MINI-FLUIDIC SILVER BASED SOLVENT EXTRACTION OF EPA/DHA FROM FISH OIL

by

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Submitted in partial fulfillment of the requirements
for the degree of Master of Applied Science

at

Dalhousie University
Halifax, Nova Scotia
January 2015

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ABSTRACT

The extraction of Omega 3 PUFA using silver ions was compared within a mini-fluidic and conventional stirred-tank reactor, with practical extraction yields of EPA –Et, DHA- Et approaching 60 to 70 Wt.%. Equilibrium was reached in less than 36 seconds in the mini-fluidic reactor at 10°C, despite stratified flow being observed rather than previously reported slug-flow profiles. The deviation in flow pattern was attributed to a measured order of magnitude reduction in the interfacial tension between fish oils and $AgNO_3$ solution relative to idealized solvent mixtures often employed in previously reported literature on this extraction. A conceptual process design for silver based solvent extraction of Omega 3 PUFA at an industrial scale was explored, and suggests that this process can be feasible with appropriate silver recycling strategies with regeneration costs approaching \$0.70 per kg of fish oil processed.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ABBREVIATIONS

Fish Oil EE	Fish Oil Ethyl Ester
Omega 3 PUFA	Omega 3 Poly Unsaturated Fatty Acids
EPA	Eicosapentaenoic acids
DHA	Docosahexaenoic acids
EPA-Et	Eicosapentaenoic acid -ethyl ester
DHA-Et	Docosahexaenoic acids–ethyl ester
LLE	Liquid–Liquid Extraction
Hex	Hexane
SPT	Spinning Drop Tensiometer
IFT	Interfacial Tension
N_2	Nitrogen in gaseous state
Aqua.	Aqueous Phase
Orga.	Organic Phase
Re	Reynolds Number
We	Weber Number
Bo	Bond Number
Ca	Capillary Number

GREEK LETTERS

μ	Fluid dynamic viscosity (Pa.s)
ρ	Fluid density (Kg/m^3)
ρH	Density of Heavy Phase (Kg/m^3)
ρL	Density of Light Phase (Kg/m^3)
σAB	Interfacial Tension (mN/m)
ώ	Angular Speed (RPM)
R	Radius of the light phase bubble (m)
U	Velocity of fluids in mini-channel (m/sec)
Q	Volumetric flow rate (ml/min)

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my thesis supervisor Dr. Adam Donaldson, assistant professor & principal investigator, Lab of Multiphase Process Engineering, Faculty of Engineering, Dalhousie University for supervising my MASc program in Chemical Engineering at Dalhousie University. Moreover, I am extremely grateful to him for he has provided full funding for my graduate studies to fulfill my dream to study in one of the oldest Canadian Universities, his passionate support and inaudible encouragement have made it possible for this work to come to a completion. Really I loved and enjoyed so much his research guidance and concepts for developing of thesis and this would not have been possible without him in my life.

Additionally, I sincerely thank him for involving me in the DSM Ocean Nutrition Engage project for my thesis and also for the industrial exposure this has provided.

I further am grateful to the Head of the Process Engineering and Applied Science department and Dalhousie for providing financial assistance through various teaching assistantship and educational support.

My sincere gratitude extends to my supervisory committee of Dr. Clifton Johnston, Dr. Amyl Ghanem and Dr. Mark Gibson.

My straightest thanks also go to Mr. Matt Kujath and Mr. Trent Hillard, whose support during the experiments contributed to the successful completion of this research work.

Finally, I am sincerely grateful to all my family members who supported and encouraged me in all walks of my life.

CHAPTER 1. INTRODUCTION

1.1. Synopsis

Liquid–Liquid Extractions (LLE) with reversible chemical reactions are encountered within a broad range of industrial applications where various design configurations such as stirred tank, mixer-settler and rotating disc contactors are adopted based on mixing requirements and mass transfer characteristics. The most commonly used extraction equipment in LLE is the stirred tank reactor which has certain pros and cons of its design and operating conditions such as inadequate mixing and high energy requirements. Other stage-wise LLE operations, such as spray, packed and sieve tray columns, rely heavily on the type of column internals and flow rate of the liquid phases involved in the extractions processes, which can often result in poor mass transfer, flow mal-distribution and inefficient extraction performance. (Kashid, Harshe *et al*. 2007).

An evolving technology in the area of LLE is the recent development of mini-fluidics/microfluidics-based contacting systems which are potentially used for mass transfer limited and/or reversible exothermic reactions. Multiphase liquid-liquid flow in mini/microfluidics and the effect of hydrodynamics on reaction conversion and mixing in the process continues to be an area of active research. While the fundamental parameters governing an extraction processes are mass transfer across the phase boundary, interfacial kinetics and mixing between light and bulk phases, these parameters may be strongly affected by geometry, orientation, temperature, contaminants, and other elements of a process which can be controlled to varying extents during scale-up.

In LLE with chemical reactions, the reaction rate depends on the interfacial area and mixing characteristics. Conventional stirred tank reactors can achieve specific interfacial areas from 100 to 1000 m^2/m^3, depending on the reactor volume and the type of mechanical agitator/impeller. In conventional CSTRs, the interfacial area of the dispersion generated by stirring is not sufficient for applications which are either highly mass-transfer limited or strongly exothermic. As a consequence, diluents and/or longer residence times may be required to achieve high conversions (de Bellefon, Caravieilhes et al. 1998). In conventional systems, gravity is the dominant force acting on a multiphase system, where buoyancy force has a significant effect on the flow pattern and hydrodynamics. In mini/microfluidic systems, the surface forces increase until gravitational forces no longer have a significant impact on the hydrodynamics of the system. Interfacial tension forces begin to play a more predominant role in determining flow properties due to the reduction of channel diameter.(Gunther, A. *et al*, 2006) Therefore, miniaturization of the systems causes a shift in dominant forces which results in the flow stabilities. Based on hydraulic diameter size, the channel are classified into conventional (D_h> 3mm), Mini-channels (200 µm< D_h < 3mm) and micro channels (10µm<D_h<200 µm).(Burns and Ramshaw 2001).

Single phase flow in mini-fluidics is typically laminar or transitional, where viscous effects dominate over inertial forces. As a consequence, active mixing devices are difficult to incorporate into a given design, prompting the development of numerous passive mixing configurations and operating strategies. Laminar flow at mini scales offers effective diffusion for mixing two immiscible phases due to the reduced path length, provided sufficient interfacial areas can be maintained. (Steinke & Kandlikar 2004). The most common flow profile for multiphase applications in these systems is slug/Taylor flow or emulsified flow, with stratified flow rarely observed due to the increased dominance of surface forces relative to gravity. These flow patterns have both pros and cons in their application. In the case of stratified flow, the flow is laminar and the mass transfer between two phases is controlled by diffusion. In the case of slug/Taylor flow pattern, the mass transfer between two fluids is improved by internal circulation which takes place in each plug of liquid. This creates good mass transfer while minimizing mass transfer between adjacent slugs, creating a plug flow profile in the reactors. For emulsion-based flow, mass transfer is excellent but separation of the finished products can be challenging. In general literature, slug flow patterns have offered better performance than other flow patterns from a practical processing perspective. One particular application where this was highlighted was for extractions involving high-value solvents, such as the removal of DHA-et from an organic solvent using silver ion solutions. This particular application as of interest to a multinational company, DSM (Ocean Nutrition) Nutritional Products, who wished to perform trial experiments to determine the technical feasibility of a silver-based extraction for commercial-scale fish-oil processing at mini scales. A number of LLE examples appears in literature using silver-salt solutions to complex with the unsaturated esters and selectively extract DHA and EPA. The challenge, often reported with this approach, is the reduced solubility of the target compounds in the aqueous phase, commonly employed as a carrying medium of the silver salts, resulting in a surface-active complexing mechanism that requires significant mass transfer areas between phases to be effective. Mini-fluidic platforms offer a potential solution to this constraint, with interfacial areas ranging from 100 to 10000 m^2/m^3 reactor volume.

1.2. Research Objective

In this investigation, a mini-fluidic platform was used to explore the feasibility of liquid-liquid extraction of omega 3 PUFA from fish oil ethyl esters using a silver nitrate solution. Moreover, the hydrodynamics of mini-fluidics contact system was investigated during this process and compared to what was observed in previous literature, as much of the previous reports focused on idealized systems containing a purified Omega 3 compound dissolved in a high-purity organic solvent. Solvent based extraction methods for separation of Omega 3 PUFA was performed both in a batch-wise stirred tank reactor and in a innovatively constructed Slug flow based mini-fluidic reactor experimental system constructed with food grade 1/16th Inch ID Tygon tubing. The scientific purpose of the work was to explore the feasibility of mini-fluidic reactor technology for extraction of Omega 3 PUFA using concentrated aqueous silver nitrate solution as alternative to either primary extraction technology (i.e. the urea precipitation process) or secondary purification technology such as chromatography or molecular distillation.

This thesis is separated into four sections: a detailed review of the current state of extraction methods for removing DHA and EPA ethyl-esters from fish oil and the role of mini-fluidic reactor technology; a description of the experimental apparatus and results of extraction experiments; an overview of flow pattern experiments and the results obtained; and a process design-based assessment of the feasibility of an industrial operation based on the LLE process tested.

The main objectives of this work were to

1. Determine the performance of silver-based LLE for recovering Omega 3 PUFA from fish oil ethyl esters and
2. To explore scaling strategies for this process, specifically as it relates to the flow pattern and the ancillary processing steps which would be needed for full-scale operation.

CHAPTER 2. HISTORICAL EXTRACTION OF OMEGA 3 PUFA

2.1. Historical Nutraceutical Omega 3 Production

Omega 3 PUFA (Poly Unsaturated Fatty Acid) has an excellent marketing potential in the field of nutraceutical and food additives, with increasing production as a pharmaceutical grade tablet and food grade product for replacement of Omega 6 PUFA. Fish oils in the category 18/12 (containing 18% EPA and 12% DHA) are available as feed stock for concentration of PUFA (Lembke, 2013), where novel extraction methods are currently being explored to increase overall EPA and DHA yield. The importance of PUFA in human nutrition and disease prevention was scientifically proven three decades ago. Both Omega 3 and Omega 6 PUFA are precursors of hormone-like compounds known as eicosanoids that are involved in many biological processes in the human body. It was found that Omega 3 poly unsaturated fatty acids play an essential role in biological function such as reduction of inflammation and lower risk of chronic diseases including heart disease, cancer, and arthritis. In addition, Omega 3 fatty acids are highly concentrated in the brain and appear to be important for cognitive functions such as memory, performance and behavioral development (Connor, 2000). Most of Omega 6 fatty acids, however, tend to promote inflammation. The typical American diet tends to contain 14 - 25 times more Omega 6 fatty acids than Omega 3 fatty acids, which many nutritionally oriented physicians consider to be excessive. In order to develop proper biological function of human body, the supplement of Omega 3 polyunsaturated fatty acids is essential in the diet (Covington, 2004).

The detailed methods of extraction of Omega 3 PUFA from fish oils with its demand and market potential has been described here. In addition to that, the potential of mini-fluidic reactor technology is discussed within the context of Omega3 PUFA extraction from fish oil ethyl esters.

It is reported that brain and heart disorders resulting from LC-Omega-3 (EPA + DHA) deficiency are a significant challenge to longevity. The necessity of additional Omega 3 PUFA in diets is gradually being recognized, with a critical shortage of nutritional lipids such as Omega 3 PUFA anticipated by 2017. Omega 3 PUFA (DHA and EPA) are the nutritional lipids that have been proven in a growing body of scientific evidence to provide various health benefits and support the growth of brain, eye and cardiovascular health. Worldwide, consumption of Omega-3 polyunsaturated fatty acids (PUFAs) is estimated at 123.8 thousand metric tons (worth US$2.3 billion in 2013), and is forecast to be 134.7 thousand metric tons valued at US$2.5 billion in 2014. By 2020, it is projected that demand for Omega-3 PUFAs globally would reach 241 thousand metric tons with a value of US$4.96 billion, thereby posting a volume CAGR of almost 10% and a value CAGR of 11.6% between 2013 and 2020. (Meester, Fabien, 2013)

2.2. Properties of Omega 3 PUFA and Structure

PUFA (Poly Unsaturated Fatty Acids) are fatty acids with more than one double bond. Several PUFAs are recognized as ‘essential fatty acids’ in the normal diet for preventing nutrition-related illnesses. It is hypothesized that there are few direct actions of these PUFAs, and their activities are mostly mediated by their transformation in a number of metabolically active compounds collectively known as ‘eicosanoids’ (tromboxanes, leukotrienes, and prostaglandins). Eicosanoids are physiologically highly active and play diverse roles in human metabolism. (Calder, P. C., 2013).

Oily fish accumulate EPA and DHA obtained in their food chain, but do not synthesize these fatty acids. Eicosapentaenoic acids (EPA: 20:5n-3, Figure 2.1) are derived from long-chain fatty acids with a long un-branched aliphatic tail (chain) of saturated or unsaturated carboxylic acid (Bruinsma and Taren, 2000). The differences in geometry among the various types of unsaturated fatty acids and between saturated and unsaturated fatty acids play an important role in biological processes (Harrison, 2007). EPA is found in fish oil, sea weeds, algae and sea food.

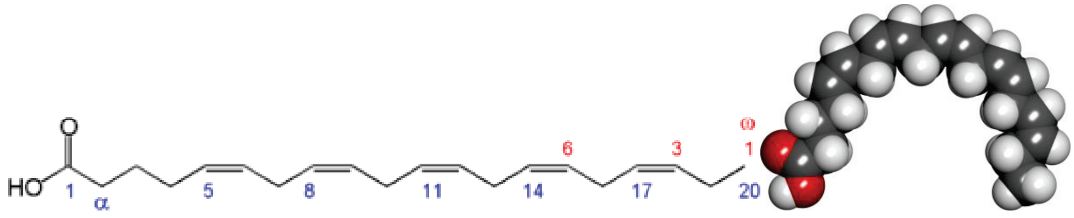


Figure 2.1. Structure of EPA and it has 5 double bonds

DHA (Figure 2.2) is synthesized from EPA by elongation and subsequent desaturation. Docosahexaenoic acids (DHA: 22:6n-3), and are long-chain fatty acids with a long unbranched aliphatic tail (chain) of saturated or unsaturated carboxylic acid (Bruinsma and Taren, 2000). DHA is found in fish oil, sea weeds, algae and sea food.

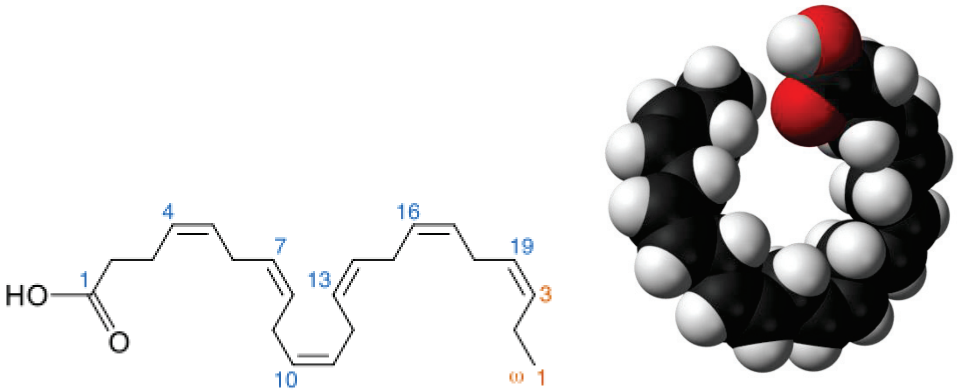


Figure 2.2. Structure of DHA and it has 6 double bond

2.3. Traditional Extraction Process Overview -Extraction of Fish Oils

Fish oils are classified into their lipid fractions such as triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG), sterylesters, phospholipids, free fatty acids and sterols. Lipids differ between various tissues, organs and species. The kind of fatty acids present as free acid or as neutral lipid depends upon the species and environments. Currently, fish oil is extracted by cooking and pressing at the commercial scale. However, solvent and enzymatic extractions are performed at the laboratory scale and are being looked at increasingly in commercial synthesis for specific applications. The most important aspect of processing fish oils is the elimination of saturated and mono unsaturated fatty acid, thereby concentrating the remaining EPA and DHA. The concentrate market name is “33/22”, roughly representing 33 wt. % EPA and 22 wt. % DHA (Lembke, 2013). An overview of the extraction process is provided in Figs. 2.3 and 2.4, illustrating the significant steps involved in processing raw fish feedstock to form concentrated esters.

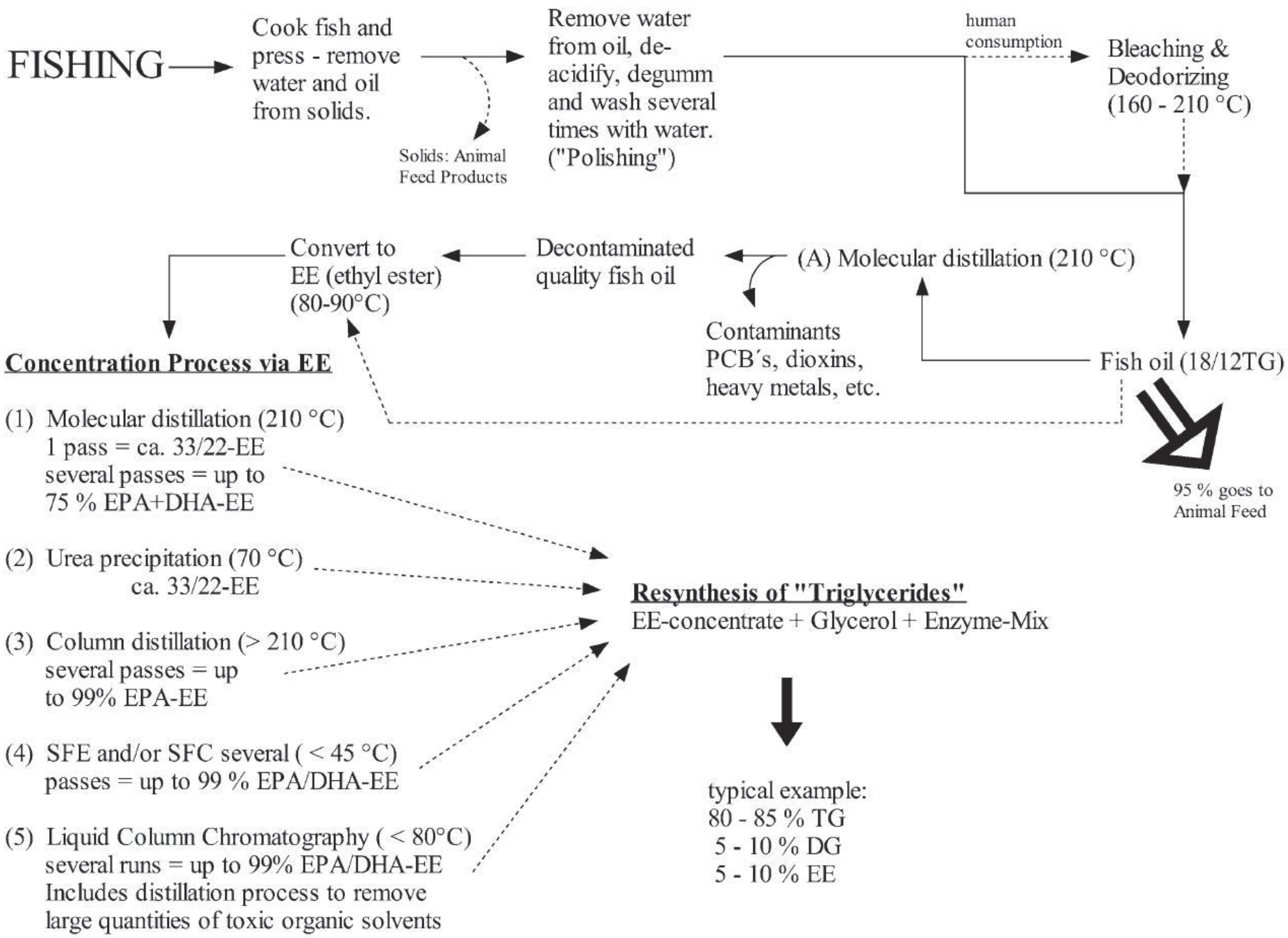


Figure 2.3. A simplified flow diagram for extraction of fish oils from the raw fish (Lembke, 2013).

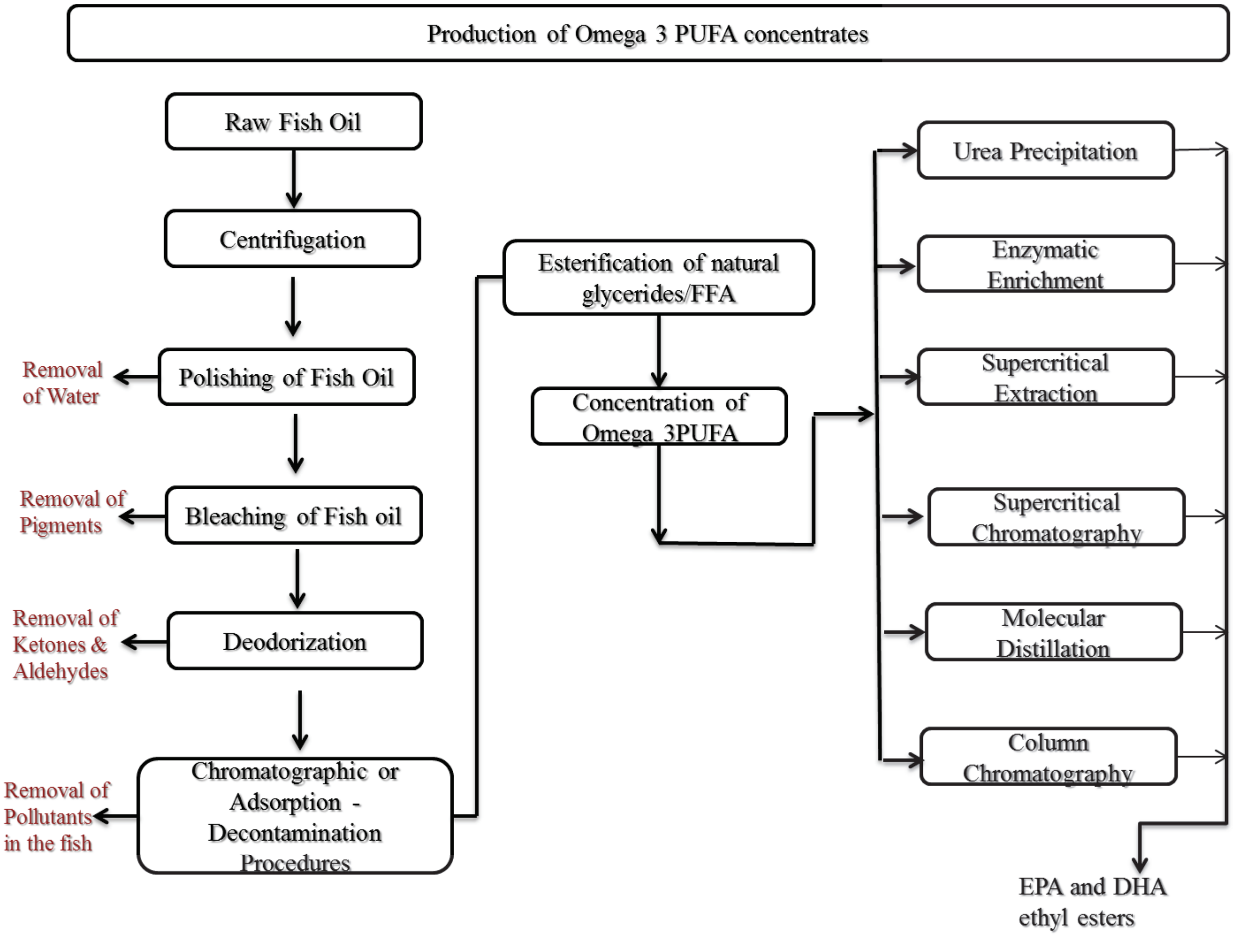


Figure 2.4. Overview of significant processing steps to produce EPA and DHA ethyl esters (Lembke, 2013).

Common examples of feedstock for fish oils and Omega 3 extracts are sardines, anchovies, menhaden and other small oily fish. After being caught, the fish are often already boiled on board the fishing vessel and then mechanically pressed to remove their body water and fat/oil. The remaining meat and bones are minced, dried and finally sold as protein rich animal feed, called fish meal. Following removal from the solids, the oil/water mixture is polished, deodorized, trans-esterified and concentrated to produce high-purity EPA and DHA. The various steps involved in the concentration of Omega 3 PUFA are explained in detail as follows and the obtained Water/Oil emulsion is first filtered and then separated into oil and water phases by large industrial centrifuges. The oil then gets washed several times with hot water which is known as polishing. Finally, the water is quantitatively removed by centrifugation and then the dried fish oil is stored for further processing for various unit operations. Bleaching is carried out in the absence of oxygen, which removes pigments from the oil with the help of adsorbents like bentonite, activated carbon and or silica. During the subsequent deodorizing step, the undesired fishy smell and taste get removed and the shelf life of the oil is prolonged. Deodorization is often a vacuum distillation process where at 170 to 220°C the free fatty acids, oxidation products like ketones and aldehydes, and other lighter boiling substances (compared to triglycerides), are stripped off. However, this process does not remove cholesterol, saturated fatty acids or contaminants like heavy metals, dioxins, pesticides etc. Supercritical technology and molecular distillation are also sometimes applied during the decontamination process, as well as chromatographic methods or selective adsorption techniques. (Rubio-Rodríguez, N. *et al*, 2010)

In order to continue concentration of fish oil, the triglycerides (TG) of fish oil are converted to the ethyl esters (EE) or free fatty acids (FFA). As a consequence, The EEs or FFAs are removed from the glycerol backbone of the original triglyceride molecule which enhances the possibility of enriching their concentration. But it reduces their digestibility and uptake rate as a nutraceutical. It is performed in a large scale reactor by treating the fish oil triglycerides in the presence of an alkaline catalyst with pure ethanol for at least an hour at 80°C. Enzymatic trans-esterification is an often-used alternative to alkali trans-esterification which minimizes the operating temperature and helps to avoid thermal decomposition and oxidation. Following trans-esterification, the PUFA's are extracted through a variety of techniques based on their intended application. (Lembke, 2013).

2.4. Processes for Concentration of PUFA from fish oils

An overview of the more predominant concentration processes encountered in literature is provided here in an effort to illustrate their relative processing parameters, advantages and disadvantages (Figure 2.3). The processes reviewed include urea precipitation, low temperature crystallization, molecular distillation, supercritical extraction, chromatographic methods, enzymatic enrichment, and solvent extraction. Much of the content of this review is based on the recent article by Lembke (2013), and is discussed for the purpose of identifying the applications for each approach.

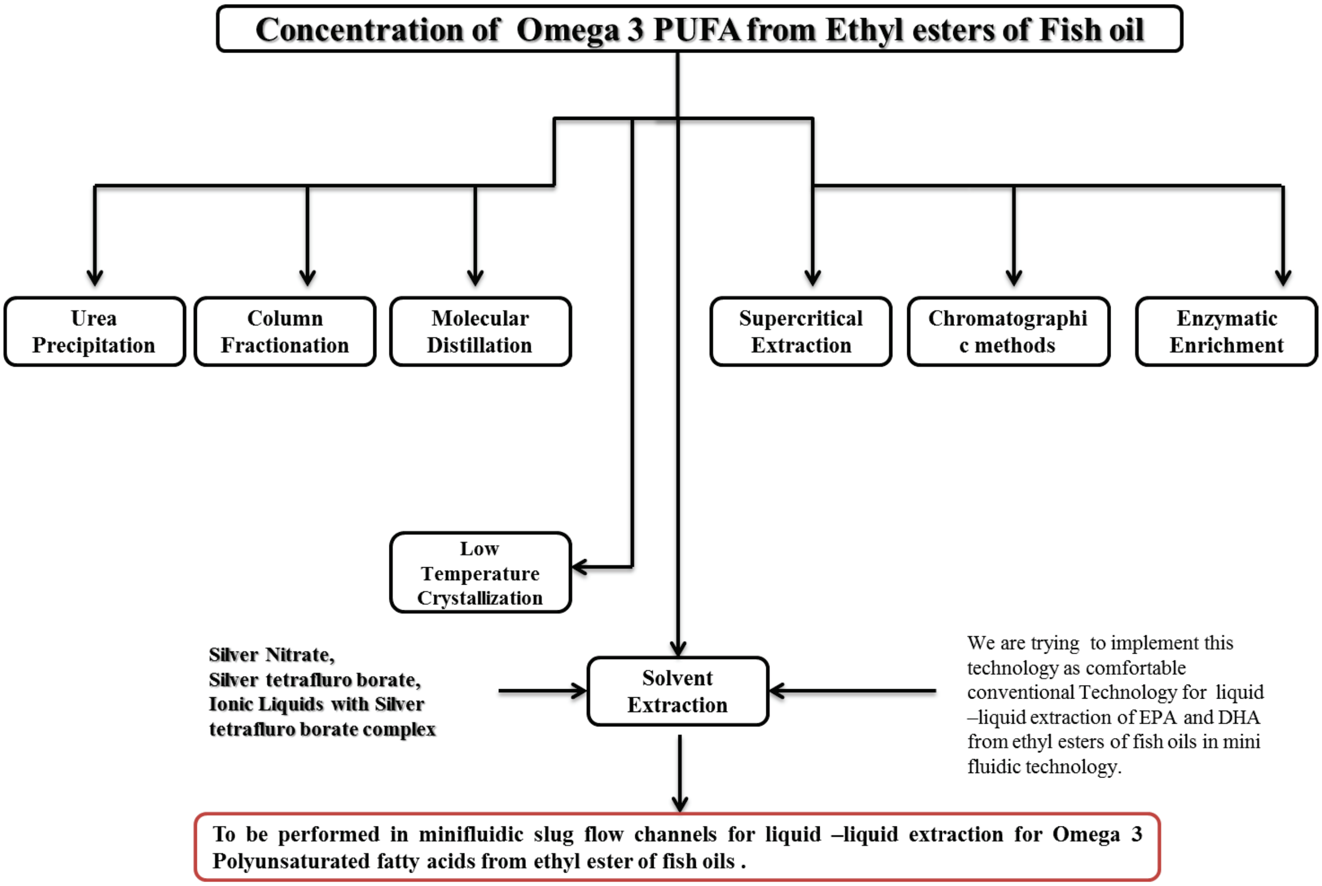


Figure 2.5. Overview of common extraction methods (Lembke, 2013.)

