2 for viscosity of dispersed phase, η_1 for continuous phase, r for droplet size, ρ_1 and ρ_2 for density of continuous and dispersed phases respectively. Increasing the viscosity of the continuous phase and/or decreases in droplet size reduce the probability of creaming.

Flocculation refers to the process whereby two or more droplets come together to form an aggregate in which the droplets retain their individual integrity. Both collision frequency and efficiency affect flocculation. Collision frequency is determined by Brownian motion, gravity and/or applied shear force. The rate at which droplets encounter each other can be reduced by increasing the viscosity of the continuous phase, but it increases at sufficiently high shear rates. Many types of biopolymers promote flocculation by forming bridges between two or more droplets, referred to as bridging flocculation. It should also be noted that increased droplet size will lead to higher collision efficiency and potentially decreased stability.

Coalescence is the process whereby two or more droplets merge together to form a single larger droplet. It is the principal mechanism by which an emulsion moves toward its most thermodynamically stable state because it involves a decrease in the contact area between the oil and water phases, and thus lower surface energy. Therefore, coalescence is the most important instability associated with a nano-emulsion. Collisions, prolonged contact between droplets, and membrane disruption can all lead to coalescence. It can also be affected by emulsifier type, which determines surface coverage, interfacial forces and barrier strength.

Ostwald ripening is the process whereby large droplets grow at the expense of smaller ones because of mass transfer of dispersed phase from one droplet to another; driven by the surface energy differences and gradients. The rate of Ostwald ripening increases as the droplet size distribution becomes wider. Thus, a homogenous distribution of droplet sizes is desired.

Phase inversion is the process whereby a system changes from an oil-in-water emulsion to a water-in-oil emulsion or vice versa. The important factors determining the susceptibility of phase inversion include dispersed phase volume fraction, emulsifier type and concentration, temperature, and external forces [4,7,62,63,75,76,98]. In general, a radical change in viscosity accompanies phase inversion. This is explained by the fact that the two fluids have switched their roles in determining system physical properties. For example, volume fraction of the new dispersed phase, which is critical in controlling the emulsion viscosity, is now that of the original continuous phase. Temperature changes affect both physical properties and rate phenomena, and are often coupled with applied forces. Cooling an oil-in-water emulsion can induce partial oil crystallization and then shearing results in fat crystallization–induced phase inversion. On the other hand, for a surfactant stabilized emulsion, heating above a certain temperature might lead to phase inversion due to rate enhancement and/or barrier property changes, and in particular, molecular structure and HLB values.

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