2.4.1. Urea Precipitation

Urea precipitation is the conventional method for the extraction of omega 3 PUFA from fish oil, used extensively for the extraction and concentration of polyunsaturated fatty acids from marine oils described initially by Domat *et al.* (1955). The urea process relies on the formation of crystals containing urea-complexes with saturated fatty acids, whereby subsequent removal of the crystals (centrifugation or filtration) produces an effluent liquid stream containing a higher concentration of unsaturated fatty acids (Fig. 2.4). Though a simple process, it suffers from disadvantages such as moderate extraction efficiencies and the potential formation of carbamate which is a highly carcinogenic compound (Canas *et al.*, 1999).

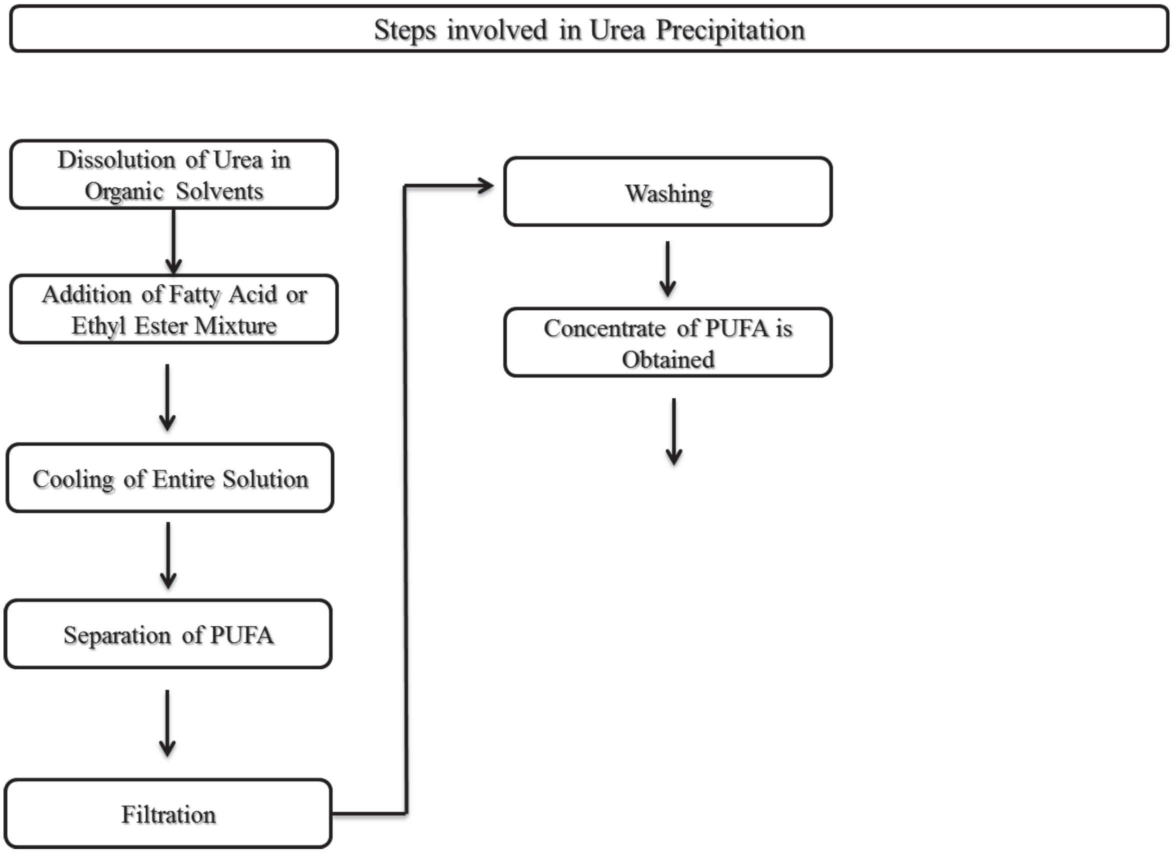


Figure 2.6. Overview of the Urea Precipitation extraction method.

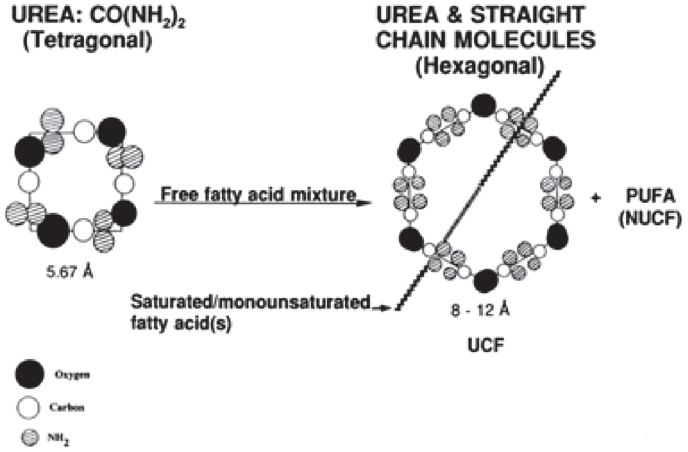


Figure 2.7. Formation of urea crystals in the absence and presence of long chain fatty acids (Wanasundara and Shahidi, 1998).

Reports of performance of the urea-based method have varied by feedstock and conditions employed. Udaya *et al.* (1999) reported a maximum Omega 3 recovery from seal blubber of 88.2% under optimum conditions for a urea to fatty acid ratio of 4.5:1. Fujita *et al.* (US Patent No 4,377,526) reported that when treated with urea, a mixture of fatty acids containing EPA could be subjected to fractional distillation in order to obtain higher yields of EPA. The fractional distillation, however, is carried out at a temperature of at least 180° C over a period at least 40 minutes. The best purity obtained by this method was 92.9%. Furthermore, substantial cis-trans conversion (as much as 20%) was observed when this method of purification was employed, which is strictly undesirable for food or pharmaceutical use. An additional study by Crexi *et al.* (2011) reported that urea complexation of fatty acids at -10°C for 20 hrs with urea: fatty acid ratio of 4.5:1 yielded a 31.4% increase in mono saturated and poly saturated fatty acids (MUFA and PUFA) and a 75% decrease of saturated fatty acids. An 85.4% increase in EPA and DHA content was also observed. The non-urea complexation fraction can be considered a rich source of MUFA and PUFA with a total amount of 88.9%. According to economic feasibility, the urea complexation process is conventional and the yield of omega 3 PUFA is around 65 % in industrial process. US patent (Breivik, H. *et al* , 2012) reported that urea fractionation sometimes may result in higher concentration factors for omega-6 fatty acids than for the homologue omega-3 fatty acids. Furthermore, urea crystallization is performed on fatty acids or ethyl esters when the saturated and monounsaturated components are removed through formation of adducts with urea, leaving behind a fraction enriched in PUFA, particularly the highly unsaturated components EPA and DHA. Although it is efficient and cheap, urea crystallization is not recommended for production of food grade ingredients because of the risk of producing carcinogenic alkyl carbamates.

2.4.2. Low Temperature Crystallization

Low temperature crystallization of lipids, especially triglycerides and fatty acids, is based on the separation of triglycerides and fatty acids or their methyl/ethyl esters according to their melting points in different organic solvents like hexane, methanol, ethanol or acetone at very low temperatures (-50°C to -70°C). The low temperature crystallization has to utilize large amounts of flammable solvents while also operating under a heavy refrigeration load, presenting serious shortcomings for large scale industrial applications (Lembke, 2013). While mini-fluidic technology may offer advantages in solvent inventory reduction, the presence of solids within such small channels is known to pose reliability concerns for the current state of technology. Thus, its use would require careful consideration of allowable solids content and purification stages to avoid process downtime due to blockages. It is, however, a possibility provided the energy duty of refrigerating the incoming ester feedstock is economically viable.

2.4.3. Molecular Distillation

The process of concentrating individual fatty acids from fish oil with the help of vacuum distillation has been known for more than a century. Furthermore, modern MD is an efficient way to produce especially semi-concentrated EPA and DHA Products. This technology makes use of the fact that the free fatty acids and fatty acid ethyl esters can be vaporized at industrially feasible temperatures if distilled under a strong vacuum (0.001 mbar). Under these conditions, at a temperature between 140-170°C, a good separation between simpler C18 fatty acids and larger C18 fatty acids can be achieved, propelling the initial EPA and DHA content from 30 % (18/12EE) to approximately 50 -60%. In this case, the classical standard molecular distillation concentrate on the market is a 33/22 EE or 33% EPA and 22 % DHA, together EPA and DHA total 55% (Lembke, 2013)

2.4.4. Supercritical Technology

Supercritical extraction technology is one of the most recent methods introduced in this field, and have been promoted for its advantages from a product quality perspective. There is, however, as an ongoing concern over processing cost. Specific advantages observed include decontamination of fish oil triglycerides, removal of polar impurities, improved color characteristics, reduced oxidation products from the ethyl ester (EE) fraction, and enrichment the C20 and C22 groups of fatty acids. In order to operate supercritical technology, compressed CO_2 is used at pressures above 73 bar (~1070 psig) and temperatures of ~32°C. Under these conditions, the CO_2 is in its supercritical state and is an excellent solvent for most lipids. Polar compounds are insoluble in CO_2 and can thus be separated. Once depressurized, the CO_2 loses its dissolving properties and the pure lipids/FFA/EE drop out of the gaseous CO_2 without the need for the secondary dilution/solvent extraction encountered when using liquid organic solvents. The solubility of fatty acids in supercritical CO_2 decreases with increasing molecular weight. The longer the fatty acid, the less soluble it is in supercritical CO_2 (Lembke, 2013)

Perretti *et al.* (2007) reported the extraction of a lipid fraction enriched in omega 3 fatty acids using supercritical CO_2 operating pressures of 150 and 300 bar. Follegatti-Romero *et al.*(2009) reported that supercritical carbon dioxide (SC-CO_2) was employed to extract omega-3 rich oil from Sacha inchi (*Plukenetia volubilis L.)* seeds and partially defatted cake. In this case, the system was operated at 300 and 400 bar and 40 to 60°C to obtain an extract with 50.5% linoleinc acid. Rubio-Rodríguez *et al.* (2009) also reported the production of omega 3 PUFA from hake *(Merluccius capensis–Merluccius paradoxus)* using supercritical CO_2. The extraction temperature was maintained at 40°C in all cases in order to avoid thermal degradation of the polyunsaturated fatty acids contained in hake oil. The results obtained indicate that internal mass transfer controlled the rate of the process under the conditions studied, suggesting that a system which enhanced inter-phase mass transport may offer specific advantages in this process. The optimum extraction conditions were found to be 25MPa, with a flow rate of 10 kg CO_2/h over freeze-dried and grounded hake. Under those conditions, more than 96% of the total oil contained in the raw material was extracted after 3 h. The oil extracted presented a high omega-3/omega- 6 ratios (around 7) and a high EPA and DHA content (about 6% and 14%, respectively, of the total fatty acids contained in the hake by-product used). Research into this process is on-going due to the significant advantages and extraction performance observed. There remains, however, the overriding cost of pressurizing both the oil feedstock and the recycled CO_2 to achieve supercritical conditions.

2.4.5. Chromatography

Liquid chromatography has been employed to improve yield of EPA and DHA from fish oils and has the highest selectivity for separating specific chain lengths and degrees of unsaturation. Liquid chromatography is, however, a dilution process, requiring the feedstock to be diluted in significant quantities of organic solvent (mobile phase) prior to injection into a separation column. Column effluents with specific residence times are then removed and re-concentrated, potentially introducing oxidative stress on PUFAs (Lembke, 2013). A number of the columns employed in this method incorporate immobilized organometallic compounds (i.e. silver) to selectively adsorb unsaturated molecules and delay their elution time from the column.

2.4.6. Enzymatic Enrichment

This method relies on the liberation of FFA from the glycerol backbone using specific enzymes that only cleave in the sn1 and sn3 position of the glycerol or that cleave specifically in the sn2 position for enrichment of free fatty acid in the fish oils. The lipase is mostly used for enzymatic enrichment of FFA with EPA and DHA in the fish oils. The FFA can easily be removed by MD, leaving a concentrated omega-3 rich 2-monoacylglycerol fraction. To this fraction previously enriched EPA and DHA-FFA could be added together with a lipase, which synthesizes an omega-3 rich reconstituted triglyceride product. In this process, the yield is slightly above 50 % (Lembke, 2013).

2.4.7. Solvent Extraction of EPA and DHA omega 3 PUFA from fish oils

Silver nitrate salt-solutions have previously been used as a solvent for the purification of the ethyl ester of PUFA (PUFA-Ets) Yazawa *et al.* (1995). In this process, silver ions interact with the double bonds in unsaturated fatty acids to produce complexes which are soluble in ionic/polar carrying phases. Since the separation is performed exclusively via a reversible chemical reaction, this technique is very simple and would be a promising method for the purification of PUFAs. Similar principles have been applied in chromatography-based separations, where Fagan *et al.* (2013) reported that the ethyl esters of EPA and DHA from tuna oils can be isolated using a polymeric silver cation exchange as the stationary phase. In this case, greater than 88% of separation occurred and suggested the potential for reasonable yields.

Limited reports are available of extraction performance using silver salts to isolate polyunsaturated fatty acids from a mixture of FFA, with previously-known methods not providing for a sufficiently selective and/or efficient process for concentrating omega-3 fatty acids. US Patent (Breivik, H *et al*, 2012) reported that silver salt solutions could be used in the extraction of PUFA from marine oils, describing a potential process that is summarized as follows:

i. Combining the fatty acid oil mixture and an aqueous silver salt (such as $AgNO_3$ or $AgBF_4$) solution to form an aqueous phase and an organic phase, wherein in the aqueous phase, the aqueous silver salt solution forms a complex with the at least one omega-3 fatty acid,
ii. Separating the aqueous phase from the organic phase,
iii. Extracting the aqueous phase with a displacement liquid, or increasing the temperature of the aqueous phase to at least 30°C, or a combination of extracting with a displacement liquid and increasing the temperature, resulting in formation of at least one extract,
iv. Combining the aqueous phase with water, or extracting the aqueous phase with supercritical CO_2, or a combination of combining the aqueous phase with water and extracting the aqueous phase with supercritical CO_2, to dissociate the complex, wherein an aqueous phase comprising the silver salt and at least one solution in which a fatty acid concentrate forms; and
v. Separating the at least one solution comprising the fatty acid concentrate from the aqueous phase comprising the silver salt.

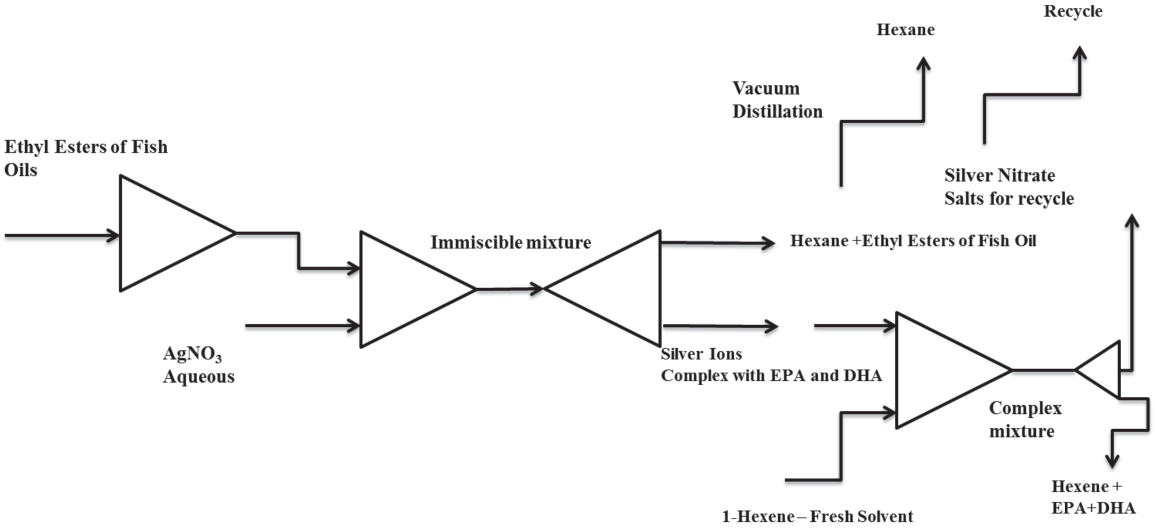


Figure 2.8. The simplified process for liquid–liquid extraction of EPA and DHA.

Within available literature, it was observed that PUFA–ME (Poly Unsaturated Fatty acids methyl esters) with high degree of saturation such as DHA-ME and EPA-ME selectively bind to the aqueous phase of silver nitrate solution. Li *et al.* (2009) reported that the extraction of poly unsaturated fatty acids from oils with hydrophobic ionic liquids (imidazolium-based) containing silver salts (silver tetra fluoroborate) as the extraction medium yielded greater extraction performance than silver nitrate based solutions. In their work, the percentage of PUFA of methyl esters in the extract phase approached 93.1%. Moreover, Li *et al.* demonstrated improved solvent recovery and salt recirculation when an ionic liquid was employed with a hexene-based secondary extraction, although the method employed may also be suitable for aqueous-based salt systems. Temperature has also been found to significantly affect solvent extraction with silver nitrate solutions. In the work of Teramoto *et al.* (1994), the distribution ratios of polyunsaturated fatty acid ethyl esters between aqueous $AgNO_3$ extraction phase and an organic phase of esters dissolved in heptane were found to increase drastically as temperature was lowered. Qualitative evidence in literature would suggest that hydrophobic ionic liquids may potentially offer advantages over water when used as the dissolving phase for the silver salts. Li *et al.* (2008) reported that $AgBF_4$ exhibited high extraction potential in the hydrophobic ionic liquids but little or no extraction capacity in the hydrophilic ionic liquids. It was observed that the increase in alkyl chain in the ionic liquid (imidazolium ionic liquid) caused better hydrophobicity with $AgBF_4$ and leads to better extraction potential and also minimum extraction time. The preparation of ionic liquid, however, is a tedious process and requires inert gas atmospheres such as argon blanketing and heating at 70°C. Additionally, there is chance of decomposition of ionic liquid conjugated silver salts under harsh environments. The ionic liquids with silver salts are also light-sensitive, and must be stored under dark conditions. A significant limitation of the solvent extraction approach is the need for large amounts of expensive silver-salts, both for high recovery and freezing point depression when an aqueous carrier phase is used at low temperatures. While $AgNO_3$ was historically employed for this method, Li *et al.* (2009) recently reported a number of other novel π-complexing sorbents for extraction of omega 3 PUFA from oils. Novel π-complexing sorbents were prepared by covalently immobilizing ionic liquids (ILs) onto silica and then coating this silica- supported ILs with silver salts, with applications primarily in chromatography. The salts explored (Table 1) do, however, offer potential alternatives for a solvent-solvent extraction system.

Table 2.1. Silver-salts for the selective extraction of unsaturated fatty acids.

Salt compound	In Water	In IL	Remarks
$AgBF_4$	US Patent 2012/038833	M.Li *et al* 2008	$AgBF_4$ in water less extraction potential than in IL.
$AgNO_3$	M.Teramoto *et al*, (1994).	M.Li *et al* 2008	Cheap silver salt compared to other options and Least performance in Ionic Liquids and Lowest extraction potential among silver salts.
$AgClO_4$	-	M.Li *et al* 2008	Poor performance than $AgBF_4$
$(CH_3)_3COO$ Ag	-	M.Li *et al* 2008	Good Extraction performance in IL
CF_3SO_3Ag	-	M.Li *et al* 2008	Good Extraction performance in IL
CF_3COO Ag	-	M.Li *et al* 2008	Good Extraction performance in IL

Min Li *et al* (2008) reported that (CH3)3COOAg, CF3SO3Ag and CF3COOAg showed moderate extraction potential and the separation performances were in the order of AgBF4 > AgCF3SO3 >> AgNO3.

Table 2.2. Comparison of most common Omega 3 PUFA concentration techniques, adapted from Lembke (2013)

	Molecular Distillation	**Supercritical Fluid Chromatography**	**Liquid Chromatography**	**Supercritical Extraction**	**Urea Precipitation**	**Low Temperature Crystallization**	**Solvent Extraction**
Selective mechanism	Chain length (Boiling Point)	Chain Length and C = C	Chain Length and C = C	Chain Length	Saturated Fats	Melting Point (Chain Length and C = C)	Silver ion reversible binds with d C = C
Process Conditions	Temp -140-220 C 0.001mbar	35-50°C >140 Bar	20-50° C 1 Bar	35-50°C >140 Bar	-10 C -90°C 1 bar	0 -70°C 1bar	20 -30°C 1bar
Use of Toxic Solvents	No	No	Organic Solvents	No	No	No	No
Extraction Efficiency	65-75%	99%	99%	75-85%	45-65%	>90%	88% -91%*
Decontamination Efficacy	Very high	Very high	High	Medium	Low	Low	Low
Product Oxidation	Low	Very Low	Possible	Very low	Possible	Possible	Very low
Operation Mode	Continuous	Semi-continuous	Semi-Continuous	Continuous	Batch	Batch	Under investigation
Capital Investment	Low	High	High	High	Low	Low	Low

* The extraction efficiency of the solvent extraction based on aqueous phase silver nitrate solution is 88–91% from concentrate. However, the separation performance is more >94% in silver salts in ionic liquids.

2.5. Mini-fluidics Approach on silver based solvent extraction of Omega 3 PUFA

To produce concentrated pharmaceutical and food grade Omega 3 PUFA, efficient extraction and concentration methods are required and also continually researched for improvement on performance of separation. Fish oils are abundant in Omega 3–PUFA and have traditionally been used as the feed stock for preparation of omega 3 PUFA concentrate. Since fish oils contain a complex mixture of fatty acids with various chain lengths and degrees of unsaturation, so that separation of individual fatty acids is challenging for production of highly concentrated Omega 3 components. With the successful implementation of a number of technologies at the industrial scale, the challenge now faced is now to develop more cost–effective procedures to produce omega 3 PUFA concentrates to meet the growing demand. (Lembke, 2013, Rubio-Rodríguez *et al.*, 2010, Shahidi *et al.*, 1998)

Recently, micro/mini-fluidic technology has been developed to enhance the performance of liquid–liquid extraction and other applications such as micro total analysis, nuclide separation systems and mini and micro chemical plants. The flow patterns generated in mini-fluidic technology have been shown to offer high mass transfer areas and consistent extraction performance, while minimizing solvent inventory requirements compared to conventional extraction systems. This has led to its adoption for a number of temperature sensitive reactions such as nitration and halogenation for transitioning from batch to continuous processing technology. (Burns and Ramshaw, 2001, Dummann, G, 2003). Given the reversible and exothermic nature of the silver-PUFA complexation reaction and the desire to operate at lower temperatures if possible, the compact framework of the mini-fluidic system should enable more efficient temperature control while reducing both solvent inventory and ambient temperature effects on operating efficiency. This was, in fact, observed recently by Kamio *et al.* (2010, 2011), who described liquid–liquid extractions of DHA-Et from an organic carrier using silver salt solutions in a micro reactor framework. In their work, contact times on the order of 10 to 20 seconds were sufficient to reach equilibrium at the conditions tested, leading to the current interest in this approach. This section explores a number of approaches on mini-fluidic technologies which have been considered for processing of marine oils, with the intent of providing a brief overview of the state of the field.

Miniturization is an alternate approach to process intensification which aids to achieve a reduction in energy used for operations, capital expenditures, plant profile in terms of height and area and further environmental benefits. (Charpentier, J. C., 2005).This method has been attempted for silver based solvent extraction of omega 3 PUFA from fish oil ethyl esters. Execution of silver-based solvent extraction in mini-channels results in significant interfacial surface area and a shift in dominant forces governing fluid flow. In fluid–fluid chemical reaction based extraction systems, the organic and aqueous phases are generally immiscible in nature. Since the two immiscible phases must contact each other before reacting, both mass transfer and the chemical kinetics impact the overall rate expression. Increased mass transfer can be achieved through either an increase in local shear (mixing) or interfacial area (mixing or geometric scale reduction), or a decrease in diffusive path length (geometric scale reduction). The net impact of these adjustments within a variety of technologies is summarized in Table 3 as overall volumetric mass transfer coefficient K_La values. A wide variety of conventional extraction equipment is accessible in solvent extraction, such as mixer settlers, centrifugal extractors, spray columns and agitated columns. However, a significant limitation in conventional systems is the inability to control the drop size precisely and the lack of plug-flow characteristics as the process is scaled to larger capacities. Mixing and solvent inventory are key issues in the separation performance in extraction, with large reactor volumes requiring significant secondary storage capacity. (Okubo, Y. Okubo, Y., 2008)

Within the past decade, mini-fluidic contacting systems have been applied to various applications of process engineering. Based on a review of extraction methods of potential benefits of operating mini-channel scales, limited analysis has been performed on solvent extraction of omega 3 PUFA. Of particular note was a lack of hydrodynamic studies evaluating the impact on yield of omega 3 PUFA. Table 4 summarized the application of mini-fluidics in silver based solvent extraction and approximate yields to which this work will be compared.

Table 2.3. K_La Values for Different Types of Contactors (Kashid, M. N. *et al* 2007)

Conventional Extractors	**Extraction System**	**K_La (x 10^{-4} s^{-1})**
Agitated vessel	water (c)-iodine-CCl_4 (d)	0.16-16.6
	sulfate ore (c)-uranium-kerosene (d)	2.8-17
Rotating disc contactor	water (c)-succinic acid-n-butanol (d)	57
	water (c)-acetic acid-methyl isobutyl ketone (d)	20-120
	water (c)-acetone-DCDE (d)	63-266
Rotating agitated column	n-hexane (c)-acetone-water (d)	0.15
	toluene (c)-acetone-water (d)	0.2 -1.0
	water (c)-furfural-toluene (d)	105
Spray column	water (c)-acetone-benzene (d)	8-60
	water (c)-adipic acid-ether (d)	20-70
	water (c)-acetic acid-benzene (d)	17.5-63
	water (c)-acetic acid-nitrobenzene (d)	7-32
Packed Column	CCl_4 (c)-acetone-water (d)	7.4-24
	kerosene (c)-acetone-water (d)	5.8-61
	methyl isobutyl ketone (c)-uranyl nitrate-water (d)	14.7-111
	toluene (c)-diethyl amine-water (d)	5-14.7
	vinyl acetate (c)-acetone-water (d)	7.5-32
Perforated plate column	water (c)-acetaldehyde-vinyl acetate (d)	28.5
Impinging streams	water (c)-iodine-kerosene (d)	15-2100
	kerosene (d)-acetic acid-water (c)	500-3000
	water (c)-iodine-kerosene (d)	560-2000
Rotating discs impinging streams contactor	water (c)-iodine-kerosene (d)	1187-3975
	kerosene-acetic acid-water (c)	1364-4456
	water (c)-succinic acid-n-butanol (d)	1311-9815
Minichannel (0.5,0.75, 1mm)	water-iodine-kerosene	1311-9815
	kerosene- acetic acid-water	4058-14730
	water-succinic acid-n-butanol	200-3200

* ID varies from 0.5 mm -1 mm**,** C- continuous phase, D- Dispersed phase

Table.2.4. Overview of Silver Ions based extraction of Omega 3 PUFA in different processes

System	Flow pattern	Residence Time	Yield	Reference
Ionic Liquids based $AgBF_4$ in Mistral Multi-Mixer	Mixing	30 mins at 20°C	80% in stripping Solvents	Min Li *et al* 2008
Imidazolium-based ionic liquids Containing silver tetra fluoroborate	Mixing	25 mins at 20°C	Distribution ratio-320	Min Li *et al* 2009
Silver Ion Loaded Porous Hollow fiber membrane	Flow through packed column	N/A	100% recovery	Akio Shibasaki *et al* 1999.
∏ Complexing Sorbents	Solid Phase extraction in Mistral Multi-Mixer	30 mins at 20°C	89-91% Stripping in solvents	Min Li,2009
Silver Nitrate Solution	Micro reactor Slug flow	20 sec		Eiji Kamio *et al* ,2009
Silver nitrate Solution	Hydrophobic Hollow fiber membrane	N/A, 303 K	N/A	Kubota *et al* ,1997
Silver nitrate solution	Slug flow* based Mini-fluidic reactor	36 sec at 10°C	80%	Adam.A.Donaldson 2013

- Adam. A. Donaldson, 2013 reported that the mini-fluidic experimental set up designed for slug flow pattern. However, in experimental conditions, a Stratified flow pattern was observed due to physical properties of the liquids.

Solvent extraction of Omega 3 PUFA can be operated on mild condition such lower temperature and is suitable of large scale production. Nishi *et al* 1991 reported that ethyl esters of PUFAs such as DHA and EPA could be successfully extracted into silver nitrate solutions since silver ion can complex with carbon-carbon double bonds of PUFAs. M**.**Teramato *et al* 1994 reported that the extractions of ethyl and methyl esters of PUFA such as EPA and DHA from various organic solvents into the aqueous silver nitrate solutions were performed. It was observed that EPA and DHA specifically bound to the silver ion as it has highest degree of unsaturation. In addition to that, the distribution ratio (D) increased drastically as temperature decreased and also by addition of water-soluble alcohols such as methanol, ethanol, and n-propanol to aqueous solutions. The distribution ratio depends on the solvent used for dissolution of organic phases.

The closest work to that presented here is an extraction of pure components of ethyl ester of Docosahexaenoic acid (DHA-Et) and Eicosapentanoic acid (EPA-Et) from an organic carrier into an aqueous silver nitrate solution in a 0.5mm T – micro reactor (Eijo Kamio *et al* 2010). In their work, extraction equilibrium was reached after 20 seconds, with large mass transfer rates observed due to the high interfacial areas present and internal recirculation within the liquid slugs. The DHA–Et reaction against silver ion follows first order kinetics with the respect to oil phase and is 2.5 orders with respect to silver ion concentration.

The creation of slug flow in the micro reactor especially in the case of omega 3 PUFA extractions offers better interfacial area and enhanced mixing between the organic and aqueous phase. The enhancement was such that at lower temperatures (268 K), slug flow pattern had faster extraction rates than emulsion-based methods without the associated phase separation difficulties. While promising in nature, the work by E. Kamio *et al* (2010) focused specifically on highly idealized mixtures, and did not explore the impact of commercial fish oils which contain a broad spectrum of organic compounds.

2.6. Thesis Scope within Prior Literature

In this work, the practical implications of extraction of Omega 3 PUFA from 18/12EE fish oils with concentrated silver nitrate solutions were evaluated in 1/16" mini- fluidic channels. The intent is to evaluate it previously observed mass transfer performance and hydrodynamic characteristics in idealized mixtures transfers to the industrial process streams. To do so, an experimental system has been constructed to contact 18/12EE fish oils with concentrated silver-salt solutions (50wt. %) in a controlled manner and determine if previous idealized system performance is observed when commercial fish oils are used. Silver-based solvent liquid –liquid extraction was carried out in 1/16" ID food-grade Tygon tubing submersed in a temperature-controlled refrigerated reservoir. The development of experimental setup is detailed in the chapter 3. Samples of the organic/aqueous phase were subsequently collected and analyzed at the Dartmouth Analytical facility of DSM, Ocean Nutrition. The organic phase was compared directly to the feedstock for each experiment to identify changes in the oil composition, while the aqueous solution was further processed in an attempt to elute and recover the extracted PUFA's. The variety of flow patterns and physical property characteristics of the system were explored, and a conceptual design analysis of the practical implementation of this technology at a commercial scale is discussed.

CHAPTER 3. EVALUATION OF SILVER BASED SOLVENT EXTRACTION

To achieve the experimental objectives of this thesis, it was necessary to carry out LLE experiments using commercial trans-esterified fish oils and silver nitrate-based solvent solutions and characterize the results. This chapter describes the experimental system, the procedure used for preparing and running each experiment, the analytical methods appropriate to the results obtained, and the subsequent tools used to evaluating flow patterns in mini-fluidic reactor and interfacial tension between these particular fluids and the effect of organic solvents on interfacial tension. Comparison is made to previous reported work which attempted the extraction of pure DHA–et using silver ions in slug flow based micro reactor which has the dimension of ID 0.5 mm.

In mini-fluidic/micro-fluidic contacting systems, there are significant reports of the slug flow (segmented flow) patterns and its advantages and disadvantages when used for liquid-liquid extraction. Stratified flow, however, has only been reported in a limited number of mini-fluidics based systems, primarily due to the tendency of surface tension dominance in these systems limiting the formation of a stable stratified layer. In this work, The LLE experiments were performed with raw fish oil ethyl esters using concentrate aqueous silver nitrate solution under conditions for which slug flow was expected.

3.1. Extraction Procedure

In solvent based extraction of omega 3 PUFA, freshly prepared concentrated aqueous silver nitrate solution is used. The chemical used are silver nitrate (ACS grade-99% Purity), sodium nitrate (Assay – 99%) from fisher scientific , 95% ethyl alcohol , deionized water, semi refined 18/12 fish oil ethyl esters from DSM Ocean Nutrition, Dartmouth, NS, Canada, and Nitrogen gas which is used to blanket and prevent the oxidation of fish oil and $AgNO_3$. Semi refined fish oil ethyl ester was provided from DSM, derived from anchovy (*Engraulis ringens)* and Sardine (*Sardinops sagax sagax*) on 29thJune 2013.

3.1.1. Preparation of Silver nitrate solution

Prior to each experiment, a fresh batch of the silver nitrate solution with 50 wt.% of $AgNO_3$ and 5 wt.% $NaNO_3$ was prepared to avoid long-term oxidation of the non-stabilized silver nitrate solution. The solution was prepared by adding 400 grams of silver nitrate (99.9% ACS grade) to 400 ml of distilled deionized water and dissolved completely. 40 grams of $NaNO_3$ was subsequently added as an ionic strength adjuster to provide a comparable composition as reported by Kamio *et al.* (2010, 2011). It seems that the liquid volume of solution is increased up to 125 % due to the addition of silver nitrate and sodium nitrate. The silver nitrate solution is then purged with nitrogen and refrigerated in a blanketed opaque container to minimize oxidation and sunlight exposure prior to use. Similar to silver nitrate solution, the 18/12EE fish oil was also nitrogen purged before and after transfer to the reservoir to avoid the oxidation of the omega 3 PUFA.

3.1.2. Winterization of Fish Oil Ethyl Esters

The 18/12 fish oil ethyl ester contains some solid matter and extraction at lower temperature forms some gelation in the fish oil ethyl esters. As a consequence, it may block flow in mini-channel. In order to avoid this problem, the fish oil ethyl ester was winterized. The winterization is the process of fractional crystallization of oils and fats followed by the separation of solids, and are often used to make high quality food oils. On heating the fish oil ethyl ester to 60˚C and cooling down to 10˚C, a gel forms within the solution and settles in the container. Subsequent analysis of non-winterized and winterized feedstock oils used in this process by Gas Chromatography confirmed that the overall DHA, EPA and total Omega 3 PUFA was not significantly modified by the winterization process.

3. 1.3. Development of Slug Flow mini-fluidic reactor experimental setup

The slug flow mini-fluidic reactor was constructed for LLE of EPA and DHA ethyl esters from semi refined 18/12EE fish oil ethyl esters. The simplified mini-fluidic experimental setup is shown in Figure. 3.1, consisting of a $1/16^{th}$ inch ID Tygon mini-fluidic channel submerged in a cooled reservoir controlled to 10°C using an external refrigerated circulating bath. The solutions from the reservoir were pumped using a double syringe pump (2 NE 4000), whereby a 60CC syringe was used for the silver nitrate solution and a 10CC syringe was used for the 18/12EE fish oil. By setting a dispense at rate of 5 ml/min for the 60CC syringe, the silver salt solution flow rate was 5 ml/min and the oil flow rate was 1.47 ml/min, thus maintaining an approximate salt to oil solution flow ratio of ~3.4:1 (Kamio *et al.* 2010, 2011). The fish oil ethyl ester and silver nitrate solution are pre-cooled in a 1.5m length of tubing prior to being contacted together in a “Y” junction, after which the immiscible fluids were allowed to contact for a set residence time before being sampled via syringe. Sampling ports were fitted into the immersion vessel by creation of holes through the side, minimizing the time which the fluids spent outside of the refrigerated environments. The holes through the vessel walls were below the water line inside the vessel, and were sealed by silicon caulking. Water inside the cooler was circulated using a submersible pump, with a copper line run through from the refrigerated bath recirculation loop.

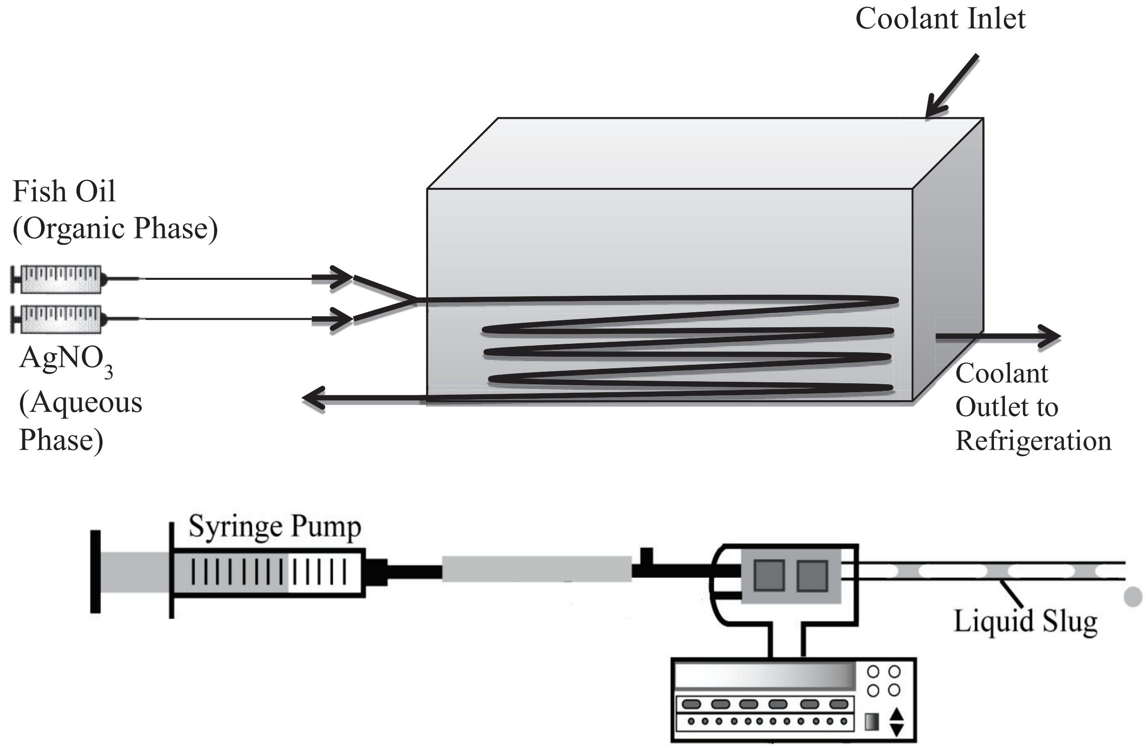


Figure 3.1. Slug flow mini-fluidic extraction experimental set up. The 1.58mm ID mini-fluidic channel is immersed in sub cooled water (10° C) in the bath which is circulated by submersible pump. In the second picture of the fig 3.1, the flow patterns produced also depends on the contactor (mixing system) in the experimental systems. So that the various flow patterns will be anticipated like slug flow/drop let flow/ stratified flow. The experimental set up has been designed for slug flow pattern.

Figure 3.2. Dual syringe Pump from Longer Instruments used to control flow into the mini-fluidic system.

Figure 3.3. Immersion vessel for cooling the mini-fluidic channels

Figure 3.4. Tygon mini-fluidic channel

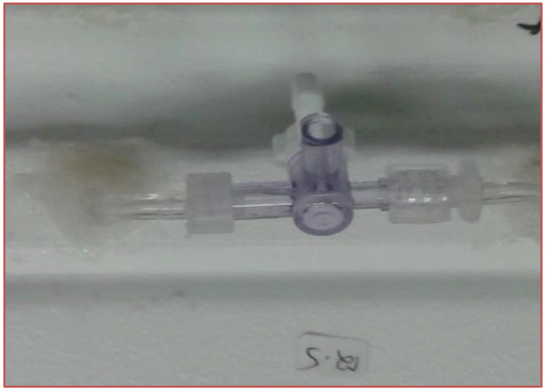


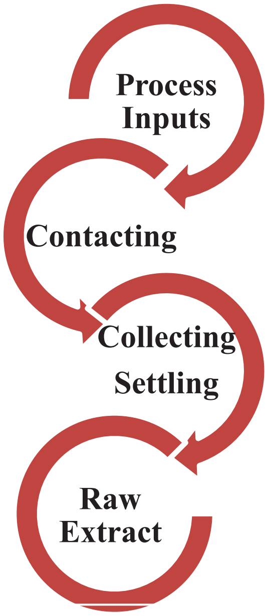
Figure 3.5. Sample Port

3.1.4. Safety Consideration

In the LLE experiments, 50 wt. % concentrated silver nitrate solution was used as extraction solvent for extraction of omega 3 PUFA from fish Oil EE. Silver nitrate is toxic and corrosive, necessitating minimum exposure to avoid immediate or any significant side effects other than the purple skin stains, but with more exposure, noticeable side effects or burns may result. Long-term exposure can cause permanent blue-grey staining (argyria) of eyes, mouth, throat and skin, and may cause eye damage. Short contact can lead to deposition of black silver stains on the skin. Besides being very destructive of mucous membranes, it is a skin and eye irritant. Silver compounds, such as silver nitrate can be extremely toxic in the human body due to the extremely high concentration of silver. (Fisher Scientific, 2014)

3. 2. LLE Operation Procedure

Extraction of PUFA's from 18/12EE fish oil with $AgNO_3$ (aque.) silver-salt solutions was performed at 10°C in accordance with the procedure outlined in Figure 3.6. The flow channels were sterilized with ethanol, washed thoroughly with deionized water and finally purged with air to remove any residual water vapor. The dual syringe pump delivered the fluids into the mini-fluidic channel through a 1.5 meter pre-cooling loop, after which time the phases came into contact in a Y-junction. The combined flow was then allowed to proceed through set lengths of tubing (0.5, 1, 2, 4, and 6 meters) before being sampled for subsequent analysis. Due to the limitations of the syringe pumps employed, flow rates above those stated were not possible for these lengths (skipping of the screw drive on the syringe pumps). The resulting residence times for each of these sample ports were 0.5, 1, 2, 3, 4, and 6 minutes. Samples were collected at each length in a 60 CC syringe and manually processed to recover the PUFA fraction by de-complexation and dewatering. The processing method was nearly identical to that use with in the batch experiments, and is thus described in the following section.



- 18/12 Fish Oils EE (Organic Phase)~1.5 ml/min
- 50%wt.AgNO$_3$ (Aqueous Phase)~5 ml/min
- Temperature = 10±0.5°C

- Residence times varies from 36 to 438 Secs
- Phase inversion observed at "Y" Junction
- Stratification of flow has been observed.

- Samples are collected at specified location.
- Gravity settling has been allowed.
- Exiting ethyl ester of fish oil–Oil layer
- Isolation of Emulsion phase (Oil +AgNO$_3$)
- Exiting silver nitrate aqueous phase enriched with Omega 3 PUFA.
- Silver ions in the solutions bound to double bond of these fatty acids (EPA/DHA).

Figure 3.6. Overview of LLE extraction in mini–fluidic experimental set up.

3.3. Liquid–Liquid Extraction in Stirred Reactor

In order to compare the performance of extraction in mini-fluidic reactor technology with a more traditional system, the extraction was carried out in a batch-wise stirred vessel and the results compared to the mini-fluidic system to determine if there was a significant difference in extraction performance. A 65 ml three neck flask equipped with outer jacket, stirrer and thermometer was initially charged with 50 ml of silver nitrate solution, blanketed with nitrogen, and allowed to cool to 10°C. Once cooled, 14.7 ml of 18/12EE fish oil was added and the fluids allowed contacting under stirred conditions for 15, 30, 60, 90 and 120 minutes. At the end of reaction time, the stir bar was shut off and the contents allowed to separate by gravity. After the removal of the oil layer formed, the aqueous layer was processed for separation of omega 3 PUFA.

3. 4. Post Extraction Processing of Aqueous and Organic Extract

After extraction of omega 3 PUFA in the mini-fluidic or batch reactors, the aqueous phase enriched with omega 3 PUFA was further processed to determine yield and purity. The processing of the extraction mixture consisted of 4 steps (Figure 3.7)

Gravity separation of oil phase, de-emulsification with hexane, de-complexation using hexane and water, and sample drying and filtering prior to analysis at DSM Nutritional Products.

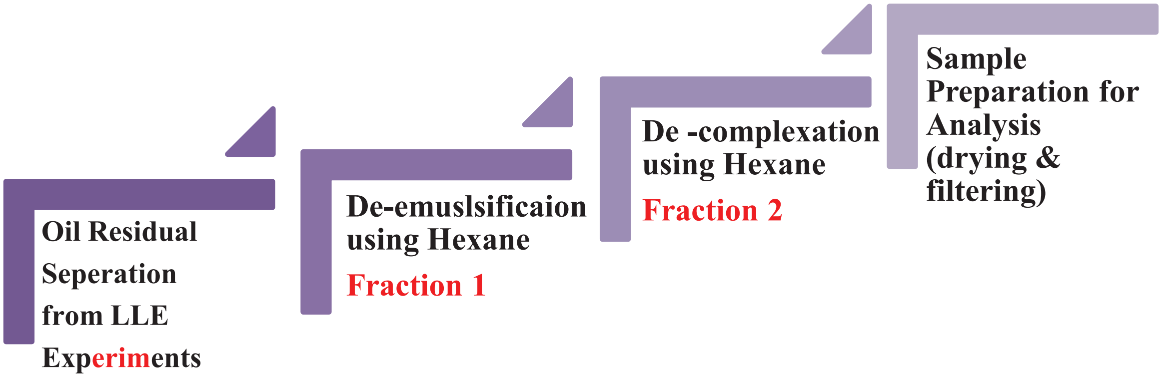


Figure 3.7. Step up process for recovaray of Omega 3 PUFA from Aqueous Phase and Emulsion phase

3.4.1. Oil Residue Recovery

In the stirred tank vessel and the sample syringes, a small residual oil phase remained following the initial reaction and gravity partitioning. In the case of the batch system, the entire contents of the batch were transferred to a seperatory funnel, where the aqueous/emulsion phase was removed and the residual oil phase collected. For the syringe samples from the mini-fluidic process, the syringe was held vertically and the oil phase discharged into a second syringe using a short interconnecting tube. The residual oil phases were then stored in a nitrogen blanketed sample vial for subsequent drying and filtration.

3.4.2. De-emulsification of Oil Phase

An emulsion phase between fish oil ethyl ester and silver nitrate solution was formed during the extraction process, allowing for fast mass transfer between Omega 3 PUFA and silver ions. This emulsion was not the complexed PUFA, and needed to be separated prior to de-complexation of the PUFA from the silver nitrate solution. De–emulsification was carried out through the addition of hexane in the ratio 1 part hexane to 10 parts sample volume. In the batch system, hexane was added to the seperatory funnel and mixed thoroughly prior to recovering the aqueous and organic fractions in separate containers. For the syringe samples, Hexane was transferred to the sample syringe containing the emulsion/aqueous phase, with contacting achieved by repeated transfer between two syringes through a short connecting tube length. In both cases, the hexane breaks the emulsion and separates into an oil phase dissolved in hexane fraction and aqueous phase. The de-emulsified hexane fraction is henceforth named as fraction 1.

3.4.3. De-complexation of Aqueous Phase:

To recover the complexed ethyl esters from the aqueous phase, the fractionated aqueous phase typically having a volume of ~50 mL was placed in a seperatory funnel where it was brought into contact with 20 ml of hexene and 600 mL of water and allowed to contact for 120 minutes. The aqueous phase was then separated off and the organic fraction was stored for analysis. The non-polar solvent (hexane) was added into the aqueous phase to weaken the bond between EPA/DHA and silver ion and to increase the volume of organic phase recovered. This final organic fraction was henceforth referred to as Fraction 2, representing the extracted PUFA.

3. 4.4. Sample Preparation and Analysis

The hexane (de-emulsified fraction) and hexene (de-complexed fraction) fractions recovered from this process were subsequently filtered and subjected to a nitrogen flow to vaporize the hexane and hexene solvents. The solvent free fractions were then sealed and transported to DSM Nutritional Products where they were subjected to Gas chromatographic analysis. The analytical results obtained from DSM for the various samples tested are provided in the appendices of this thesis for future reference. These values were used in conjunction with the mass of each of the collected fractions from these experiments to perform an approximate material balance to determine the wt% yields.

While the exact analytical procedure used within DSM's facilities for determining EPA and DHA content cannot be included within this thesis, a suitable method was identified from open literature which offers a brief description of what would be required. In order to find the content of EPA/DHA–Et and omega 3 PUFA in fractions, A BPX70 capillary column phase designed specifically for the analysis of FAMEs could be used, utilizing the phase technology with the aromatic group (silphenylene) on a siloxane backbone. To give the resolution required for such a complex mixture of Omega 3

PUFA, the analysis of fractions is routinely performed with a 30m length, 0.25mm i.d. BPX70 column (phase ratio, b = 250). The analysis time is approximately 20 minutes. A shorter BPX70 column with a smaller i.d (where b=250) can considerably reduce the analysis time for FAMEs from 20 minutes to a fast 8 minutes and still maintain excellent separation of the critical components, such as saturated (C16:0 and C18:0) and monounsaturated (C18:1n9 and C18:1n7) fatty acids. The content of fractions of GC chromatogram is expressed in terms of peak area percentage of each component in the fractions, which is mentioned in Appendix A3.

3.5. Interfacial Tension Investigation of Fish Oil Ethyl Ester

Due to reduction of channel dimension, an interfacial force plays a significant role in the formation of flow patterns. Fish oil ethyl ester–$AgNO_3$ is a rare binary system which lacks information about interfacial system details. In previous literature, the flow patterns observed using idealized fish oil analogues was slug-flow, with significant discussions presented as to why this flow pattern is beneficial. Within this work, a stratified flow pattern was observed, which has significant implications on relative residence times of the oil and aqueous phases and overall system performance. This work thus explored the interfacial surface force variations with practical fish oil/solvent/silver nitrate mixtures using spinning drop tensiometer to explore the observed variation in flow pattern.

Interfacial tension between organic and aqueous phases plays a major role in LLE experiments. The larger the interfacial tension, the more difficult it is to form emulsions but the easier it is to separate immiscible phases by coalescence. Low interfacial tension aids dispersion and thus improves contacting mass-transfer efficiency, but can make subsequent separation difficult. At high interfacial surface tensions, slug flow is the predominant flow pattern observed in mini-fluidics. As most of the previous analogous fish oil systems where slug flow profiles were observed used pure organic alkanes as a solvent with quantities of EPA or DHA dissolved, it is expected that the interfacial tension in these systems is higher than that present when commercial fish oils are employed.

In order to evaluate the impact of solvent addition on interfacial tension, Properties of fish oil and silver nitrate solutions, non-polar organic solvents such as hexane and hexene were added to the fish oil ethyl esters in the following ratios:

1. 100 wt.% 18/12EE Fish Oil -EE
2. 10 wt.% Hexane and 90 wt.% 18/12EE Fish Oil -EE
3. 50 wt.% Hexane and 50 wt.% 18/12EE Fish Oil -EE
4. 10 wt.% Hexene and 90 wt.% 18/12EE Fish Oil -EE
5. 50 wt.% Hexene and 50 wt.% 18/12EE Fish Oil -EE

The interfacial tension was evaluated for 18/12EE–water, 18/12EE–silver nitrate solution, hexane-18/12EE–silver nitrate solution and hexene–18/12EE–silver nitrate solution at 25 °C. The experiments were carried out in a spinning drop tensiometer (SITE 100) with Drop Shape Analysis Software (DSA2). This technique is frequently used in the petroleum industry for determining interfacial tensions between oils and brines, relying on elongation of a fluid drop suspended in a confining horizontal tube are spun about its longitudinal axis. At low rotational velocities (ώ), the fluid drop will take on an ellipsoidal shape, but when ώ is sufficiently large, it will become cylindrical (Figure 3.8). Under these conditions, the radius (R) of the cylindrical drop is dependent on the interfacial tension, the density difference between the drop and the surrounding fluid, and the rotational velocity of the drop. As the result, the interfacial tensions are calculated from the Vonnegut equation.

$$\gamma = \frac{(\rho_{heavyPhase} - \rho_{lightphase})\omega^2 R^3}{4} \tag{3.1}$$

The spinning drop tensiometer SITE 100 is a computer controlled system for measuring low interfacial tensions of two immiscible liquid-liquid systems. The motor allows rotational speeds up to 15000 RPM (Standard Instrument) or even 20000 RPM. The heavy bulk phase (silver nitrate solution) is inserted into a capillary which is open to an inlet and an outlet. The drop of the light phase is dispensed into the capillary through a septum with a syringe. The measuring cell, inside which the capillary rotates, is temperature controlled with oil that also serves as lubricant for the rotating parts. A challenge encountered in this work was the propensity of fish oil droplets in suspension to break when rotational speed increases beyond 6000 RPM, sometimes with the droplet never quite reaching the requisite ratio of the drop length to diameter *(L/D)* greater than 4 needed for the Vonnegut Equation to apply (Currie and Nieuwkoop, 1982).

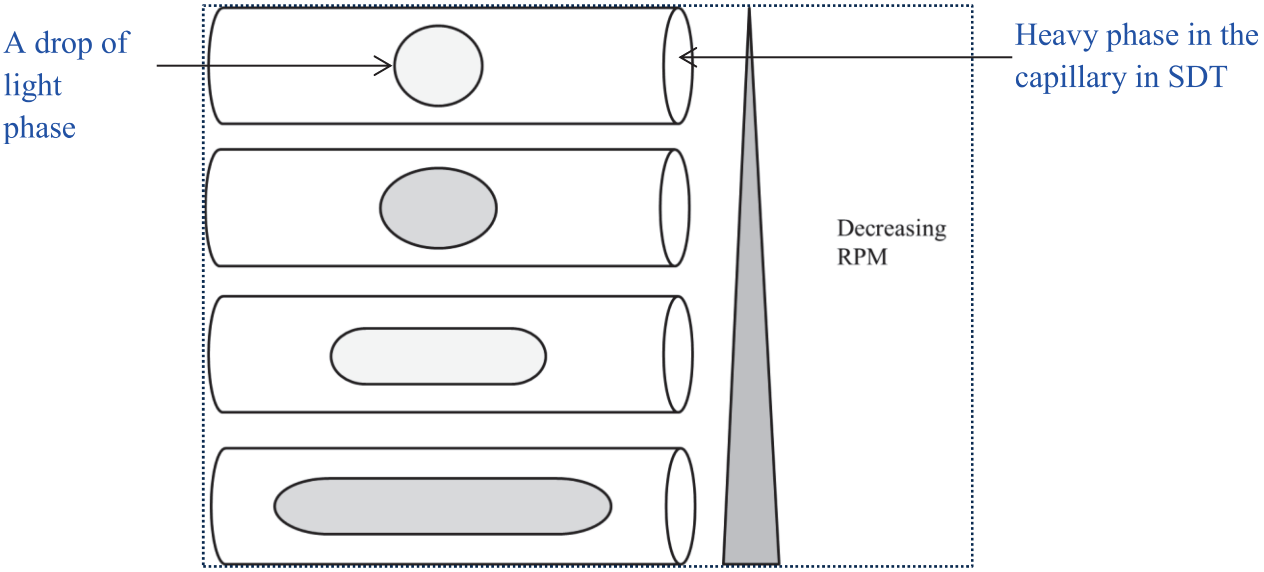


Figure 3.8. Spinning drop tensiometry. In this method, the light phase is injected into the heavy phase and forms droplet in the capillary. The drop of fish oil ethyl ester in a narrow capillary tube elongates as the tube is spun along its long axis demonstrating to satisfy the Vonnegut equation.

During each test, the bulk phase (silver nitrate solution) was injected into the capillary tube of the spinning drop tensiometer, the motor was turned on to run at an rpm of 8500 and the capillary tube was tilted to detect any air bubbles in the tube. Care was taken to remove any air bubble present, as air bubbles can result in horizontal movement of droplets of interest as the RPM increases. A drop of light phase (fish oil/solvent mixtures) was then injected into the capillary tube. The rotation rate was gradually increased and the diameters of the light phase noted. The temperature was then set at 25°C to evaluate IFT. Once the temperature which was set on the refrigeration unit was in equilibrium with the temperature of capillary in the spinning drop tensiometer and when the drop was elongated an enough length to satisfy the Vonnegut condition, the interfacial tension was measured. Measurements were taken at two minute intervals for 10 minutes. After obtaining the IFT measurements the capillary tube was cleaned with ethanol and hot water before carrying out the experiments with other solutions.

CHAPTER 4. EXTRACTION YIELD IN CONTACTING SYSTEM

4.1. EPA/DHA Yield and IFT Trial Results

The analytical results provided from DSM for the various sample's tested are provided in the appendices A3, as well as the raw mass balance data used in determining the yields discussed herein. In addition, the analyses of Omega 3 PUFA in the feedstock, residual oil, fraction 1 (hexane de-emulsification) and fraction 2 (de-complexation and recovery of final product) are also provided. Based on analytical results, the EPA-Et, DHA-Et and total Omega 3 PUFA in various fractions are calculated, where the latter represents unsaturated fatty acids other than EPA and DHA. The analytical results and a sampling of gas chromatograms are included in the appendix A3 and A4.

4.2. PUFA Recovery Results and Analysis

The actual composition of the 18/12-EE fish oil used as feed stock in the extraction investigation was determined for both non-winterized and winterized oil (Table 4.1). The EPA-Et and DHA-Et content in the fish oil did not change over the winterization process performed, confirming that precipitation of waxes within the feedstock vessel did not artificially concentrate the 18/12EE fish oils relative to the process stream which was initially sampled to provide the tested feedstock. The extraction performed at 10°C was also at a temperature slightly above the normal refrigerated storage temperature of the feedstock, so no additional gelation or precipitation occurred during the actual extraction process. The GC analysis of Fish Oil Ethyl Ester and Winterized fish oil ethyl ester are included in Appendix A3. The values provided are representative of both the non-winterized and winterized feedstock in Table 4.1.

Table 4.1. Composition Analysis of the 18/12EE fish oil feedstock.

Organic Phase	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt. %
Non Winterized Fish Oil-EE	15.0	10.1	30.9
Winterized Fish Oil EE	14.9	10.6	30.7

The mass of each sample collected was recorded during the post-extraction process (Tables 4.2 and 4.3), both for the purpose of performing a rough yield calculation and to determine the approximate volumes of each phase which might be expected. As can be seen in each of these tables, the sum of the oil fractions recovered was typically less than that of the initial oil fed (excepting 1.2, 2.4 and 7.3 minutes within the mini-fluidic system, primarily due to oil residue losses on the glassware and syringes used in the post-extraction processing. For those three tests with positive material losses (i.e. mass gain), small droplets of water were observed in the sample vials following solvent removal after de-complexation.

Table 4.2. Sample masses after solvent evaporation, in grams, for the mini-fluidic tests. Positive material losses attributed to residual water present in Fraction 2.

Process	0.6 min	1.2 min	2.4 min	4.8 min	7.3 min
18/12 Feedstock	10.85	10.28	10.24	11.38	11.03
Residual Oil	7.6324	6.922	7.204	5.527	7.799
De-emulsification Fraction 1	0.4386	0.5554	0.3151	0.8790	0.2470
De-complexation Fraction 2	2.4574	3.0519	2.972	2.6906	3.2425
Material Losses (Extracts–Feedstock)	0.3216	-0.2493	0.255	2.2865	-0.2557

Table 4.3. Sample masses after solvent evaporation, in grams, for the batch reactor tests.

Process	15 min	30 min	60 min	90 min	120 min
18/12 Feedstock	13.21	13.21	13.21	13.21	13.21
Residual Oil	6.741	6.2916	6.741	5.3928	6.2916
De-emulsification Fraction 1	2.2373	0.8762	1.112	1.6498	1.3822
De-complexation Fraction 2	3.8835	4.5633	4.0605	3.8718	4.0396
Material Losses (Extracts–Feedstock)	-0.3482	-1.4789	-1.2965	-2.2956	-1.4966

Moving sequentially through the extraction process, the first organic fraction obtained was the residual oil phase which is neither complexed nor emulsified at the end of the reaction time. From an extraction and yield perspective, the results in Table 4.4 appear quite promising, with 85 to 95% of the Omega 3 extracted based on compositional analysis (i.e. 1 to 5 wt.% remaining of the initial 30 wt.% present).

Table 4.4. Omega 3 PUFA content in the Residual Oil Layer

Mini-fluidic Reactor				**Batch Reactor**			
$t_{Residence}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt. %	$t_{Reaction}$ (mins)	EPA–Et Wt.%	DHA–Et Wt.%	Ώ 3 Wt.%
0.6	1.15	0.17	4.50	15	0.33	0.06	2.19
1.2	1.14	0.15	4.38	30	0.19	0.06	1.05
2.4	1.21	0.18	4.47	60	0.41	0.06	2.34
4.8	0.58	0	2.80	90	0.46	0.06	2.42
7.3	0.35	0.02	2.39	120	0.74	0.10	3.10

The next stage of post-extraction processing is involved with de-emulsification of the aqueous phase using hexane, producing Fraction 1. The wt. % compositions of this fraction for both the batch and mini-fluidic experiments are provided in Table 4.5. These values were determined from peak areas and interpolated calibrations from the raw oil and hexene fraction calibrations. It is important to note that the volume of Fraction 1 typically recovered was quite small, especially for the mini-fluidic studies, so the extrapolation of these values to an overall yield calculation does introduce some error into the final analysis. An interesting observation with respect to the emulsion layer is that for the batch reactor, the emulsion appeared to consist primarily of a less-concentrated oil phase in terms of EPA and DHA, but had a significant fraction of the other Omega 3 compounds extracted. Meanwhile, the mini-fluidic system's emulsion phase appeared to contain an oil phase which was more concentrated in EPA and DHA relative to the feedstock. The most likely reason for this difference is that in the batch system there is significant agitation of the immiscible phases, providing significant opportunity for some of the less "soluble" oils to be emulsified, while in the mini-fluidic system the stratified flow profile observed resulted in minimal agitation, so less raw oil emulsification occurred (i.e. more of the emulsion phase consisted of partially solubilized oils). This would also explain the lower yield within Fraction 1 for the mini-fluidic setup (approximately 1/3rd the mass of oil compared to batch wise operation). Following de-emulsification, the silver nitrate solution was de-complexed through watering out and hexene was added to increase the volume of oils which could be recovered from the seperatory funnel. The final EPA, DHA and Omega 3 weight percentages in this fraction following solvent removal and filtration are provided in Table 4.6.

Table 4.5. Yield of Omega 3 PUFA in Hexane Fraction 1 after de-emulsification.

Mini-fluidic Reactor				**Batch Reactor**			
$t_{Residence}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt. %	$t_{Reaction}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt.%
0.6	16.2	17.2	-	15	3.72	1.8	22.4
1.2	15.6	16.4	-	30	3.35	5.3	26.1
2.4	18.4	19.7	-	60	5.04	2.52	25.2
4.8	8.1	9.8	-	90	5.3	2.27	25.5
7.3	10.8	10.6	-	120	-	-	-

Table 4.6. Weight percent EPA/DHA/Total Omega 3 in Fraction 2 collected at different contact times from LLE experiments.

Mini-fluidic Reactor				Batch Reactor			
$t_{Residence}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt. %	$t_{Reaction}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt.%
0.6	42.3	30.5	81.3	15	41.5	27.1	81.8
1.2	39.8	29.0	77.5	30	41.6	27.0	81.9
2.4	40.4	29.2	78.5	60	39.8	25.9	78.9
4.8	37.9	26.8	73.7	90	42.0	27.4	82.5
7.3	40.3	27.8	78.5	120	40.1	26.6	78.7

While the results in Table 4.6 show slight variations between the mini-fluidic and batch reactor with time, these results indicate that equilibrium extraction levels can be achieved within 0.6 minutes in the mini-fluidic system, and 15 minutes in the batch reactor. Despite the change in flow pattern from slug to stratified, the time scale for extraction coincides with the previous work of Kamio et al. (2010, 2011). In their work, pure DHA or EPA dissolved in an organic solvent reached equilibrium with a silver salt solution in 20 s with a silver ion concentration of 1000 mol/m^3 at 268 K, and 10 s with a silver ion concentration of 2000 mol/m^3 at 288K. For the purpose of process feasibility, looking at shorter residence times was not required as most of the batch-wise processes would operate for longer than 15 minutes due to filling and emptying considerations, and a 36 second residence time within a continuous process provides an upper bound of what might be needed.

4.3. Yield of Omega 3 PUFA from Hexane Fractions:

The approximate net yield or wt. % of EPA/DHA/Total Omega 3 recovered within Fraction 2 was subsequently determined based on the mass of Fraction 2 collected, the measured wt. % of each component, and the initial material fed based on either the reactor loading (batch) or the relative fluid flow rates (mini-fluidic). The results are provided in Table 4.7.

Table 4.7 - Weight percent EPA/DHA/Omega 3 recovered in Fraction 2, relative to the initial feed used in each of the experiments.

Mini-fluidic Reactor				**Batch Reactor**			
$t_{Residence}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt. %	$t_{Reaction}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ώ 3 Wt.%
0.6	64.2	68.5	59.9	15	81.8	79.1	78.2
1.2	79.3	85.7	74.9	30	96.4	92.8	92.0[1]
2.4	78.6	84.4	74.2	60	82.0	79.0	78.2
4.8	60.0	63.0	56.7	90	82.5	79.7	78.7
7.3	79.3	81.2	75.1	120	82.2	80.9	78.3

1. The value shows higher than the expected due to improper handling of the sample and gravimetric methods.

The variations in yield of EPA and DHA at 0.6 min and 4.8 min for the mini-fluidic system and at 30 minutes in the batch system are seen as potential outliers in data. The 30 minute batch data point had an unusually high mass of Fraction 2 (likely water droplets present in the sample), while the 0.6 min mini-fluidic experiment had a comparatively low Fraction 2 volume and the 4.8 minute mini-fluidic experiment had an unusually high material loss (Table 4.2). These values were thus likely due to problems in handling the samples in the vial during nitrogen evaporation of hexane or recovering the organic fractions between syringes. Looking at the remaining values which had typical recoveries of 75 to 78 wt. % Omega 3 (on average 28.9% of the initial oil mass) with concentrations of ~78 wt. % total Omega 3. This was compared to lab-scale urea complexation results over a 20 hour period (S.Liu *et al*, 2008), where the total content of DHA and EPA from tuna oil was increased up to 85.02% with a liquid recovery yield of 25.10% of the weight of the original tuna oil.

The recovery estimates are to be viewed as conservative estimates, as they represent only the actual mass of Omega 3 PUFA's collected after sample losses. For the CSTR, the amount added was well defined due to the batch-wise operation, limiting potential errors in yield calculations to losses during handling. For the mini-fluidic system, an unanticipated flow pattern within the channels could have potentially affected the relative flow rates of the two species in question (and the contact times), creating additional uncertainty in the yield. It is worthwhile noting that the typical sample masses obtained for the oil fractions were 5.5 to 7.5 grams, while the hexane fractions after solvent evaporation were 0.5 to 1.5 grams. This is compared to a mass of 2 to 4 grams of concentrated oil obtained following de-complexation and solvent removal. Based on these values, the maximum recovery of EPA and DHA based on mass retained in the residual oil would approach just over 90% (based on 8 grams of residual oil, 1.2 wt. % EPA content, vs. 13.2 g of fed oil at 15 wt. % EPA).

4.4. Flow patterns in Slug Flow Based Mini-fluidic experimental system:

A notable difference between this study and previous mini-fluidic studies was the formation of a stratified flow profile in the mini -fluidic channels, where a slug flow profile was expected. The stratified flow is characterized by a complete separation of the liquid-liquid interface, where the relative residence times of both phases may vary depending on the relative cross-section occupied by each phase. It is quite odd to observe stratified flow under these conditions, as one of the characteristic properties of mini-fluidic channels is the predominance of surface forces over gravity forces, which should limit the formation of stable stratified flow. The dimensionless parameters relevant to flow at this scale are provided in Table 4.8. A comprehensive discussion regarding the flow patterns observed is given in the following chapter, which explores the effects of tube wettability and fluid properties on flow pattern. For now, it is important to note the high Bond number present under these conditions with the IFT of this particular system, as the use of commercial fish oils seems result in a sufficient reduction in IFT relative to analogous mixtures to cause a change in flow dynamics in the system. For now, a comprehensive discussion on the flow patterns is mentioned below and various forces involved in flow pattern are commented.

Table 4.8. Influence of Various forces on Flow pattern at "Y" mixer

Dimensionless numbers	Definition	Formula	Values
Weber Number	$\frac{\text{Inertial force}}{\text{Interfacial tension force}}$	$We = \frac{d_H \rho u^2}{\sigma}$	21.78
Capillary Number	$\frac{\text{Viscous Force}}{\text{Intenfacial tenstion force}}$	$Ca = \frac{\mu u}{\sigma}$	0.45
Bond Number	$\frac{\text{Gravity Forces}}{\text{Interfacial Tension}}$	$Bo = \frac{\Delta\rho g d^2_H}{\sigma}$	54.94
Reynolds Number	$\frac{\text{Inertia force}}{\text{Viscous force}}$	$\mathrm{Re} = \frac{d_H \rho u}{\mu}$	48.35

The physical properties such as density and viscosity were evaluated at temperature of 15±1°C. Interfacial tension was evaluated at 25°C. The flow rate of aqueous phase is 5 ml/min and the organic phase is 1.47 ml/min.

The "Y" mixing section in experimental set up is used for creation of slug flow pattern which is anticipated. However, raw fish oils contained numerous compounds which reduce the interfacial tension relative to that of idealized solvent mixtures, resulting in a stratified flow within the channels used in this study. In addition to that, the interfacial reaction between fish oil ethyl ester and silver nitrate systems might cause reduction of interfacial tension between them. This system is an anomaly within the context of mini-fluidics, as very few fluids form stable stratified flow in millimeter-scale geometries. Despite this flow pattern being present, the overall extraction performance did not appear to suffer from the change in flow dynamics.

4.5. Interfacial Tension

The fish oil / silver nitrate system poses an interesting problem in the context of IFT and fluid flow. The system as a whole represents a heterogeneous mixture of organics and aqueous salt solutions, with active complexation occurring on the interface which should result in similar dynamics as is observed with surfactants. Adsorption of extraction molecules at the interface lowers the interfacial tension and makes it easier to disperse one phase into the other. Based on reports of slug flow in 0.5mm channels when alkane solvents are used in the place of fish oils, the addition of non-polar organic solvents into the fish oil ethyl ester could elevate the interfacial tension between fish oil and $AgNO_3$ and potentially lead to a change in predominant flow pattern. This would potentially be desirable as slug flow is known to have improved mass transfer characteristics and the presence of the alkane solvent could potentially limit the formation of emulsions during contacting (thereby simplifying post-extraction processing).

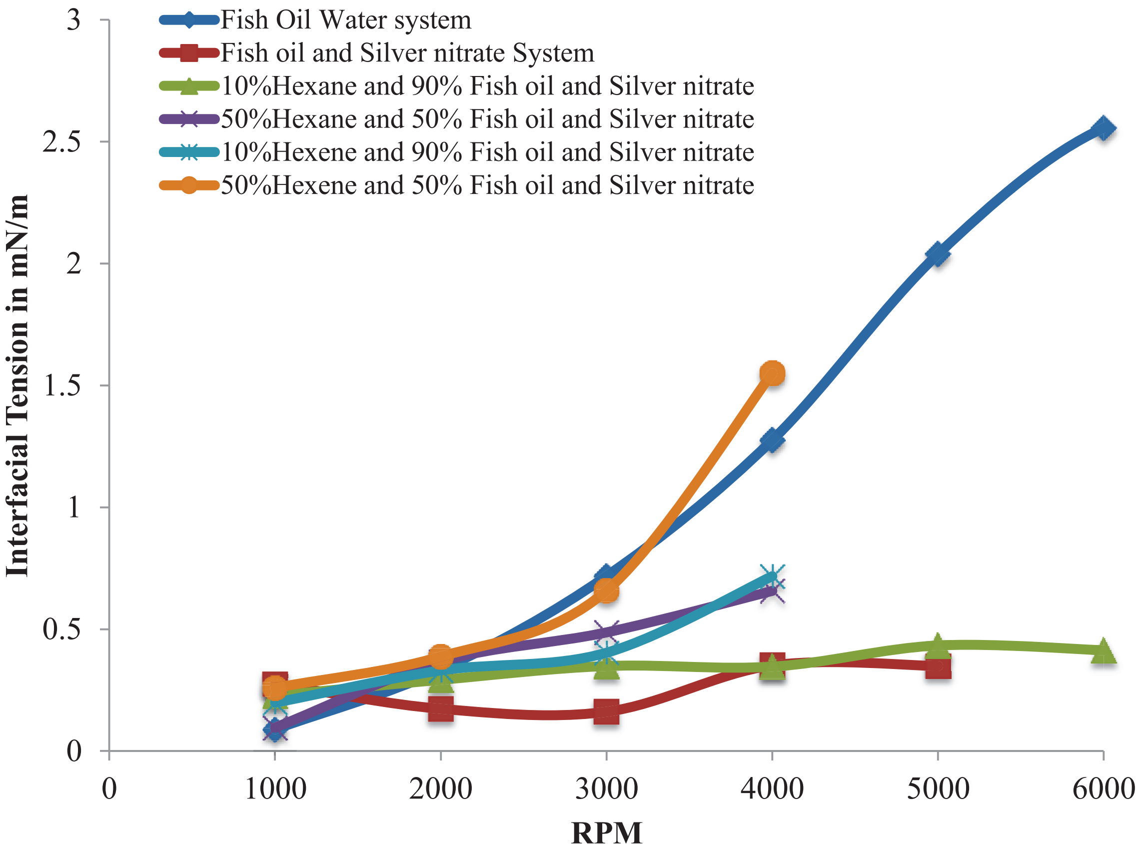


Figure.4.1. IFT vs. RPM obtained using Spinning drop tensiometry (SDT). In this method, interfacial tension is typically the stable value obtained as RPM is increased. Light phase droplet breakup at higher RPMs limited the determination of IFT of experimental fluids.

Figure 4.1 illustrates the IFT determined via spinning drop tensiometry for various mixtures of 18/12EE, organic solvents, water and silver nitrate solutions. The data would suggest a significant variation in interfacial tension depending on whether or not silver nitrate is present in the solution, and whether or not an organic solvent was employed. The fish oil/distilled water mixture had an IFT which was in the upper limit of the instrument's ability to measure, as illustrated by the steady rise in calculated surface tension with RPM (where RPM's above 7000 to 9000 can create some difficulties in the equipment used). In contrast, fish oil in silver nitrate solution was at the lower end of the instrument's capabilities, where the interface would breakup up rapidly at rotation rates above 5000 RPM before stable surface tension measurements could be obtained.

The presence of the silver ions and the complexation process resulted in at least a single order of magnitude reduction in interfacial tension.

The addition of non-polar organic solvents into the fish oil ethyl ester generally increases the interfacial tension between fish oil/solvent solution and the silver nitrate solution. The 10 wt. % hexane/hexene addition resulted in an apparent increase in interfacial tension. The 50 wt. % of hexane/hexene had a significant increase in the interfacial tension, rapidly moving past the instrument's ability to measure accurately. In all cases where organic solvent was added, capillary instability occurred in the spinning drop tensiometry unit, limiting the maximum rotation rate which could be applied before droplet breakup or coalescence occurred. These trends are important for future processing considerations, both in recognizing that the low surface tension between fish oil and silver nitrate will facilitate contacting and mass transfer, while possibly making separation difficult. The addition of hexane or, preferably, hexene, could be used to help in separating the two phases in the design of either mini-fluidic technology for liquid-liquid extraction of EPA/DHA from fish oil ethyl ester or on a more general basis in de-emulsification.

The following two chapters explore the impact of IFT and solvent addition on flow pattern formation with in the mini-fluidic channels, and the relative merits of a silver-based extraction process for fish oil production given the performance metrics and procedures used here.

CHAPTER 5. HYDRODYNAMICS BEHAVIOR OF MINI-FLUIDIC SILVER BASED EXTRACTION OF EPA/DHA

5.1. Role of Hydrodynamics in Mini-fluidic channels.

To improve the performance of slug flow mini-fluidic reactor technology, it is necessary to investigate flow patterns of the fluids at mini-scales and various dominating forces to control the flow patterns. Hydrodynamics is the key role in controlling the mass transfer and kinetics in any type of chemical reaction. In mini–fluidic channels, specific flow patterns are observed for two immiscible fluids, including stratified flow, wavy annular flow and slug/plug flow. Usually the flow patterns at mini/micro scales are well-defined, and thus the interfacial areas of the flows are uniform, allowing simple and precise modeling of numerous chemical processes. Among the various flow patterns, slug flow offers promising advantages in mini/micro-fluidic liquid-liquid extraction because of the large interfacial area-to-volume ratio and an increased mass transfer coefficient due to liquid recirculation within each slug (Kamio, E *et al* 2011). This enhances the rate of extraction in the mini–fluidic contacting system and micro-fluidics system.

As mini-fluidic contacting system has the dimension in the range of 0.5 mm<ID<3mm, interfacial tension and viscous forces dominate the effects of gravity and inertia forces. Three dimensionless numbers, the Weber, Capillary and Bond numbers, are significant in characterizing multiphase phenomena in such mini scale flows. The Bond number is often used to justify whether flow is dominated by interfacial tension force or gravity force, thereby defining the point at which geometry is “mini” or “micro” scale. However, it depends on the nature of binary liquid systems, but it affects the formation of flow patterns in the mini-channel. There are two types of liquid-liquid two phase flows that can result when working with liquids at the micro- and mini-fluidic scales: slug flow and parallel flow. The capillary number can be used to predict the presence of such flow. A capillary number less than 1 indicates that the forces of interfacial tension dominate viscous forces, resulting in a decrease in the interfacial area. Depending on device geometry, a decrease in interfacial area could lead to slug flow or parallel flow. A capillary number greater than 1 often results in parallel flow due to the viscous fluid’s resistance to shear which extends the interface down the length of the channel. At low Bond numbers this results in the eventual formation of slug flow, while at high Bond numbers stratified flow can result. The Weber number is used to predict when inertial forces become so significant that they lead to instabilities at the interface of two phase flow, which can also contribute to a transition from slug to stratified flow. A Weber number greater than 1 is indicative of flow instabilities. These dimensionless numbers are used to analyse the flow patterns of fish oil ethyl ester and silver nitrate systems at mini scale dimension.

Table 5.1. Dimensionless number used in fluid flow

Dimensionless Numbers	Definition	Formulae	Implications
Reynolds number	$\frac{\text{Inertia force}}{\text{Viscous force}}$	$\mathrm{Re} = \frac{d_H \rho u}{\mu}$	Re<2100 – Laminar flow Re>2300- Turbulent Flow
Weber number	$\frac{\text{Inertial force}}{\text{Interfacial tension force}}$	$We = \frac{d_H \rho u^2}{\sigma}$	We < 1 → stable interface We > 1 → unstable interface
Capillary number	$\frac{\text{Viscous Force}}{\text{Intenfacial tenstion force}}$	$Ca = \frac{\mu u}{\sigma}$	Ca<1 - reduce interfacial area Ca > 1 → parallel flow
Bond Number	$\frac{\text{Gravity Forces}}{\text{Interfacial Tension}}$	$Bo = \frac{\Delta \rho g d^2{}_H}{\sigma}$	Bo >1 , Gravity Force dominates Bo < 1,Interfacial tension dominates

The reported literature on extraction of Omega 3 PUFA in mini-fluidic contacting system has been attempted with extraction of only DHA dissolved in n-Heptane solvents with silver nitrate solution, typically with slug flow or emulsion flow patterns present (Table 5.2). In contrast, a practical evaluation of extraction performance using commercial 18/12EE fish oils yielded completely different flow behavior than the idealized systems. Stratified flow is quite rare within these systems due to the increased dominance of surface forces at these scales, which tends to result in the formation of slug flow. In the case of slug flow, two mechanisms are known to be responsible for the mass transfer between two immiscible liquids: internal circulation within each slug, and the concentration gradients between adjacent. In the case of stratified flow, the flow is laminar and the transfer of molecules between the two phases is supposed to occur only by diffusion.(Vir, Fabiyan et al. 2014). We would thus expect a lower performance in a stratified flow system than that observed within a slug-flow system.

Limited reports are available of extraction performance using silver salts to isolate polyunsaturated fatty acids from a mixture of FFA, with previously known methods not providing for a sufficiently selective or efficient process for concentrating Omega-3 fatty acids. (Breivik, Thorstad et al. 2012) reported that silver salt solutions could be used in the extraction of PUFA from marine oils, describing a potential process which is summarized as follows. Table 5.2 describes the flow patterns involved in extraction of Omega 3 PUFA with silver nitrate solutions in mini-channel.

The objective of the present work has to be determined the influence of liquid-liquid flow patterns in Y shaped mixing section in Tygon and PFA minichannel. To improve the performance of mini-fluidics, it is necessary to study the hydrodynamic behavior of fish oil silver nitrate system in the channel, identifying the reason for the shift in flow behavior observed and the impact this would have on mass transfer performance. The addition of non-polar organic solvents into the fish oil ethyl ester was attempted to change the flow pattern of fish oil silver nitrate system by elevation of interfacial tension. Flow pattern maps are presented in terms of Ca, Re and We, with three regions of interest identified within the context of this specific system.

Table 5.2. Extraction of Omega 3 PUFA in mini-channel

Organic Phase	**Aqueous Phase**	**Minichannel Configuration and Diameter**	**Flow pattern**	**Reference**
DHA Et	$AgNO_3$	0.005 m Connected "Y" Junction to mix the Organic and Aqueous Phase	Slug Flow	E.Kamio *et al,*2010
DHA Et	$AgNO_3$	0.005 m Connected "Y" Junction to mix the Organic and Aqueous Phase	Slug Flow	E.Kamio *et al,*2011
DHA Et	$AgNO_3$	"Y" Shaped Micro channel	W/O Emulsion Based drop flow	E.Kamio *et al,*2011
EPA Et DHA Et	$AgNO_3$	"T" Shaped Micro reactor	Slug Flow	Yu Seike *et al,*2007
Fish Oil Ethyl Ester	$AgNO_3$	1.58mm Tygon mini-channel connected to "Y" Mixing and Contacting section	Stratified Flow	A.A.Donaldson,2013*

*In that work, the experimental set up is designed for Slug flow pattern. However, the experimental conditions favor stratification of flow.

5.2. Evaluation of physical properties of experimental fluids

5.2.1. Evaluation of Density of Silver nitrate Solution and Fish Oil Ethyl ester:

ASTM D1480 set forth the procedure to estimate the density and specific gravity of liquids using a Bingham Pycnometer. The technique is limited to liquids with vapor pressure less than 600 mmHg and viscosity less than 40000 cSt (mm^2/s) and specifically designed to conduct experiments at any temperature between 20 and 100° C. This procedure allows measuring density at high temperatures on the compromise of the precision (ASTM Standard 1480, 2013). A pycnometer is a simple glass vessel with a long glass stopper consisting of a capillary hole to accommodate a fixed volume. The density is measured by weighing the sample filled pycnometer. The capillary determines the exact volume consequences the high resolution of density measurement. Initially, the empty vessel is weighed, followed by the weighing of distilled water filled vessel to measure the volume of the vessel. Finally the pycnometer is filled with sample liquid whose density is to be determined and weighted with high precision weighing machine. The density is estimated as the ratio of the mass of the sample liquid and volume of the liquid (Halit, 1999). The specific gravities of Silver nitrate solution, fish oil ethyl ester, 10% Hexane 90 Fish Oil Ethyl ester, 50% Hexane 50% Fish Oil ethyl ester , 10% Hexene 90 Fish Oil Ethyl ester, 50% Hexene 50% Fish Oil ethyl ester are evaluated using the density obtained.

In order to verify the experimental value of density, the value is predicted by various correlations. The specific gravity of the samples was determined using the specific gravity bottle method. Two bottles were used for each test simultaneously and repeated twice. The mass of the bottles and fuels were determined using a Mettler 110 Analytical balance. This test was performed according to ASTM D5355-95. The Density correlation between fish oil ethyl ester has been reported and the correlation was valid over the temperature range of 20 – 300°C.

$$\rho = -1.24T + 862.69 \tag{5.1}$$

The Andrade Equation for Kinematic and Dynamic viscosity correlation for fish oil ethyl ester has been reported and the correlation was valid in the temperature range of 10–300°C.

$$\ln(\nu) = -0.453 - \frac{739}{T} + \frac{415{,}238}{T^2} \tag{5.2}$$

The physical properties of 50% Wt. silver nitrate solutions are calculated based on various literatures.(Appendix A2). The final density measured at experimental conditions was 1751 Kg/m^3. The Viscosity of 50 Wt. % concentrate silver nitrate solution is 0.0015 Kg/m.sec. Similarly, the density and viscosity of fish oil ethyl esters are 898 Kg/m^3 and 0.005732 Kg/m.sec.

The IFT of experimental fluids is evaluated by spinning drop tensiometry and reported the values in the chapter 3. However, in this section, the plot for IFT is reported at highest value of RPM.

5.2.2. Experimental System for Flow Visualization Studies

A schematic of the experimental setup is shown in Figure 5.1. In the experimental set up, each phase was pumped through a 1.5 meter length of tubing submersed in a temperature controlled reservoir at 10°C to pre-cool the solution, after which time the phases were contacted in a Y-junction. Then Y-junction acts mixing section between two immiscible phases and the outlet of Y-junction is connected to a mini-channel which comprises of a length of 1 m and inside diameter 1/16th inch. Tygon material and PFA minichannel have been selected as the material for flow patterns investigations. The flow patterns for different combinations of flow velocities (rates) are observed visually and photographed by a high speed camera (PCO Dimax). The high speed video camera was set to capture images of process fluids flow at a frame rate of up to 650 frames per second.

The flow regimes noticed during experimentation covers the aqueous phase dispersed and organic continuous phase patterns as well as the zone of transition between them.

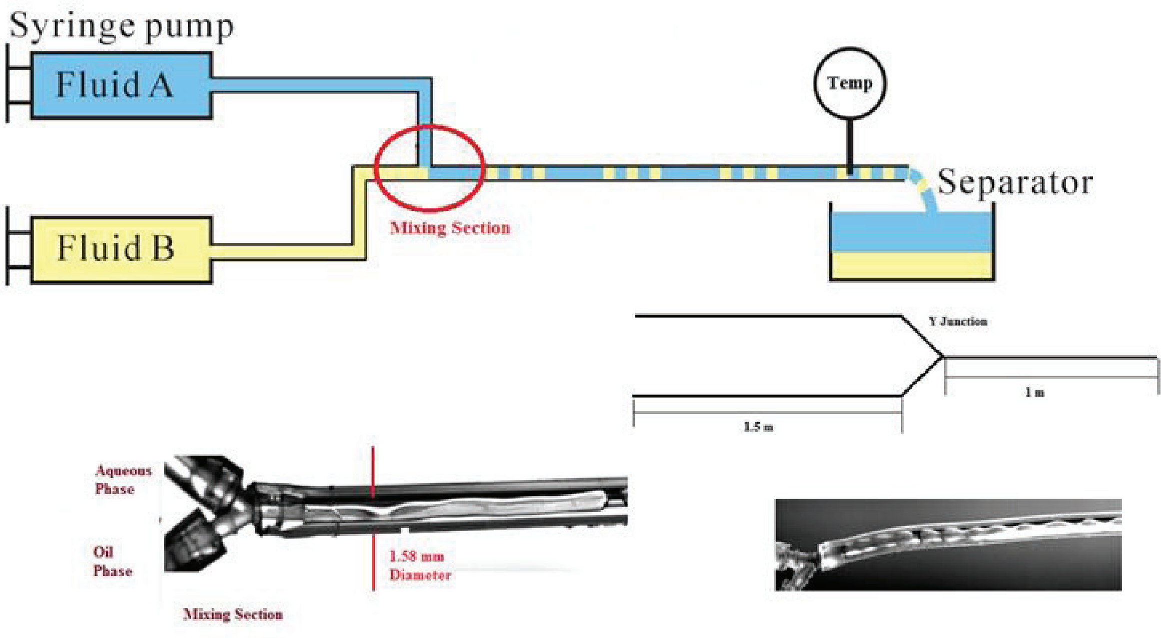


Figure 5.1. Experimental set up and mixing section. The flow patterns at "Y" the mixing section have been captured by high speed camera.

5.3. Flow pattern analysis:

5.3.1. Properties of Experimental fluids:

The physical properties of the experimental fluids are tabulated in table 5.3. It is used in the evaluation of dimensionless numbers for flow patterns analysis and construction of flow patterns map for fish oil silver nitrate system.

Table 5.3 Properties of Experimental fluids at 15°C

Experimental Fluids	**ρ Kg/m^3**	**μ Kg/m.sec**	**σ mN/m**
Fish Oil EE	898.8	0.0057	17.5
Silver Nitrate Solution	1751.4	0.0015	77.4
Water	999.7	0.0013	74.2
Hexane	695	0.00036	20.4
Hexene	673	0.0002	20.5
10% Hexane 90% Fish Oil EE	872.4	0.0051	
50% Hexane 50% Fish Oil EE	811.2	0.0030	
Fish Oil Water	969.4	0.0029	2.5
Fish Oil Silver Nitrate	N/A	N/A	0.34
10% Hexane 90% Fish Oil -Silver Nitrate	N/A	N/A	0.34
50% Hexane 50% Fish Oil Silver Nitrate	N/A	N/A	0.65
Hexane–Silver Nitrate	N/A	N/A	56

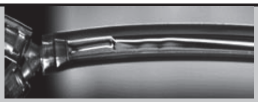
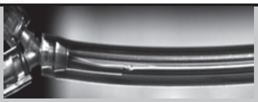
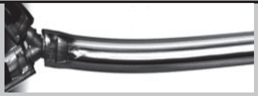
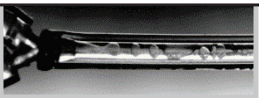
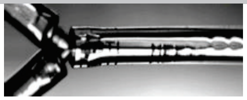
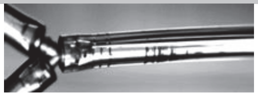
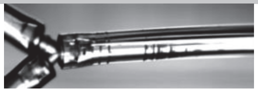
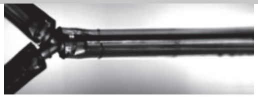
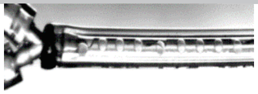
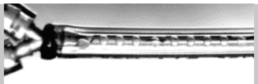
5.3.2. Flow pattern observed in liquid –liquid extraction of Omega 3 PUFA from fish oil ethyl ester:

The various kinds flow patterns in mini channel was observed and tabulated in the figure 5.2 and 5.3.One notable difference between this study and previous mini-fluidic studies was the presence of a stratified flow profile in the channels, where a slug flow profile was anticipated. Slug flow is characterized by alternating “slugs” of the two immiscible phases which travel through the channel at comparable rates. These slugs typically occupy the entire cross-section of the channel except for a wetted film layer on the wall, and are characterized by recirculation within each slug which significantly enhances mass transfer. The flow profile in these reactors approaches that of idealized plug flow reactors, with a number of other benefits in terms of surface area per unit volume. (Burns and Ramshaw 2001). This profile has been observed in previous studies which used an idealized “fish oil” mixture consisting of purified EPA/DHA dissolved in n-heptane solvent. Actual fish oils contained numerous compositions of various fatty acids which reduce the interfacial tension relative to that of idealized solvent mixtures, resulting in a stratified flow within the channels used in this study. Another reason for stratification the flow is the drastic density difference between fish oil ethyl ester and silver nitrate solution. This is extremely odd, as very few fluids form stable stratified flow in millimeter-scale geometries. In an attempt to quantify the degree to which the surface tension is modified by the addition of hexane and hexene solvents, surface tension measurements were performed by spinning drop tensiometry. When two immiscible liquids are pumped into the Y-junction, the downstream section of Y-junction is connected to the minichannel. The Y-junction acts as mixing section of organic phase and aqueous phase and the various flow patterns were observed in the flow of two immiscible liquids in minichannel at various flow rates especially in fish oil water system whereas fish oil silver nitrate system offers stratified flow at various flow rates. The figure 2 and 3 are shown various flow patterns observed in 1/16th Inch ID of Tygon and PFA minichannel for various systems.

Different flow patterns have been observed when organic phase and aqueous phase mixing together in Y-junction and flowing in the mini-channel. In this work, it is noted that the mini-channel was wetted with fish oil in Tygon channel and PFA channel.

Moreover, interfacial tension between organic phase and aqueous phase plays an major role in controlling the flow pattern in minichannel. However, due to drastic difference in density between fish oil and silver nitrate solution, startified flow pattern were oberved. The figure 2 and 3 shows how the flow rate of organic phase and aqueous phase flowed in different patterns in minichannel. In contrast with fish oil water system and hexane/silver nitrate system, fish oil/silver nitrate system, 10 Hex 90 FO silver nitrate solution and 50 Hex 50 FO silver nitrate solution, stratifed flow pattern in Tygon and PFA minchannel were oberved even at low flow rates.

Figure 5.2 and 5.3 are shown the different flow patterns observed in minifludic contact system. Droplet, plug and slug flow, annular, wavy annular flow and stratified flow have been observed. In this work, the organic phase plug and slug were distingused according to their length, if the average length of the organic pahse segements was equal to or less than 5 channel diameters (1.25 mm long), then the flow was classified as a plug flow. If the length of the organic phase segment greater than 5 channel diameter, the flow was classified as slug flow. As mentioned earlier, for each experimental fluid, the flow rate of both phase is varied. It is observed that stratifed flow pattern exists at flow rate of 10 ml/min and even in lower flow rate in the case of fish oil silver nitrate system.

Q_{Oil} ml/min	Q_{Aq} ml/min	Fish Oil Water System	Fish Oil Silver nitrate System	10% Hexane 90% Fish Oil Silver Nitrate System	50%Hexane 50%Fish Oil Silver Nitrate System	Hexane –AgNO$_3$
3.33 3.33 3.33 3.33	10 10 10 10					
1.47 1.47 1.47 1.47	5 5 5 5					
1.47	4					
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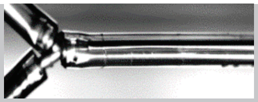
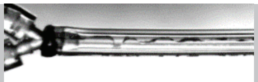
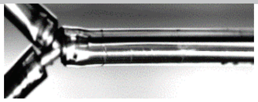
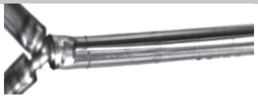
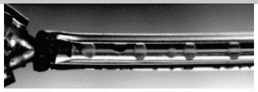
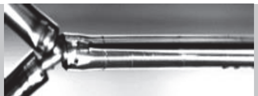
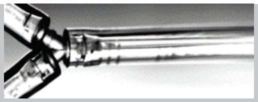
1.00 1 1	3 3 3					
0.83 0.83 0.833	2.5 2.5 2.5					
0.67 0.67 0.67 0.67	2 2 2 2					
0.333 0.333 0.333	1 1 1					

Figure 5.2. Flow patterns Observed in 1/16th ID of Food Grade Tygon mini-channel. Oil Phase enters from bottom at various flow rate and Silver nitrate enters from top in “Y’ mixing junction at various flow rates.

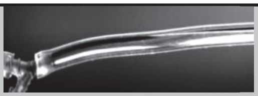
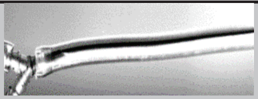
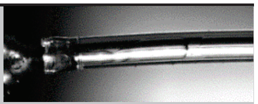
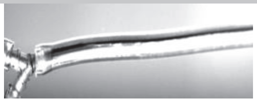
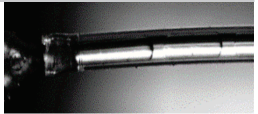
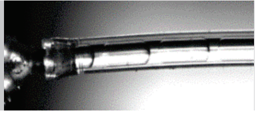
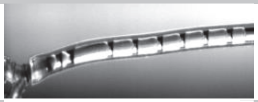
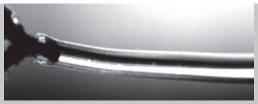
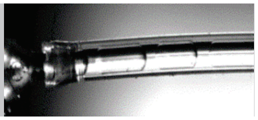
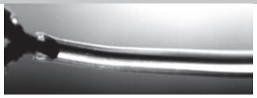
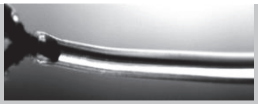
Q $_{Oil}$ (ml/min)	Q $_{Aque}$ (ml/min)	Fish Oil Water System	Fish Oil Silver nitrate System	10% Hexane 90% Fish Oil Silver Nitrate System	50%Hexane 50%Fish Oil Silver Nitrate System	Hexane- Silver Nitrate system
3.33 3.33 3.33	10 10 10					
1.47 1.47 1.47 1.47	5 5 5 5					
1	3					
0.833	2.5					
0.666 0.666 0.666 0.666	2 2 2 2					
0.5 0.5 0.5	1.5 1.5 1.5					
0.33 0.33 0.33	1 1 1					

Figure 5.3. Flow patterns observed in 1/16th ID of PFA mini-channel. Oil Phase enters from bottom at various flow rate and Silver nitrate enters from top in "Y' junction at various flow rates. In Fish Oil Water system, Stratified, Wavy annular and plug, slug flow patter were observed. The stratified flow patter only observed in Fish Oil Silver nitrate system, 10 Hexane 90 Fish Oil –Silver nitrate System, 50Hex50 Fish Oil Silver Nitrate system. But In Hexane Silver nitrate system, Slug flow pattern was observed at 5 ml/min flow rate of silver nitrate and 1.47 ml/ min of Hexane.

In contrast with fish oil-water system and hexane-silver nitrate system, the flow was stratified at higher flow rate. However,with decrease in flow rate of both phase, a flow transition is followed from stratified flow to wavy anunular flow and then transition to slug flow at 3.5 ml/min of water and 1 ml/min of fish oil in fish oil water system and 5ml/min of silver nitrate and 1.47 ml/min in the case of hexane /silver nitrate system. However, the stratified flow were formed in fish oil/silver nitrate system , 10 Hex 90 fish oil silver nitrate system and 50 Hex 50 fish oil silver nitrate system.

The occurance of different flow patterns is attributed to the competition between interfacial, inertia, gravity and viscous forces among organic phase and aqueous phase. The interfacial force tends to minimize the interfacial energy by decreasing fish oil ethyl ester and aqueous phase interfacial area i.e. formation of droplets and slugs. The inertial force tends to extend the interface in the flow direction and keep the fluid continuous. Also, Due to velocity difference between organic phase and aqueous phase across the interface, the interface could become wavy. In the case of Fish Oil Ethyl Ester – Water system, the water and oil densities difference are close, the diameter of channel is 1.58 mm, the bond number Bo, defined below is small (Bo<1) which indicates the gravitational effects on the flow pattern could be ignored at flow transition. In the range of oil and water flow rate tested in this investigation, flow pattern transitions occurred mainly with the change in the flow rate of 3.5 ml/min water and oil flow rate of 1 ml/min.

5.3.3. Destabilization of stratified flow:

At the flow rate of 3.33 ml/min of fish oil ethyl ester and 10 ml min of Water, stratification is observed once flow rate is constant and stabilized. When the syringe pump is off, the stratified flow starts to destabilize and decrease in velocity of fish oil and water. In addition, a change in density difference between fish oil and water and interfacial tension causes destabilization of stratified flow. Furthermore, oil water interface where the velocity profile at the interface is discontinuous and causes in stability in flow. Once flow is stabilized and constant velocity is reached, coalescence of the oil slugs and droplets giving rise to a continuous film. In these cases, destabilization of stratified flow is caused by a change in the density of the fluid between two phases and decrease in velocity of fish oil and water phase after pump shut off. It is observed that the flow position of oil will not affect the flow pattern. In the case of liquid-liquid extraction of omega 3 PUFA from fish oil ethyl ester with silver nitrate solution in mini-channel contactor, A flow rate of 5 ml/min for aqueous phase and 1.47 ml/min for oil phase are fixed and designed for mini-fluidic slug flow reactor according to stoichiometry requirement for reaction between Silver nitrate solution and fish oil EE. Therefore, the stratification of flow in the Tygon and PFA at flow rate of 5 ml/min is observed. However, stratified flow forms once the flow is stabilized and then once the pump stop. Destabilization of stratified flow occurs and also while in destabilization of flow, sometime wavy annular flow are observed with fish oil water system at this flow rate.

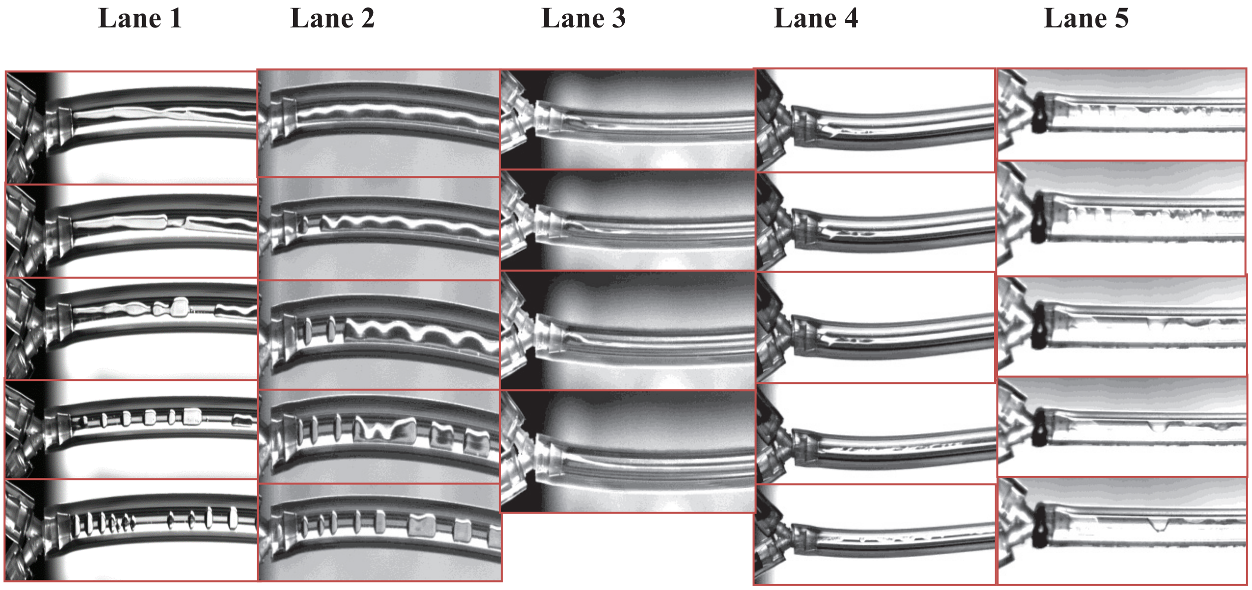


Figure 5.4. Destabilization of Stratified flow after pump is stopped. Oil Phase on Bottom in the “Y” Junction and the aqueous phase in top in “Y” Junction and the destabilization of stratified flow that are observed in a 1/16th Inch of internal diameter Tygon minichannel. The organic phase enters from bottom at 3.33 ml /min and aqueous phase enters from top at 10ml/min.

Lane 1: Fish Oil/Water system (FO on Bottom in Y junction),
Lane 2: Fish Oil Water System,
Lane 3: Fish Oil /Silver Nitrate System,
Lane 4: 10% Hexane90 FO Silver nitrate system
Lane 5: Hexane/Silver Nitrate System

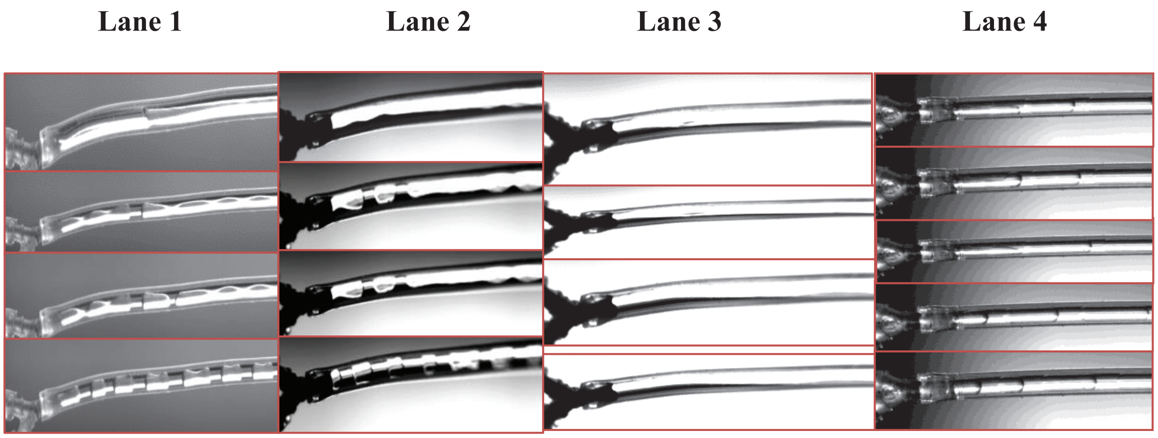


Figure 5.5. Destabilization of Stratified flow after pump is stopped. Oil Phase on Bottom in the "Y" Junction and the aqueous phase in top in "Y" Junction and the destabilization of stratified flow that are observed in a 1/16th Inch of internal diameter PFA minichannel. The organic phase enters from bottom at 3.33 ml /min and aqueous phase enters from top at 10ml/min.

Lane 1: Fish Oil/Water system (FO on Bottom in Y junction),
Lane 2: Fish Oil /Silver Nitrate System,
Lane 4: 10% Hexane90 FO Silver nitrate system
Lane 4: Hexane/Silver Nitrate System

5.3.4. Phase Inversion Phenomena:

The flow of two immiscible liquid into the Y-junction and mixing together in the mini-channel exhibits the phenomenon of phase inversion in which dispersed phase inverts to form continues phase and vice versa with slight changes in the operating conditions. Phase inversion of dispersed phase (fish oil ethyl ester) is defined as the transition of a phase from being dispersed to continuous. At high flow rate, phase inversion observed in fish oil/water and fish oil/silver nitrate system.(Piela, Delfos et al. 2009). From the physical properties, viscosities of organic and aqueous phases seem to have greatest effect. However, interfacial tension and density differences among two phases, the viscosities of the phases play an important role on the phase inversion point. In two phase flow in minichannel, a phase inversion phenomenon in Tygon minichannel has been observed at high flow rate at 10 ml/ min.

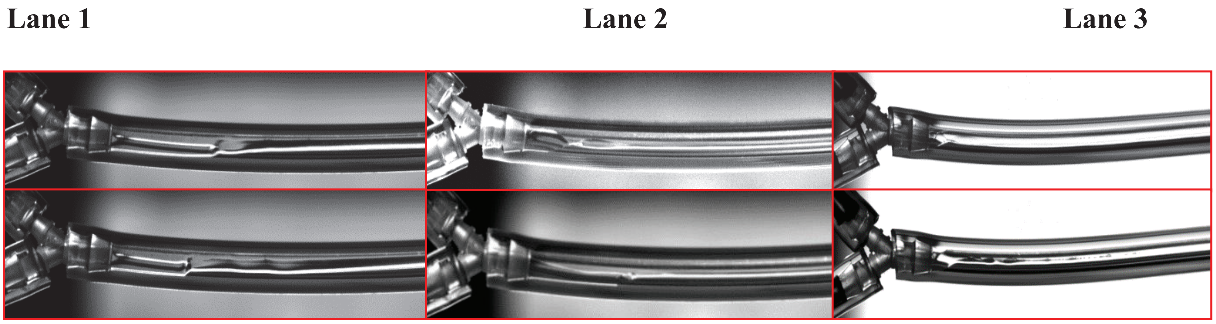


Figure 5.6. Phase Inversion Observed in 1/16th inch of Internal Diameter of Tygon channel. Lane 1- Fish Oil water system, Lane 2: Fish Oil Silver Nitrate system, Lane 3: 10 %Hexane 90% Fish Oil Silver nitrate system.

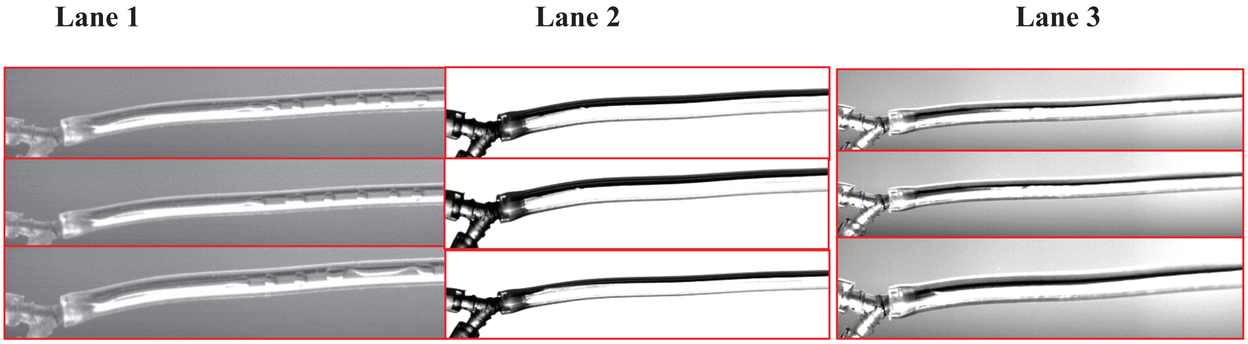


Figure 5.7. Phase Inversion Observed in 1/16th inch of Internal Diameter of PFA channel. Lane 1- Fish Oil water system, Lane 2: Fish Oil Silver Nitrate system, Lane 3: 10 %Hexane 90% Fish Oil Silver nitrate system.

5.3.5. Analysis of flow patterns based on Bond Number:

In the point of process intensification, reduing the dimensions of the channel geometry has an great impact on dominating forces on the fluid flow. In general, the interfacial forces play the most dominant forces especially in two immisible phase flow in minichannel. To compare the gravity, viscous, and intertia forces with interfacial forces,dimensionless numbers will be used. However, in multiphase flow in minifluidic channels, gravity forces has no effect in capillaries for Bo<3.37. Furthemore, in micro/minichannels, interfacial forces often dominate gravity forces Bo < 1. As can be seen in Figure 5.8, the interfacial tension results in Bond numbers less than 1 only for the fish oil & water and hexane and silver nitrate fluid pairs. For all other systems, the interfacial tension is too low to give a bond number less than 1 at 1/16” hydraulic diameter.

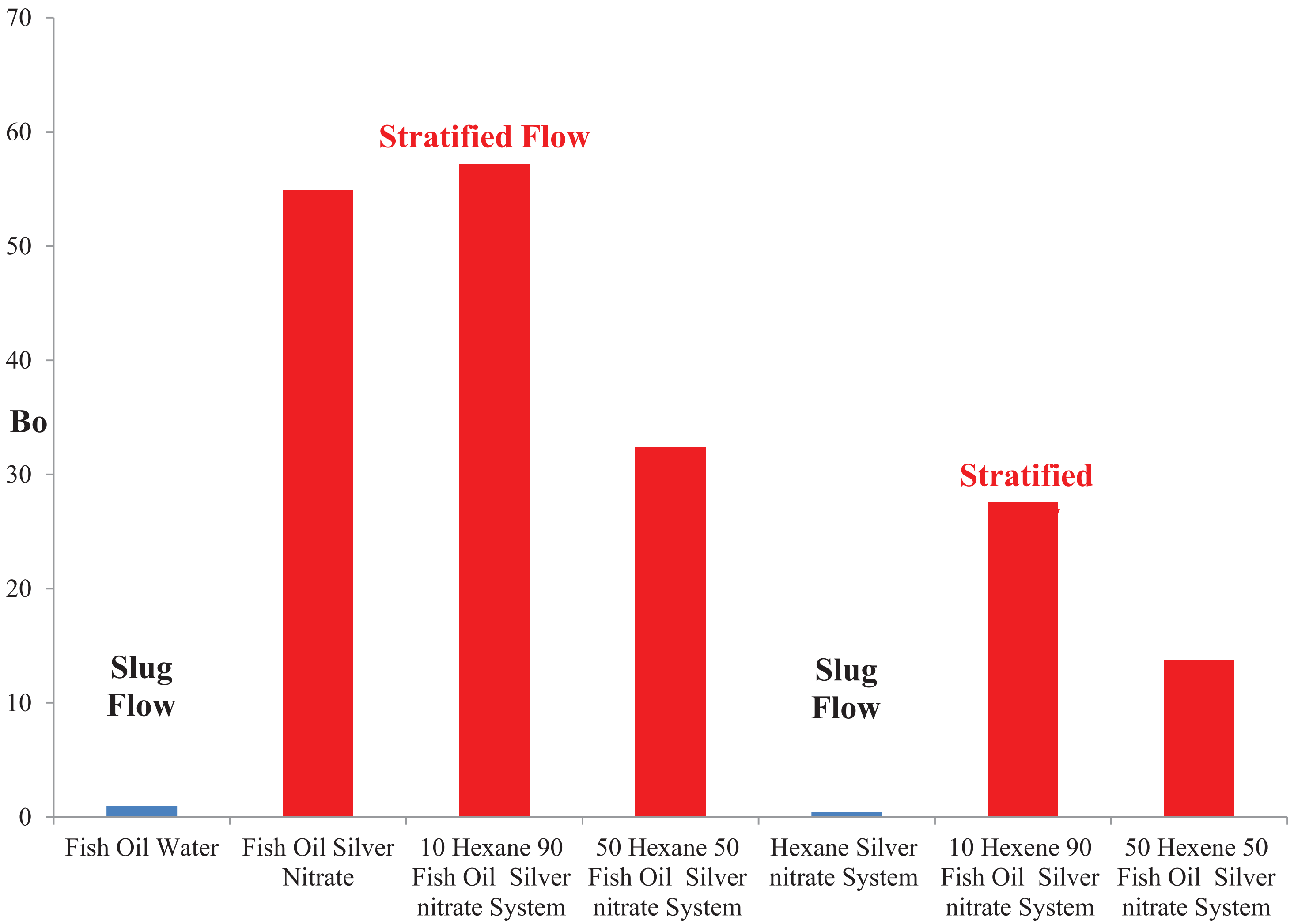


Figure 5.8. Bond Number (Bo) are plotted versus the different experimental fluids. The interfacial tension are predicted at 10°C using spinning drop tensiometry .

5.3.6. Analysis of flow patterns based on Capillary number and Weber number:

The ratio of inertia force to interfacial force is expressed by weber number (We) and the ratio between viscous force and interfacial force is expressed by Capillary number. Moreover, the analysis of flow pattern at mixing of aqueous phase and organic phase in Y-junction in mini-channel, The Weber number and capillary number are calculated for mixed phase (aqueous and organic) and the various forces action on the flow patterns are commented.

In fish oil water system and hexane silver nitrate system, the flow transition from stratified flow to Slug flow ($We < 1$) is observed and at this point, inertial forces are negligible ($We < 1$) and interfacial tension force dominates the inertial forces and cause onset of slug flow in the mini-channel whereas in the case of Fish Oil $AgNO_3$ System, 10 Hex 90 Fish Oil $AgNO_3$ System, 50% Hex 50 % fish Oil $AgNO_3$ system ($We > 1$), Inertial forces dominate interfacial tension forces due to the increase of density of silver nitrate solution and cause stratification in flow. Similarly, in the view of capillary number for hexane silver nitrate system, $Ca < 0.002$, Interfacial tension forces dominant the viscous forces which cause the slug flow in minichannel at flow rate of 5 ml/min. The plot between Log (Ca) vs Log (We) for fish oil water system, fish oil $AgNO_3$ system, 10 Hex 90 Fish Oil $AgNO_3$ systems, 50% Hex 50 % Fish Oil $AgNO_3$ system, and Hexane $AgNO_3$ system flow in 1/16th inch ID of Tygon and PFA mini-channel is shown below.

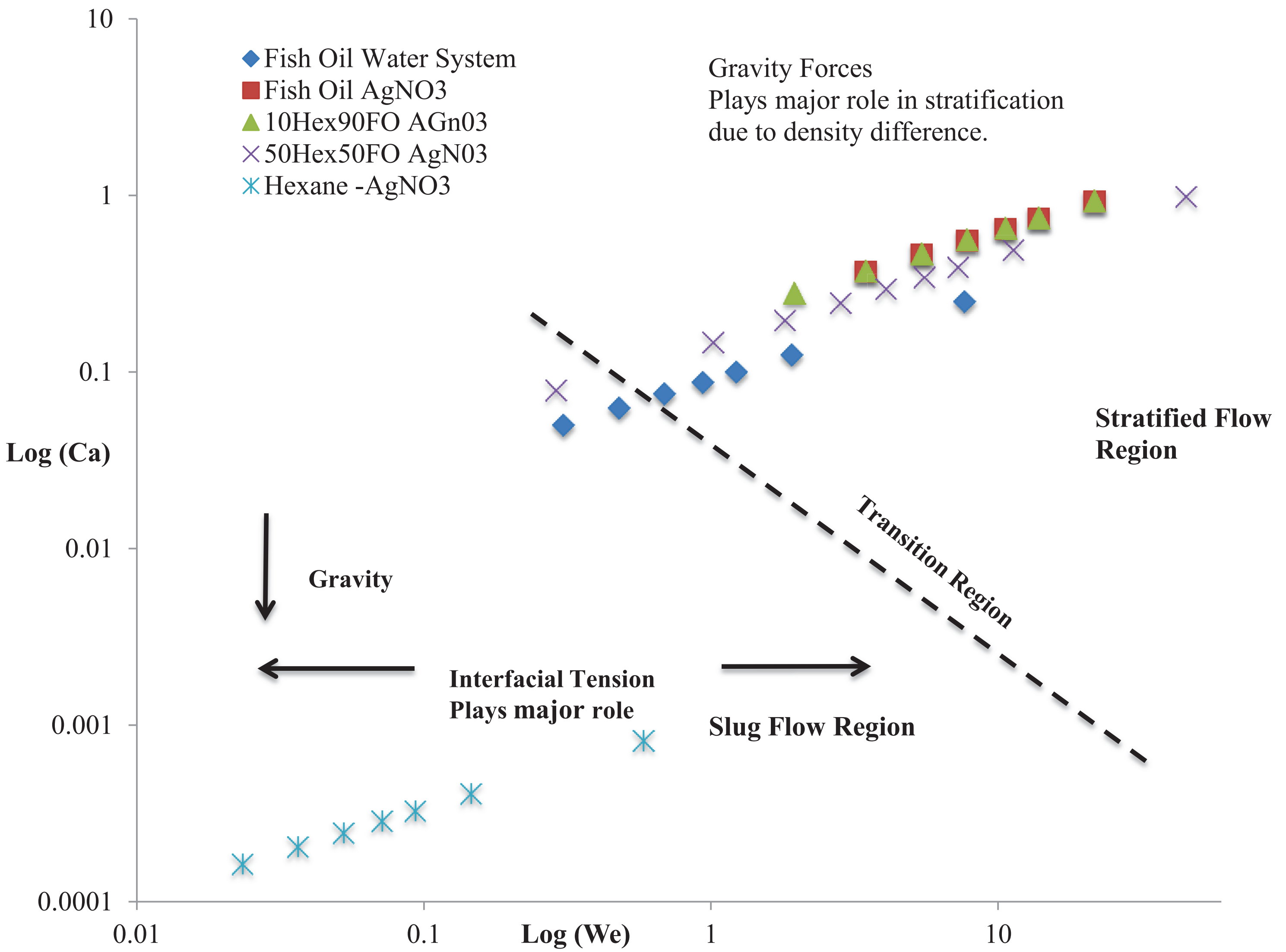


Figure.5.9. Log of Capillary number vs. Log of Weber number

5.3.7. Analysis of flow pattern based on Capillary number and Reynolds number:

In order to define quantitatively the influence of the interfacial tension and other inertia and viscous force in flow, the analysis of flow pattern map is based on capillary and Reynolds number of mixed phases has been investigated. Generally, mini-fluidic technology operates at low Reynolds number. In multiphase systems, the Reynolds numbers are low, but the flow can be nonlinear due to interactions at the interface between the immiscible fluids. In order to study flow patterns between fish Oil and water/ silver nitrate system, Dimensionless numbers used to explain the competition between interfacial tension and gravity forces between fish oil /water and Fish Oil/silver nitrate system. Therefore, the interfacial tension, viscous force and gravity forces control flow patterns because they depend on the channel diameter.

The capillary number is chosen for flow pattern analysis because the viscous forces and the interfacial tension are the dominating forces in flow pattern in mini-fluidic channel at lower flow rates. The addition of hexane decreases slightly the capillary number in comparison to the fish/water system due to the effect of the interfacial tension for the identical linear velocity. Therefore, the addition of hexane in the organic phase increases the interfacial tension in comparison to viscous forces. However, the stratified flow pattern doesn't change after addition of organic solvent. If the flow of two immiscible fluids is dominated by the interfacial tension, slugs are formed because the interfacial tension reduces the interfacial area(Joanicot and Ajdari 2005).

The addition of non-polar organic solvent into the organic phase (fish oil) increases the mean capillary number in comparison to the fish oil/silver nitrate system. This means that the viscous forces increase in comparison to interfacial tension. Viscosity produces a resistance to shear (Atencia and Beebe 2005) and the viscous stresses act to extend and drag the interface downstream (Joanicot and Ajdari 2005) . If the viscous stress is the main forces, stratified flow arises. It is observed that viscosity of fish oil/silver nitrate system is higher than fish oil water system and the addition of organic solvents to the organic phase slightly drops the viscosity however stratified flow is observed.

An increase in the linear velocity leads to an increase in the viscous forces and produces the stratified flow. As can be observed in Figure 5.10, the inertia forces tend to produce stratified flow. More precisely, inertia counters parallel movement creating turbulence. For all the experiments reported, the mean Weber numbers are smaller than 1. Therefore, the interfacial tension dominates the inertia forces producing slugs. (Zhao, Chen et al. 2006). It showed that if inertia forces dominate (We>10), the turbulence appears and annular flows are observed. So, the observed flow patterns can be explained by a competition between the main forces due to interfacial tension and viscosity. The plot between Log of Capillary Number Vs Reynolds Number for Fish Oil Water System , Fish Oil $AgNO_3$ System, 10 Hex 90 Fish Oil $AgNO_3$ System, 50% Hex 50 % Fish Oil $AgNO_3$ System, and Hexane $AgNO_3$ System flow in 1/16th inch ID of Tygon and PFA minichannel.

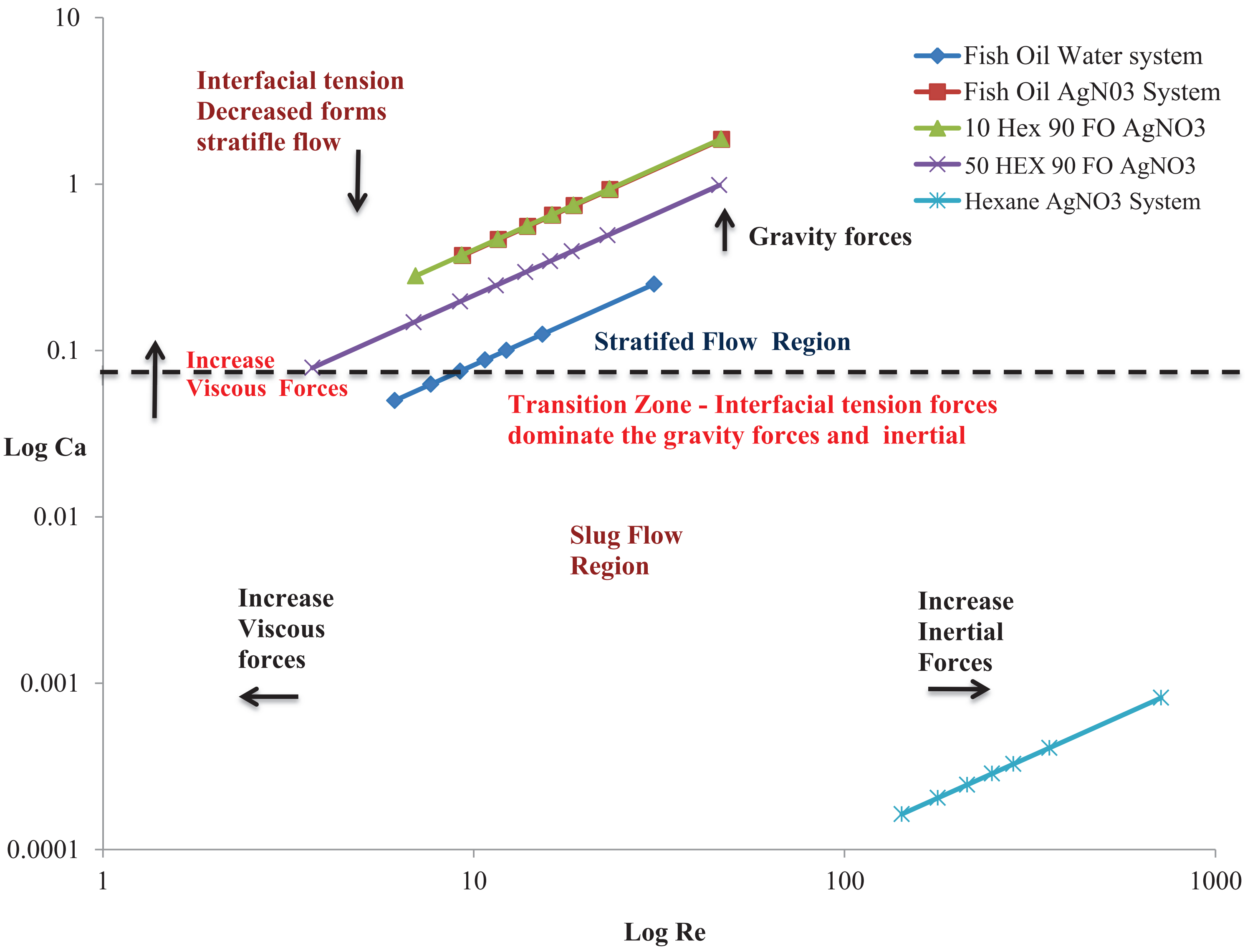


Figure 5.10. Log of Capillary Number Vs Reynolds Number

5.3.8. Analysis of Flow patterns based flow region

The flow pattern map can be divided into three different zones based namely, buoyant force dominant, transition, and interfacial tension force dominant in flow patterns. To develop a flow pattern map, care must be taken at which axial location flow patterns are observed. It is necessary to explain various dominant forces which affect the flow patterns in minichannel. Gravity forces vs interfacial tension is commented.

5.3.8.1. Interfacial dominant flow pattern:

The low values of capillary and Weber numbers confirm that the interfacial forces dominant in slug flow pattern. Due to interfacial effects, water formed a dispersed phase of slugs in the continuous fish oil stream ethyl esters. The water slugs had a bullet shaped (Fig. b and c) and were surrounded by the oil film. The viscous forces sheared and deformed the water slugs, preventing them from touching the channel wall. If the viscous forces were negligible, the dispersed phase could fill the entire cross section of the mini-channel. The formation of slugs shows that these flow patterns are controlled by the interfacial tension. However, in the slug flow pattern, the inertia force also plays major role since the slugs are long in the case of fish oil/water system and hexane/silver nitrate system.

5.3.8.2. Transition Zone where interfacial force = Inertial force flow pattern

In fish oil water system, both slug and wavy annular flow were observed in transition zone. When the inertia force controlled the flow pattern, the core flow was continuous. On other hand, when interfacial forces controlled the flow pattern, the core flow become discontinuous and dispersed. In wavy annular flow pattern of fish oil water system, though the water core was continuous, wavy shaped deformation of the oil water interface were seen. The wavy-shaped interfacial deformation was caused by an interfacial tension effect in the reduction of flow rate of water. At transition zone, the low values of capillary number $Ca<0.0456$ and $We<0.9338$, indicate that the interfacial tension effects were strong enough to disturb the interface. Furthermore, Interfacial tension force dominant in the limit of $We \ll 1$ and dispersed flow were observed in this region. The transition from wavy annular flow and slug flow to stratified flow continuous flow pattern with increase in capillary number is observed. It is observed that an increase in the capillary number would shift the flow pattern from the slug flow pattern to stratified flow region.

5.3.8.3. Buoyancy force dominant region:

In this region, stratified flow pattern was observed. Appearance of stratified flow indicated that the inertia, viscous and gravity force dominant and overcomes the interfacial tension force by high flow rate. Moreover, the viscous fish oil was more wetting compared to water and silver nitrate solution and formed stable film around the entire inner wall of the mini-channel. Also the oil and water/silver nitrate solution forms a stratified flow pattern because of a drastic density difference between oil phase and aqueous phase and gravity force dominant and overcomes the interfacial tension force due to high density of silver nitrate solution.

In micro/mini-fluidic channel, usually two characteristic flow regimes observed are stratified flow and slug in various liquid –liquid extractions. In the case of fish oil-$AgNO_3$,10 Hex 90 FO–$AgNO_3$, 50 Hex 50 FO–$AgNO_3$, stratified flow were observed and the liquid–liquid interface is flat and negligence of surface tension induced curvature and gravity force and inertia force were dominant in the stratified flow region.

Furthermore, the velocity of oil phase and aqueous phase is difference in residence time and fully developed flow and better mass transfer. A. A. Donaldson (2013) confirmed that that the shortest residence time of the developed mini–fluidic system with stratified flow was ~36 seconds, far in excess of the 15 sec required to reach equilibrium in the previous work where slug flow was observed in Kamio *et al.* (2010, 2011). In the aspect of performance of mini-fluidic system with stratified flow pattern, the yield of EPA/DHA was around 75-80 %. Though the stratified flow pattern was observed in mini-fluidic system in fish oil silver nitrate system, such flow pattern has low Reynolds number, small diffusion path and large interfacial area.(Anil B. Vir et. al., 2014). The addition of non-polar organic solvent hexane into the fish oil doesn't change the stratified flow pattern into slug flow. In addition to fluids property, the material of the wall plays a critical role in determining flow regime. Food grade Tygon mini-channel is wetted well by Oil Phase.

5.4. Role of Interfacial Tension in Flow pattern:

The data would suggest a significant variation in interfacial tension depending on whether or not silver nitrate is present in the solution, and if hexane and hexene are added to the fish oil, the fish oil and distilled water had interfacial tension which was in the upper limit of the instrument's ability to measure, as illustrated by the steady rise in calculated surface tension with RPM (where RPM's above 7000 to 9000 can create some difficulties in the equipment used). In contrast, fish oil in silver nitrate solution was at the lower end of the instrument's capabilities, where the interface would breakup up rapidly at rotation rates before stable surface tension measurements could be obtained. The 10 Percent by wt., of hexane/hexene addition resulted in an apparent increase in surface tension. 50% of hexane/hexene had a significant increase in the interfacial tension, rapidly moving past the instrument's ability to measure accurately. However, the addition of hexane either 10 % or 50 % to fish oil with silver nitrate system does not change the flow pattern in the mini-channel and cause only stratified flow pattern

These trends are important for future processing considerations, both in recognizing that the low surface tension between fish oil and silver nitrate will facilitate contacting and mass transfer, while possibly making separation difficult. The addition of hexane or, preferably, hexene could use to help in separating the two phases in design of mini-fluidic technology for liquid-liquid extraction of EPA/DHA from fish oil ethyl ester. In SDT, Interfacial tension is a function of spinning drop tensiometer rotation. It is noted that the stable value of IFT obtain, as RPM increasing represent the actual surface tension.

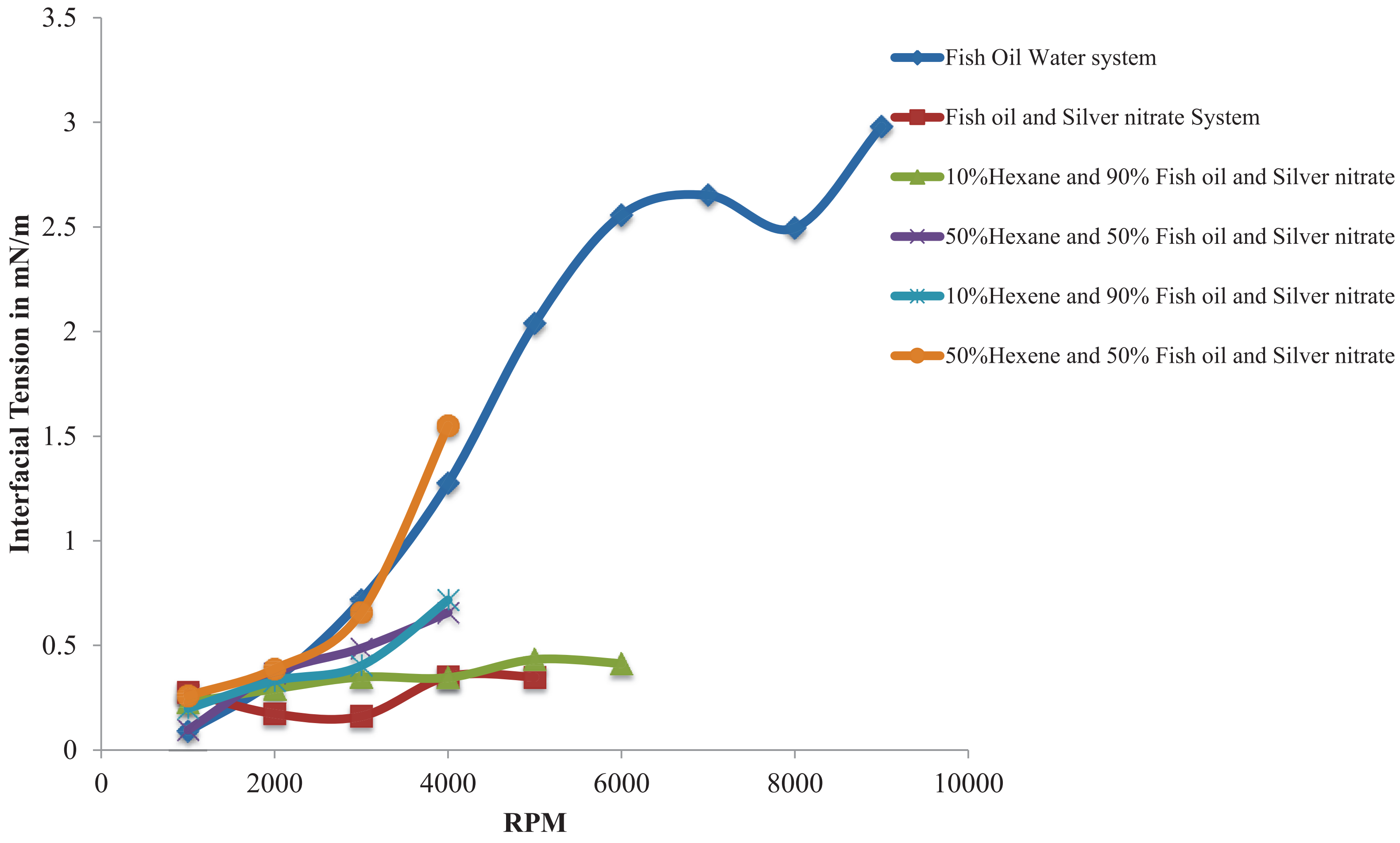


Figure .5.11. Interfacial tension studies for experimental fluids by spinning drop tensiometry.

5.5. Conclusion and recommendations

Multiphase flows in mini-channel have a wide range of applications in chemical and biochemical processing especially in the case of liquid- liquid extraction for omega 3 PUFA from fish oil using silver nitrate solution. An experimental study of viscous fish oil ethyl ester –water/silver nitrate solution flow in Tygon and PFA minichannel with an ID of 1.58 mm have been performed. Different flow patterns were observed with constant flow ratio of 3 with change in flow rate of fish oil and silver nitrate solution. Since the Tygon mini-channel and PFA mini-channel were initially saturated with fish oil, the stratified flows were observed. The various flow patterns of fish oil ethyl esters and various aqueous phases in two types of mini-channel such as food grade Tygon channel and PFA channel have been investigated. Furthermore, the flow patterns would affect the extraction processes in food grade slug flow minichannel. It is noted that the stratified flow forms in the Tygon channel at the flow rate of 5 ml/min. The flow pattern maps were presented based on Bo, We, Ca and Re. Three different zones were distinguished in flow pattern map namely:

1. Interfacial tension force dominant – Slug flow region
2. Transition region
3. Buoyancy force dominant – Stratified flow region

Furthermore, the addition of organic solvent into the fish oil ethyl ester increase the interfacial tension between fish oil and silver nitrate system, However, There was no transition from stratified flow into slug flow in the fish oil silver nitrate system by addition of hexane into oil phase. In order to design slug flow based mini-fluidics contactor for extraction of EPA/DHA from fish oil ethyl ester using silver nitrate solution, it is important to understand the hydrodynamics of the fish oil silver nitrate system and the addition of organic solvents into the system in the mini-channel. Stratified flow pattern was observed in the designed mini-fluidic system.

CHAPTER .6 .CONCEPTUAL PROCESS DESIGN OF SILVER BASED SOLVENT EXTRACTION OF OMEGA 3 PUFA

6.1. Introduction

In the process of concentration of Omega 3 PUFA, Fish oil ethyl ester is used as the feed stock which has contains complex mixtures of fatty acids with varying degrees of unsaturation. The separation of fatty acids of interest is difficult for the production of highly concentrated components. While a number of processes for the concentration and separation of Omega 3 PUFA are being researched in an effort to improve extraction efficiency, only a few are suitable for large scale production. The available methods include adsorption chromatography, fractional or molecular distillation, enzymatic splitting, low-temperature crystallization, super-critical fluid extraction and urea complexation. Each technique has its own advantages and drawbacks, and was described in detail in Chapter 2.

The recovery of Omega 3 Poly Unsaturated Fatty acids (PUFA) from fish oil ethyl esters via liquid –liquid extraction (LLE) using aqueous concentrated solution of silver nitrate was previously evaluated as an alternative extraction technology to conventional concentration and extraction processes of Omega 3 PUFA (i.e. Urea Precipitation and molecular distillation). In this work, the conceptual process designs for extraction of Omega 3 PUFA from fish oil using a batch-wise stirred tank reactor is compared to a mini-fluidic contactor in continuous operation. Laboratory investigation showed that the yield of Omega 3 PUFA in silver LLE is 75 % when compared with conventional processes and the data from the laboratory investigation is used for developed for conceptual process design for silver based solvent extraction. Furthermore, the process design for recovery silver from the waste is developed and discussed within the context of economic feasibility given the high cost of this solvent type.

Based on the economic analysis, the silver based solvent extraction in mini-fluidic contacting system is only feasible when nearly complete recovery of silver nitrate from the waste is completely possible, with mini-fluidics only providing moderate advantages if the oxidation of silver ions is independent of the number of heat/cool cycles.

6.2. Process Description

Solvent extraction is the common separation method for separation of various biological compounds from biological material, and also considered as potential conventional separation technique. This mass transfer technology has been applied to concentration and separation of Omega 3 PUFA from fish oil ethyl esters. In this solvent extraction, the concentrate aqueous silver nitrate salt solution is used as a solvent for extraction of omega 3 PUFA from fish oils as silver ions reversible form complexes with the double bond of unsaturated fatty acid. As a result, the purification of the ethyl ester of PUFA (PUFA-Ets) may be possible based on the complex between double bonds of PUFA-Et and silver ions. This silver based solvent extraction was reported in laboratory scale as new method by K.Yazawa *et al* (1991), and has since been explored by a number of other authors using idealized extraction systems and lab-scale analysis (M.Li *et al* 2008). Since the separation is based exclusively on a fast reversible chemical reaction, the simple method would be a promising technology for the purification of PUFAs.

6.2.1. Liquid –Liquid Extraction of Omega 3 PUFA using Silver Nitrate Solutions

The reported experiments on extraction using silver salts are limited and in this investigation, the polyunsaturated fatty acids from a mixture are isolated. However, previously-known methods do not provide for a sufficiently selective and/or efficient process for concentrating omega-3 fatty acids. The potential process for application of this technology, consist of 5 key steps that are described in Chapter 2. The above process is detailed in step by step in the figure 6.1, and was the basis for this comparison.

In silver-based solvent extraction, silver ions in the aqueous phase plays a major role in the extraction processes and bind with the PUFA's double bond form complexes in the aqueous phase. In the study with silver, the complexes are of the charge-transfer type in which the unsaturated compound acts as an electron donor and the silver ion as an electron acceptor (Traynham and Sehnert, 1956).

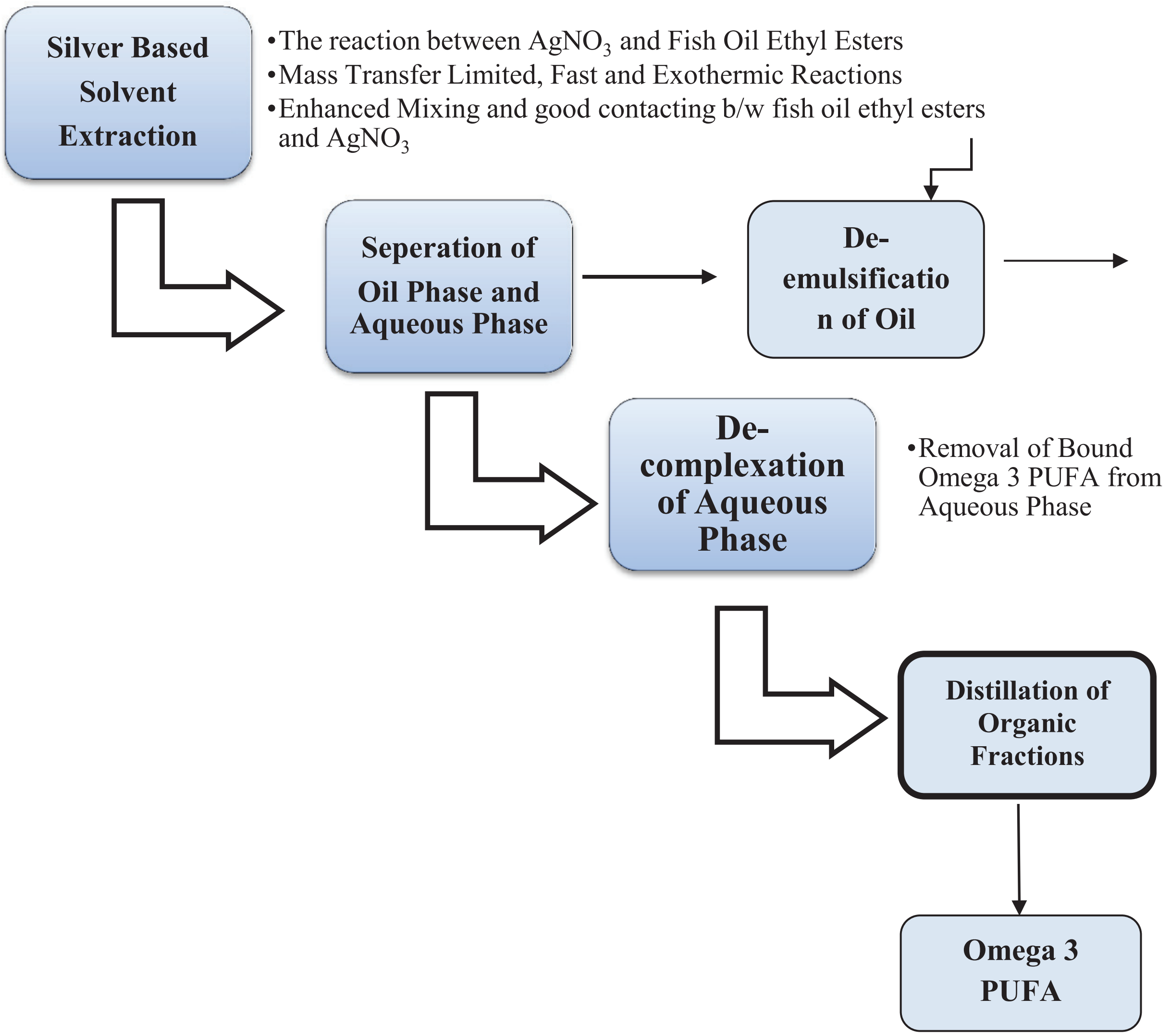


Figure 6.1. Overview of silver based solvent extraction of Omega 3 PUFA from fish oil EE.

$$\mathbf{DHA/EPA\text{-}Et + AgNO_3 \longrightarrow DHA/EPA{:}Ag^{2+}\ Complex + Fish\ Oil\ \text{-}Et}$$

The formation mechanism of complex between double bonds and silver ion is still a topic of interest receiving significant attention within scientific research intensively, since these complexes play an important role in process development based on solvent based Liquid–Liquid Extraction and chromatography based methods. However, quantitative data (e.g. equilibrium constants) exist only for a number of short chain mono-and di-olefins. The stability of complex with organic compound decreases with the increasing chain-length. Generally, the rate of complexation is very rapid but the complexes are unstable and exist in equilibrium with the free form of the olefin. The coordination forces between Omega 3 PUFA and silver ion seem to be very weak. These particular properties of complexation between a double bond and a silver ion are favorable for use in chromatography/LLE. (Traynham, and Sehnert, 1956) A significant unknown within this bonding process is the relative ratio of Ag ions required to cause one mole of DHA and EPA to solubilize within

1 mole of DHA (356g) + 6 Moles Silver ions = DHA-et –Ag complex

1 mole of EPA (330g) + 5 moles Silver Ions = EPA-et – Ag complex.

6.2.2. Development of conceptual process design:

A hierarchical conceptual process design will be applied to identify the best process mode whether batch process or continuous process to concentrate the omega 3 PUFA using solvent extraction process approach. The design phases of conceptual process design involve the following steps, based on laboratory experiments, the conceptual design have been developed in this case. (Mukoma, P., 2006)

1. Problem Definition
2. Knowledge acquisition
3. Synthesizing alternatives at different design levels: Process structure – Batch process and continuous process, Unit Operations and equipment's
4. Analysis of Behavior
5. Evaluation of performance. (Malwade, C. R.,2013,)

6.2.3. Base Case Definition and Basis of Design:

The base case is a simplification of the reality where the Omega 3 PUFA are separated from the fish oil ethyl esters by silver based solvent extraction. To compare different process options for solvent extraction of omega 3 PUFA, a base case is developed, taking into account common unit operations and process parameters in the production of omega 3 PUFA. This base case is an integrated process of three main units that are involved in the production of DHA/EPA–et: Solvent extraction, De-emulsification, de-complexation and silver recovery and its recycle to solvent extraction process. Furthermore, the selection of reactor technology plays major role in the concentration of Omega 3 PUFA from fish oil ethyl ester and minimizing the residence time for solvent extraction. Based on the laboratory LLE experiments, the mini-fluidic reactor technology has 80% extraction performance in 36 seconds of residence time whereas stirred tank system has 90% extraction performance in 15 min. Therefore, the stirred tank system is considered as batch process and mini-fluidic reactor technology is used as continuous processes. It is worth noting that Teramoto *et al*. have reported the distribution ratios of polyunsaturated fatty acid ethyl esters between aqueous AgNO3 extraction phase and organic heptane phase. The distribution ratios were found to increase drastically as temperature was lowered.

In the first stage of the base case, Fish oil ethyl ester and concentrated aqueous are fed into the reactor called solvent extraction systems and then the omega 3 PUFA binds with silver ions in the aqueous solution. As a result, emulsified oil phase is formed. The emulsified oil phase is treated with hexane to proceed de-emulsification process in the mixer settler. The hexane fraction is subjected to a separator to isolate the Oil phase and hexane which is recycled to the settler.

In the second stage of the base case, de-complexation is applied to release the bound Omega 3 PUFA from the aqueous phase by treated with hexane for an hour and then combined with a 1:10 ratio of deionized water for an hour. The organic fractions are fed into the separator to isolate Omega 3 PUFA and organic solvent is recycled. The water out aqueous phase is passed for silver recovery. In the third stage of the base case, as silver nitrate salts is expensive , it is necessary to recover the silver salts from aqueous phase from de-complexation processes and it is mentioned these.

The designed capacity of producing omega 3 PUFA is 10 tons of fish oil per day. The operating time per shift is 8 hours. A plant life of 10 years is assumed for economic analysis and it is expected that the total plant investment is recouped before this period. The system boundaries for the evaluated omega 3 PUFA production processes, including the base case, are presented in Figure 5. In order to simplify the system, it is assumed that silver recovery from wastewater is treated outside the process. However, as one of the objectives of the new process configuration is the reduction of waste salts, the disposal price of this waste is taken into account for the final economic evaluation. In addition to that, the yield of Omega 3 PUFA from each stage of LLE in mini-fluidic reactor and stirred reactor are mentioned here. Based on laboratory experiments, the conceptual process design for LLE of omega 3 PUFA have been developed.

Table 6.1. - Recovery of Omega 3 PUFA from each stage of LLE experiments.

Processing Step	Recovery % In mini-fluidic contacting system	Recovery % In idealized system	Remarks
Extraction	34.55	52.38	In Idealized system, The process is batch process.
Oil Layer isolation	69.67	47.62	
De-emulsified Layer	6.944	10.99	In idealized system, The contact between organic and aqueous phase is better and the volume is fixed.
Oils bound to the Aqueous phase	34.55	52.38	Complexation with Silver Ion depends on the mixing performance.
De-complexed Omega 3 PUFA from Aqueous phase	80.33	56.38	
Recovery of Omega	75	78	

In the above table, the yield and recovery of Omega 3 PUFA from each stage of the process is mentioned and the omega 3 PUFA yield is 75% in mini-fluidics contacting system where as in idealized system is 78%. In addition to that, the conceptual process design for batch process and continuous process are based on laboratory experiments. The mass balance and sizing of equipment based on laboratory yield of Omega 3 PUFA from fish oil ethyl esters.

6.3. Process Design Options

6.3.1. Batch Process – Equivalent Scale of Continuous Processes

In order to compare the batch process with continuous process, the batch process has been designed in same flow rate and the CSTR are operated in multiple and in parallel mode. The plant capacity for this batch process is 10 Tons per shift. Each shift is operated for 8 hours and is compared with continuous process which has run time of 8 hours. In this case, the CSTR are operated in parallel to meet the equivalent production of continuous process.

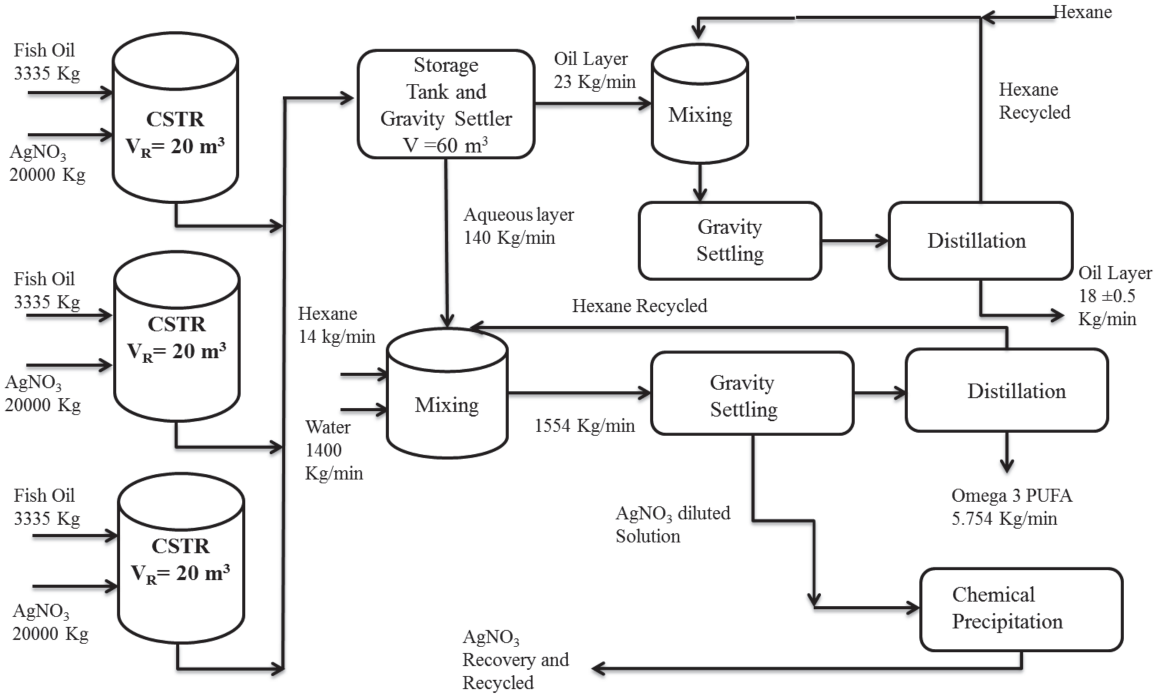


Figure 6.2. Multiple CSTR reactors in Parallel in order to meet production capacity of continuous process.

In this case, the multiple CSTR in parallel is operated to meet capacity of continuous process (mini-fluidic reactor). However, the operation of reactor in parallel mode is only in batch process and the reactions mixture is stored in the storage tank which is blanketed with nitration to avoid the oxidation of omega 3 PUFA. After this stage, the post extraction processing is in continuous processes which are equivalent to flow rate of continuous processes.

6.3.4. Continuous Processes Using Mini-Fluidic Contacting System:

Based on laboratory investigation of LLE in slug-flow mini-fluidic reactor, it requires least short residence time to reach the equilibrium concentration and has capable to extract the omega 3 PUFA from fish oil ethyl esters by continuous process. In addition to that, the extraction potential of mini-fluidic reactor technology is better than conventional extraction system such as stirred tank reactor. The simplified conceptual process flow diagram is shown below.

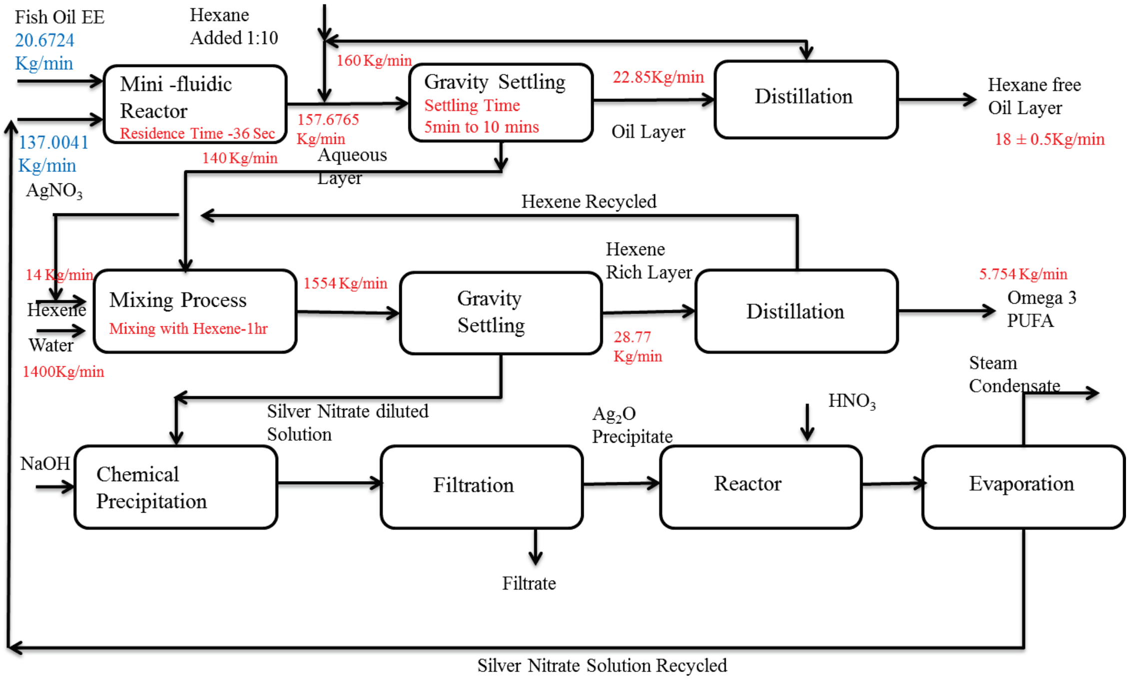


Figure 6.3. Continuous process for Omega 3 PUFA extraction using silver based solvent extraction.

The application of mini-fluidic based extraction of omega 3 PUFA using concentrate silver nitrate solution is promising because of their simplicity, shortest residence time and improved mixing than conventional system. Moreover, it is process intensified and simple in design. This facilitates continuous processing of Omega 3 PUFA and solvent inventory. In conceptual continuous process design, mini –fluidic reactor is considered as plug flow reactor or double pipe heat exchanger and offer continuous processing of omega 3 PUFA extracted from the fish oil ester. This conceptual continuous process design is based on the processing of 10 tons of fish oil ethyl ester per day. Therefore, it has capable to extract 2.5 tons of omegas 3 PUFA from 10 Tons of Fish Oil ethyl ester.

6.4. Solvent Inventory Analysis between batch process and continuous processes:

In LLE process of Omega 3 PUFA, the use of concentrated 50 % by Wt. silver nitrate solution as a solvent is expensive and its minimal consumption is expected in process development in order to decrease the operating cost of the extraction process. The solvent inventory analysis of proposed conceptual process design either batch process or continuous process are performed. Based on the process design, Solvent inventory in continuous process is less than batch process. Additionally, the solvent required for batch process is ~35 m^3. In contrast with continuous process, the required solvent inventory is ~ 16 m^3. Based on this information, the operating cost of continuous process would decrease by requiring less solvent for LLE.

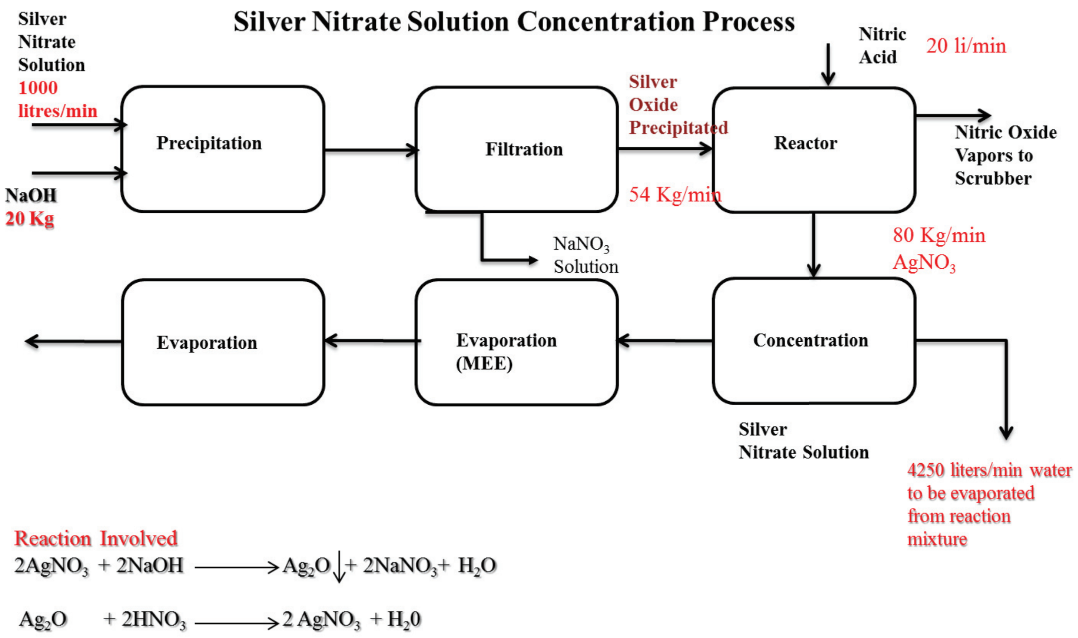
Using a standard bulk cost of AgNO3 of ~$700 per kg, a reduction in inventory by this amount could correspond to a savings of ~23 million $.

6.5. Recycle of Silver Nitrate from the aqueous Solutions:

Silver based solvent extraction of Omega 3 PUFA from fish oil ethyl esters is a cost expensive process as the cost of silver nitrate is expensive. Even though it is effective process in the separation aspects, Recovery of silver nitrate from aqueous phase after de-complexation processes is expected to minimize the operating cost of the process. There are some recoveries of silver nitrate from waste solution mentioned here. (Murphy, J.A, 1991 and Rawat, J.P, 1986).

6.5.1. Recovery of Silver Nitrate By concentration processes.

In Case 1, Diluted silver nitrate solution is reacted with sodium hydroxide solution. As a result, the silver oxide is precipitated and collected and transferred to react with concentrated nitric acid and then silver nitrate is formed in the form of solution and concentrated in multiple effect evaporators.



Reaction Involved

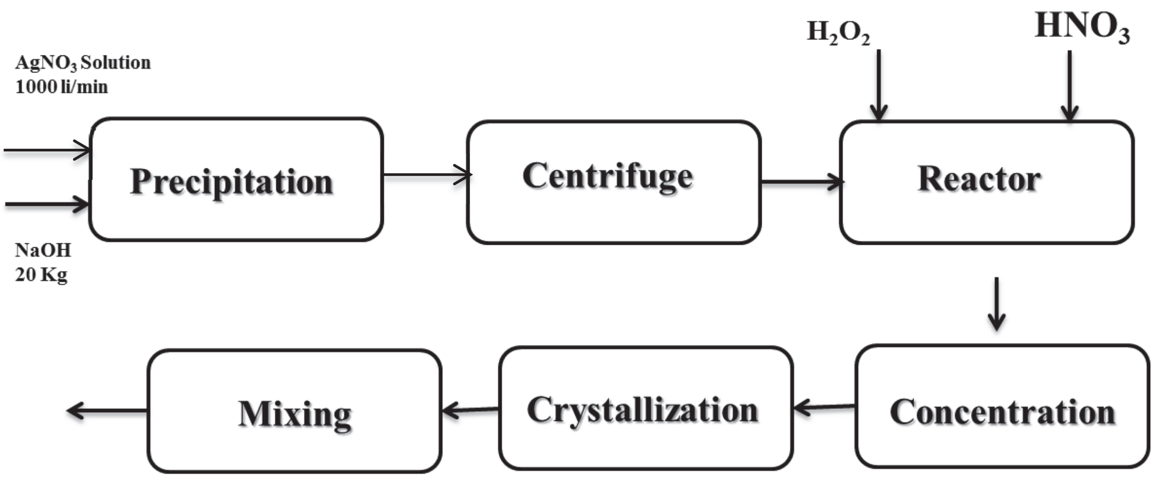
$$2AgNO_3 + 2NaOH \longrightarrow Ag_2O\downarrow + 2NaNO_3 + H_2O$$

$$Ag_2O + 2HNO_3 \longrightarrow 2\,AgNO_3 + H_20$$

Process Conditions:
Silver Nitrate Concentration : 80 Kg in 1000 liters
NaOH concentration: 20 Kg

Figure 6.4. Process Design for Silver nitrate concentration process

6.5.2. Electrochemical Oxidation Process



Silver Nitrate Solution recycled
to the liquid –liquid Extraction
of Omega 3 PUFA

Figure 6.5. Process Design for Electro chemical Oxidation Process

In Case 2, Diluted silver nitrate solution is reacted with sodium hydroxide solution and as a result, silver oxide is precipitated and allowed to reduce with hydrogen peroxide. After a brown precipitate of silver oxide is formed:

$$2AgNO_3 + 2NaOH = Ag_2O + 2NaNO_3 + H_2O.$$

$$Ag_2O + H_2O_2 = 2Ag + H_2O + O_2$$

When added H_2O_2 to silver oxide, it is at once converted into black metallic silver, with brisk evolution of oxygen. If a further quantity of H_2O_2 is added, it is catalytically decomposed by the finely divided silver. The metallic silver is reacted with concentrate nitric acid to produce silver nitrate solution and nitrous oxide.

6.5.3. Chemical Reaction Based Methods

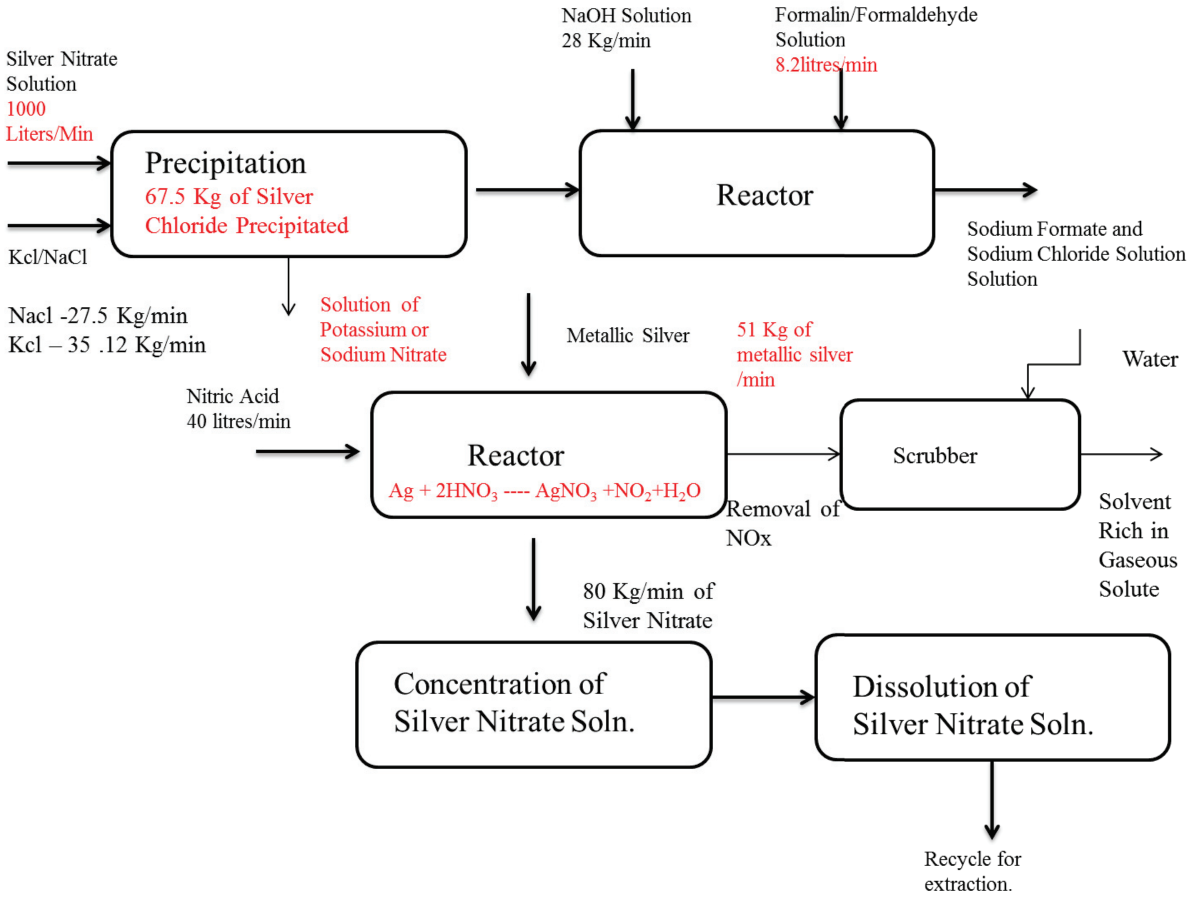


Figure 6.6. Process Design for chemical reaction based methods

In Case 3, Diluted silver nitrate solution is reacted with sodium chloride to form silver chloride which is precipitated. The precipitated silver chloride reduced with formalin/formaldehyde to form metallic silver.

$\mathbf{Ag_2NO_3 + NaCl\ or\ KCl = 2AgCl + NaNO_3}$

$\mathbf{2AgCl + 3NaOH + HCHO = 2Ag + HCOONa + 2NaCl + 2H_2O}$

The reduced metallic silver is reacted with concentrate nitric acid to prepare silver nitrate for LLE operations.

6.5.4. Silver Reduction by formaldehyde

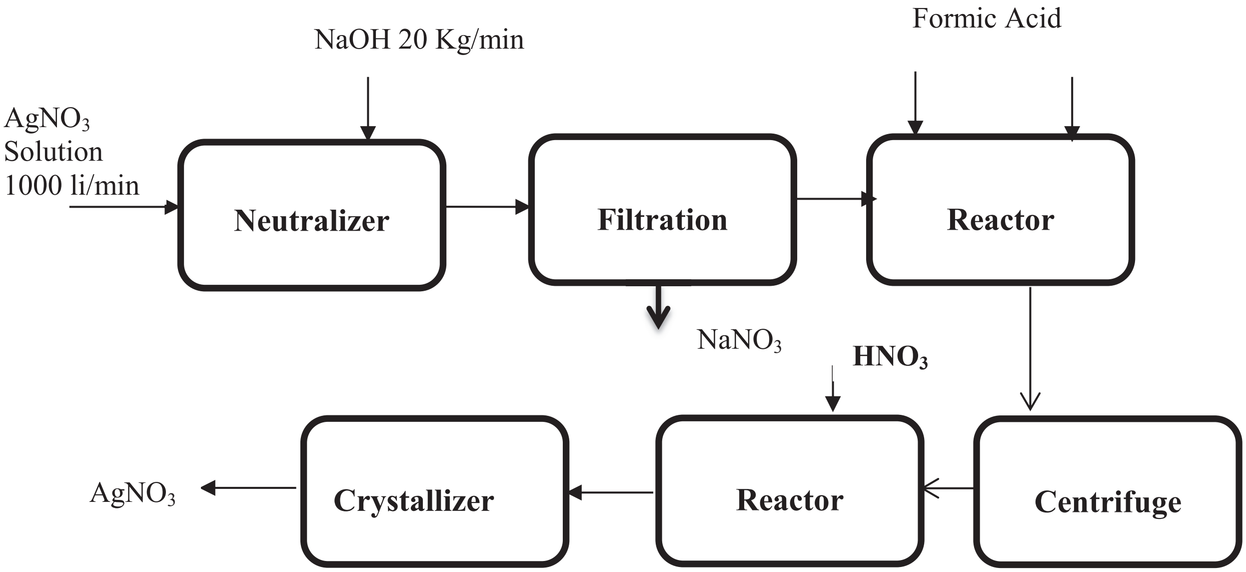


Figure 6.7. Process Design for Silver Reduction by formaldehyde

In Case 4, dilute silver nitrate solution is reacted with sodium hydroxide solution and precipitated silver oxide is reduced with formic acid to form metallic silver. The metallic silver is reacted with concentrated nitric acid to form silver nitrate solution. The conceptual process design for silver recovery this case is based on the following chemical reaction.

$$\mathbf{2AgNO_3 + 2NaOH \longrightarrow Ag_2O + 2NaNO_3 + H_2O}$$

$$\mathbf{6Ag_2O + 2HCHO \longrightarrow 6Ag + 2HCO_2 + H_2O}$$

$$\mathbf{3Ag + 4HNO_3 \longrightarrow 3AgNO_3 + 2H_2O + NO}$$

Another approach for silver recovery in the same case is use of sodium formate instead of formaldehyde as it is harmful chemical.

6.6. Process Economics

The total investment cost of all the cases in LLE and recovery of silver from waste water are calculated and tabulated. The fixed capital investment for both various cases of batch processes and continuous process were calculated. Table 6.2 and 6.3 show the fixed capital costs in the batch process are considerably higher than continuous process. In the case of Case 1 and Case 2, Batch process (Operating CSTR 20 m^3 in parallel) increased TCI in that Case. However, other process equipment's are in equivalent size. Even though the batch process requires three parallel reactors to be able to meet an equivalent production flow rate, the reaction mixture could be stored in Storage tank which acts as mixer settler within the extraction process. In contrast with the continuous processors, batch process Case 1 requires more process equipment that leads to more TCI. In contrast with Continuous process, it requires mini-fluidic contacting system in replace of CSTR and a separate mixer-settler system for downstream separations, and so has additional equipment which decreases the cost in Case 2.

The equipment cost difference between batch and continuous process is caused by the fact that the base case requires continuous run and the cost of stirred tank reactor is expensive than that of mini-fluidic contacting system (double pipe heat exchanger). It is observed that the mini –fluidic based continuous process has the advantage of reduction of 30% reduction in TCI and less solvent inventory. Additionally, Case 2 is economically viable as it requires less TCI than Batch process and also it may require only less operating cost.

The conceptual design for silver recovery process is developed based on continuous process design. Case 1 is the best case and requires less raw materials cost that confirms requirement of least operating cost.

Table 6.2.Process Economics for Batch Process and Continuous Processes:

Item	Case 1 (CSTR in parallel) US $	Case 2 Continuous Processes US $
Total Direct Plant Cost[a]	8,500,000	6,053,800
Total Indirect Cost	1,496,220	1,066,500
Total Direct and Indirect Plant Cost[b]	10,000,000	7,120,200
Fixed capital investment	11,500,000	8,190,000
Working Capital	2,300,000	1,638,000
Start up	1,000,000	655,100
Total Capital Investment	15,000,000	10,481,000

*Based on preliminary cost analysis, the continuous process has high fixed capital investment. However, it would be low production cost due to continuous process. The main component of the continuous process is mini-fluidic contacting system which considered has double pipe heat exchanger which has active heat transfer area around 8 m^2 . However, the batch process (Case 3) which is the case of operating 3 CSTR reactors in parallel is equivalent to continuous process capacity. Includes purchased equipment (PE), installation equipment (47% PE), instrumentation (36% PE), piping (68% PE), electrical (11% PE), buildings (18% PE), yard improvements (10% PE), and services facilities (1.05% PE). Includes engineering and supervision (33% PE), construction expenses (41% PE), legal expenses (4% PE), contractors fee=0.05(D+I), and contingency (0.1% D+I). Working Capital is 20 FCI and start up is 8% of FCI.

Table 6.3. Process Economics for Silver Recovery by different processes:

Item	**Case 1**	**Case 2**	**Case 3**	**Case 4**
Total Direct Plant Cost	2,627,150	2,125,500	2,312,730	2,275,595
Total Direct and Indirect Plant Cost	3,145,930	2,545,220	2,769,420	2,724,960
Fixed capital investment	3,617,820	2,927,000	3,184,900	3,133,700
Working Capital	723,600	585,400	637,000	626,800
Start up	289,500	234,200	254,800	250,700
Total Capital Investment	4,630,800	3,746,600	4,076,600	4,011,200

Table 6.4. Operating Cost for Silver Recovery

Raw materials Price	Case 1	Case 2	Case 3	Case 4
NaOH Tons/shift Cost in US$	9.6 $4032	9.6 $4032	9.6 $4032	9.6 $4032
HNO_3	14.5 $3123	14.5 $3123	14.5 $3123	14.5 $3123
H_2O_2	N/A	3.9 $2093	N/A	N/A
NaCl			13.2 $660	
Formaldehyde			3.4 $ 12157	
Net Cost per shift	$7200	$9200	$20000	$7200

In silver recovery from diluted aqueous solution, silver can be recovered easily in case 2 and 4 where installation cost is least.

The raw materials cost for all the cases on recovery of silver nitrate from continuous process (1m^3/ min of waste water contains 80 Kg of silver nitrate) is estimated. These are the raw material cost required to process the waste water per shift (8 hrs). In every case, 38.4 Tons of $AgNO_3$ is recovered. In case 1, the cost of processing 1 ton of Silver nitrate is 188 US$. For processing of 10 Tons of Fish Oil, The 60 tons of silver nitrate required is needed for LLE. The cost of silver recovery per ton of fish oil ethyl ester processed is ~1130 US$. This corresponds to an approximate cost of $11.30/kg to purify EPA and DHA from an 18/12 EE format to a 40/28 EE format.

6.7. Conclusion

A number of conceptual designs have been presented here for the processing of 10 tons per day of fish oils into concentrated Omega 3 products. The varied components within the process include the type of reactor (batch STR, and mini-fluidic system), and the silver nitrate extraction and recovery process. Of the reactor platforms, the mini-fluidic system is the only one which provides an opportunity to reduce total solvent inventory within the process, by effectively decreasing the volume of the reactors which must be filled during operation. Considering the elevated cost of silver nitrate, this could represent a significant reduction in startup costs associated with this process, as well as the size of ancillary systems and reprocessing equipment. There would be a limited effect of net material usage during the regeneration process though, as regardless of the reactor platform used, a feed ratio of silver nitrate to fish oil must still be maintained at a comparable rate.

The anticipated capital cost of setting up a 10 ton/day facility with a continuous mini-fluidic type system and a case 1 based recovery system is expected to approach 4.5 million dollars. Assuming minimal silver loss within the process, recovery of the silver ion's activity will require approximately $ 7000 in raw materials for every 10 tons of fish oil processed, corresponding to the minimum recovery cost. Additional expenses associated with the extraction and distillation phases are anticipated to be less than that of current molecular distillation technology applied in industry.

CHAPTER 7. CONCLUSION

7.1. Conclusion

The investigation on LLE results for EPA/DHA recovery from 18/12 EE fish oils using ~50 wt. % $AgNO_3$ at 10°C.,was carried out in both a 1/16" ID tubing mini-fluidic system and in a 65 ml volume CSTR, The following conclusion were suggested as part of the thesis.

EPA/DHA recoveries after ~36 Seconds of contact time in the mini-fluidic system were similar to those obtained from a CSTR after 900 secs, approaching conservative estimates of nearly 80% based on actual mass collected after sample losses, and approaching 90% based on remaining EPA/DHA in the residual oil fractions. Typical concentrations within the extract were 38 to 42 wt.% EPA, 27 to 30 wt.% DHA, and a total Omega 3 content between 78 and 82 wt.%. In the case of stirred tank systems, typical concentrations were 40 to 42 wt.% EPA, 26 to 27 wt.% DHA, and a total Omega 3 content between 78 and 82 wt.%.

While processing-related challenges need to be explored further, these values are comparable or better than supercritical CO_2 extraction, Urea Precipitation, or Molecular Distillation.

The observed flow patterns in the mini-fluidic contacting system were stratified due to reduction of interfacial tension between fish oil and aqueous concentrated silver nitrate solution. However, the formation of oil/aqueous slugs originally were anticipated based on previous literature where LLE performed with DHA/EPA dissolved in organic solvent with silver nitrate solution. Based on qualitative interfacial tension measurements performed on the fish oil/$AgNO_3$ brine mixtures, it is observed that there is a significant reduction in interfacial tension between those fluids relative to a comparable mixture containing quantities of hexane or hexene solvents. In LLE, the low interfacial tension among organic/ aqueous phase would be beneficial to mass transfer. It does raise some separation concerns and appears to change the flow regime relative to what has been previously reported in literature for idealized mixtures of purified EPA/DHA in hexane/heptane solvents. Furthermore, in order to improve the performance of mini-fluidics, the study of its hydrodynamic behavior of fish oil silver nitrate system in the channel has been performed. Even though different flow patterns influence mass transfer and mixing which indirectly impact the separation efficiency of omega 3 PUFA from fish oil, the addition of non-polar organic solvents into the fish oil ethyl ester has been attempted to bring the slug flow pattern of fish oil silver nitrate system by elevation of interfacial tension. The formation mechanism of slug and stratified flow was studied by a competition between the gravity, inertia, viscous forces and the interfacial tension forces. The flow patterns maps is presented in terms of Ca, Re and We and identified the three region namely buoyancy force dominant region, transition region and interfacial force dominant region. Furthermore, the role of Bond number in flow pattern formation is commented and justified about stratification of flow in mini-fluidics device. It should be noted that the material of construction of the contactor may have a significant impact on this, and experiments in a stainless steel plate more likely to be encountered within an industrial process system may exhibit different behavior.

De-complexation strategies and solvent retention are going to be a key optimization concern in this process, which is why we are currently exploring extractions using only solvents and temperature variations. The physical separation of the oil and aqueous phases may also be possible through modifying the wetted material in the contactor, potentially allowing for in-situ phase separation. These are areas I would like to continue looking into as part of the development of a fully integrated system for testing both process-oriented extraction and long-term solvent stability and losses.

To sum up, solvent extraction of Omega 3 PUFA ethyl ester (Both EPA/DHA) was performed with an aqueous silver nitrate solution in mini-fluidic reactor technology. The extraction performance in terms of yield of Omega PUFA and residence time in mini fluidic technology is compared with idealized reacting system. It is demonstrated that the experimental results that EPA and DHA ethyl esters could be satisfactorily separated from fish oil ethyl esters. The shortest residence time to achieve the equilibrium in mini-fluidic reactor is ˜36 seconds. In addition, the stratified flow pattern is observed in the mini-channel and commented. It was concluded that the extraction performance was satisfactory when compared with stirred tank system. However, the steps have been recommended to change the flow pattern in the mini-channel by addition of non-polar organic solvents. Furthermore, it is seen that the reason for stratification of flow is dominant of gravity force than interfacial tension force. The preliminary investigation confirms that silver based solvent extraction of Omega 3 PUFA in mini-fluidic reactor technology is promising and an alternate to other convention technologies. The economic analysis of conceptual design using mini-fluidic reactor technology is feasible.

7.2. Recommendation

In silver based LLE, The de-complexation process is a crucial step for recovery of Omega 3 PUFA currently uses dilution with water to elute bound ethyl esters, making recycle of the solvent stream difficult. To improve processing economics associated with solvent-based extractions, it would be ideal to de-complex the bound EPA and DHA without irreversibly modifying the silver nitrate solution (i.e. by combining a temperature increase with a secondary stripping agent). Temperature appears to have a dramatic effect on extraction. While the results of the 10°C results are promising, equilibrium has been reached in less than 36 seconds in the mini-fluidic reactor, suggesting that even shorter flow paths could be implemented. Given the increase in viscosity at these lower temperatures, minimizing channel length is important for limiting energy dissipation. The Tygon tubing currently used is also not suitable for long-term use, softening over time with increased exposure to trans-esterified fish oil which acts solvent. This will necessitate a transition to a more process-based plate & frame design constructed of a suitable material. There are also indications that hexane/hexene addition either before or after the initial contacting could be used within a processing strategy to control/alter flow patterns or, if coupled with wettability-based separation, be used to limit external vessel requirements. Based on these observations, there are a number of recommendations to improve process design of LLE,

1. A different material of construction (i.e. stainless steel could be recommended) significantly affect/alter the flow pattern observed (i.e. transition from stratified flow to slug flow due to wettability differences)
2. To recycle the silver nitrate solution after recovery of silver from chemical precipitation method. The addition of food based anti-oxidant addition to the aqueous solution could improve stability, and will the anti-oxidants remain in the aqueous solution or be partitioned between the residual oil and concentrated ethyl esters.

3. To investigate the equilibrium concentration of EPA/DHA affected by both the silver nitrate solution concentration, ionic strength and operating temperature. The current aqueous: organic volumetric feed ratio is approximately 3.3:1, which does require excess solvent. To design the optimum ratio for a mini-fluidic flow-based system for these applications.
4. To evaluate the fundamental mechanism by which the silver ions and EPA/DHA bond. The two compounds in question have multiple double-bonds which are capable of complexing with the ethyl esters, raising the question of how these bonds form, the number which is required to make the compounds soluble in organic solution, and how the extraction and de-complexation conditions can be manipulated to transition between solubility and insolubility with minimal energy input.
5. To develop models for each stage of silver based solvent extraction of Omega 3 PUFA for better conceptual design and process design and cost estimation and to evaluate detailed investigation of physical properties of Omega 3 PUFA for process development and scale up studies for LLE.

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APPENDCIES

A1 .Physical Properties of Fish Oil Ethyl Ester (18/12):

In order to evaluate the flow transition in mini channels, the viscosity and density of fish oil ethyl esters are evaluated using reported values and correlation. Based on this graph,

The equation is Density = -1.24 T + 862.69. (Tate, R.E. *et al* 2006)

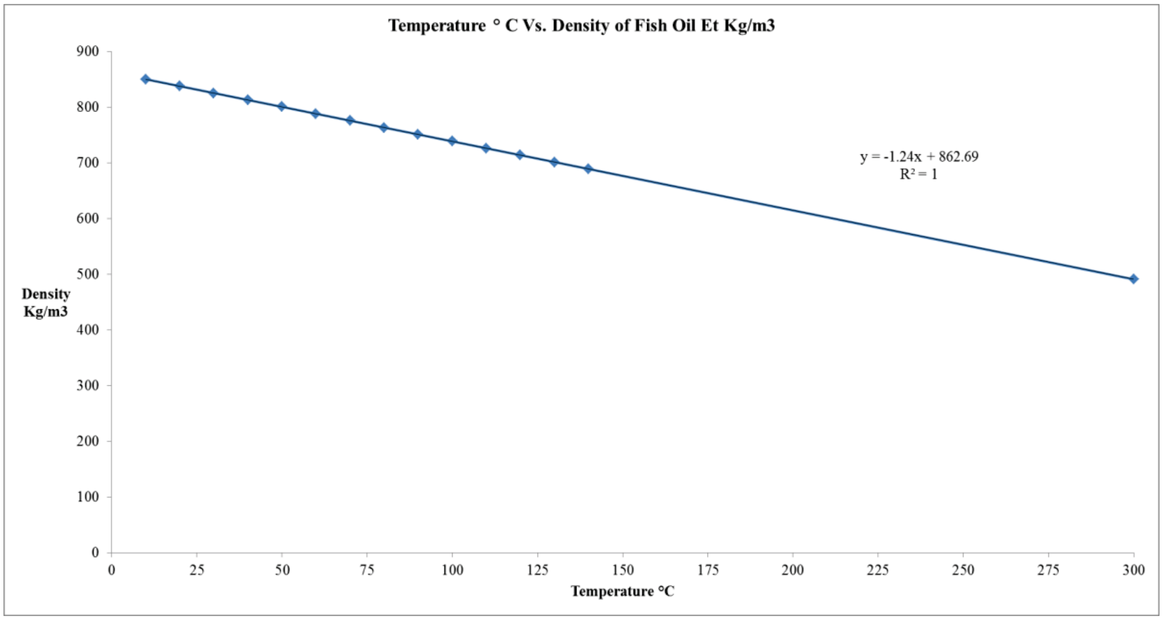


Figure A1. 1- Density of Fish Oil ethyl esters Vs Temperature in °C.

A1.2.Viscosity of Fish Oil Ethyl Ester

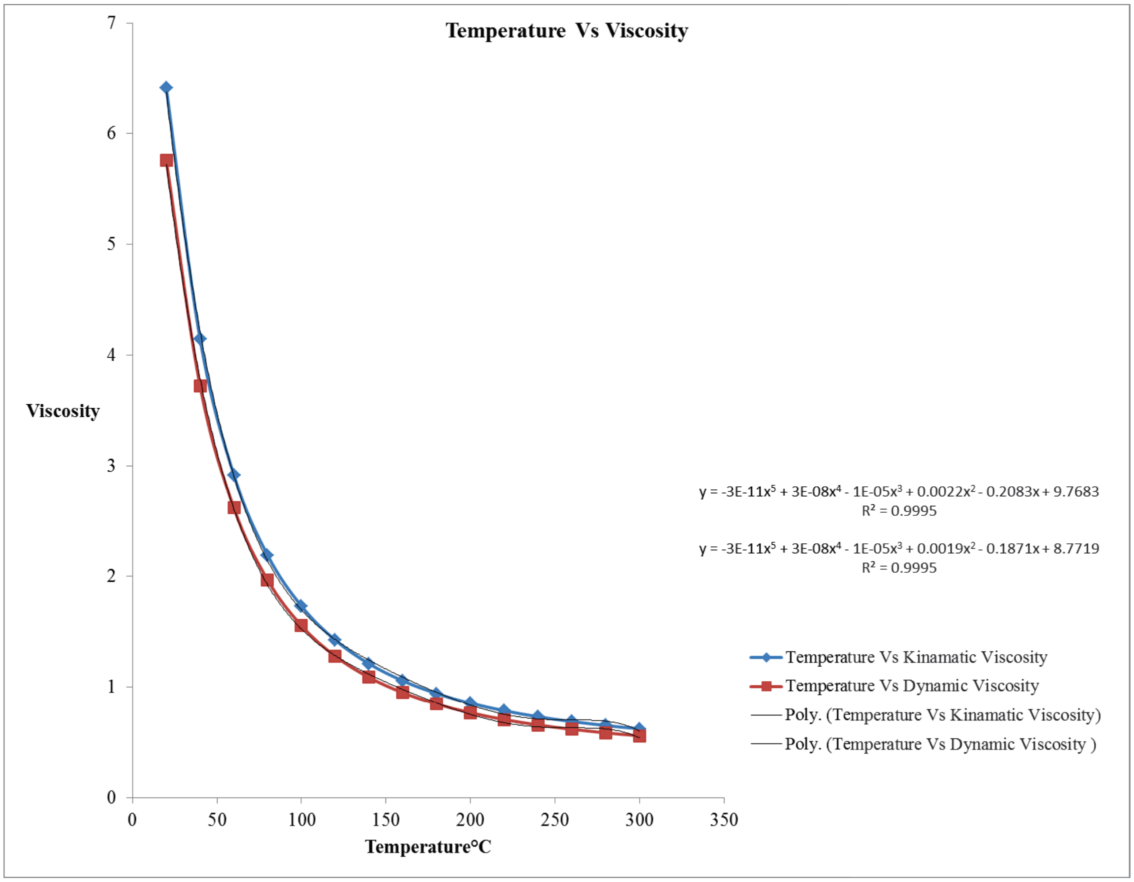


Figure A1.2. Viscosity of fish oil ethyl esters Vs. Temperature in °C (Tate, R.E. *et al* 2006)

A2. Physical Properties of Aqueous Silver nitrate solution

A2.1. Density of Silver Nitrate Solutions

The density and viscosity of silver nitrate solution are evaluated for flow transition analysis and construction of flow transition map for betterment of extraction process in mini –fluidic channels.(Campbell and Singh, 1959, Sohnel, O., and Novotny, P., 1985)

Table A2.1. Physical Properties of Silver Nitrate Solutions

Concentration	**Density**	**Concentrations**	**Relative Viscosity**	**Viscosity**	**Dynamic Viscosity of Solvent**
Wt.%	g/cc	moles /liter		Kg/m.s	Kg/m.s
1.68	1.011	0.1	1.009	0.000899019	0.000891
7.835	1.0658	0.4916	1.033	0.000920403	0.000891
15.618	1.1427	1.0515	1.078	0.000960498	0.000891
23.12	1.2288	1.6723	1.133	0.001009503	0.000891
26.702	1.2743	2.0163	1.168	0.001040688	0.000891
34.265	1.3806	2.7833	1.26	0.00112266	0.000891
40.978	1.4888	3.5913	1.382	0.001231362	0.000891
46.237	1.5874	4.3256	1.464	0.001304424	0.000891
50.333	1.6713	4.9518	1.632	0.001454112	0.000891
50.557	1.676	4.988	1.638	0.001459458	0.000891
58.08	1.8566	6.3476	1.942	0.001730322	0.000891
59.162	1.8855	6.566	1.99	0.00177309	0.000891
64.03	2.0267	7.6388	2.264	0.002017224	0.000891
68.38	2.1713	8.76	2.740	0.00244134	0.000891

The density of 50 Wt. % of Silver nitrate solution is 1.6713. But due to addition of sodium nitrate in the solution is increased the density of solution to 1.75 g/cc.

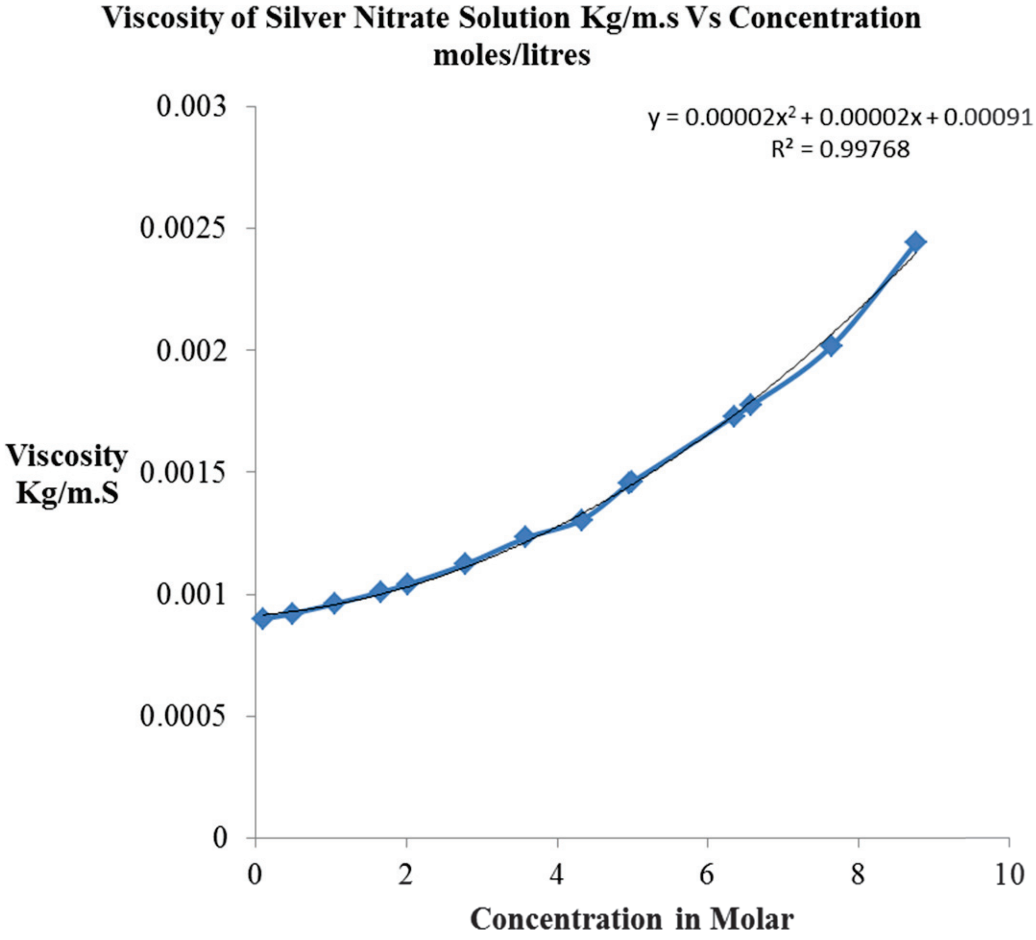


Figure A2.1. Viscosity of Silver nitrate solutions Vs Concentration in Molar

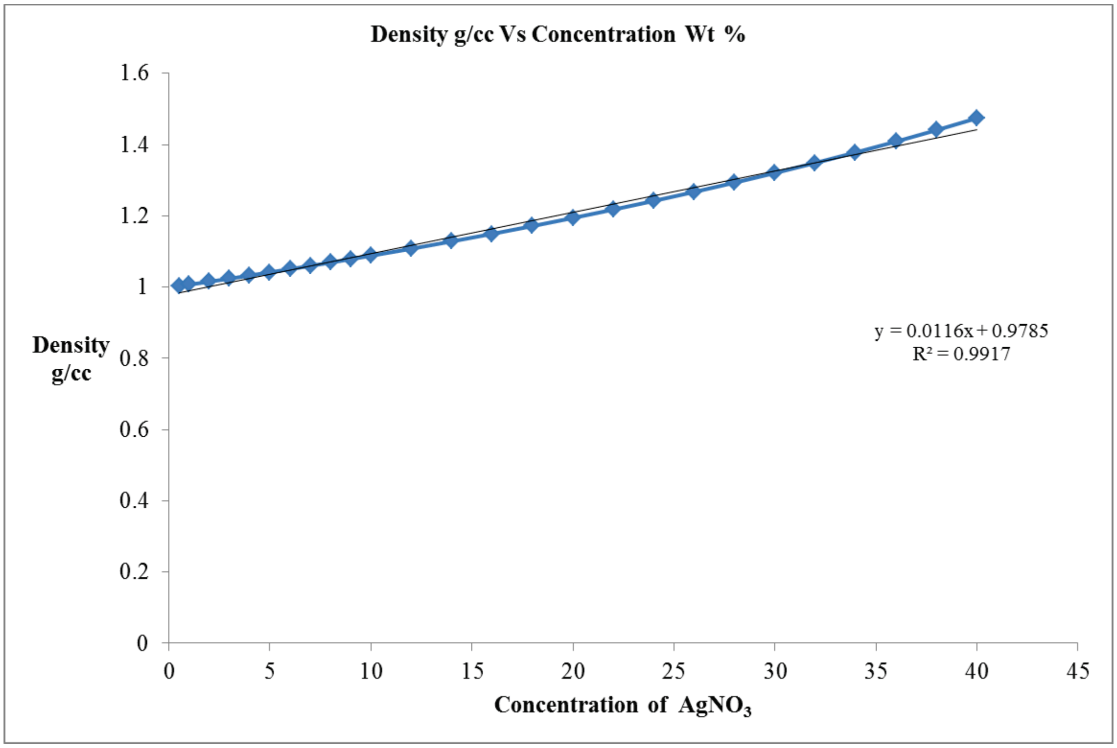


Figure A2.2. Density of Silver nitrate solutions Vs Concentration in Molar

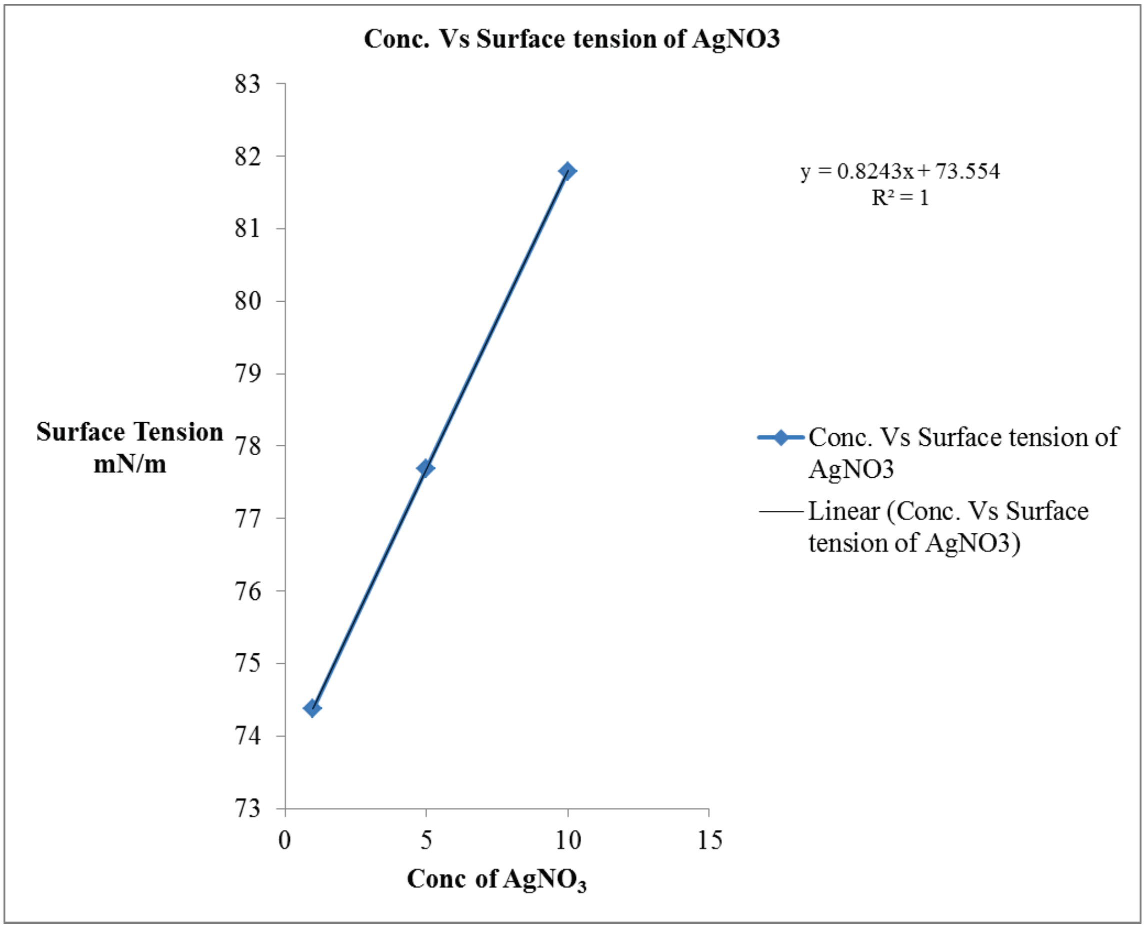


Figure A2.1. Surface Tension of Silver nitrate solutions Vs Concentration in Molar

A3. Gas Chromatography Results

Gas chromatography results provided by DSM Ocean Nutrition, Dartmouth, NS, Canada. The area under the GC chromatogram is directly proportional to concentration of fatty acids in the fraction.

Table A3.1. Area Percentage from GC Chromatogram in slug flow reactor

	EPA		DHA	
Distance (m)	E-fraction	Aqueous fraction	E-fraction	Aqueous fraction
0.5	2.5	43.9	0	31.6
1	1.5	42.7	0	30.1
2	35.6	45.9	4.1	34.7
4	14.8	46.1	1.2	33.6
6	22.6	46	2.8	33.8

Table A3.2. Area Percentage from Slug Flow Mini-fluidic Reactor

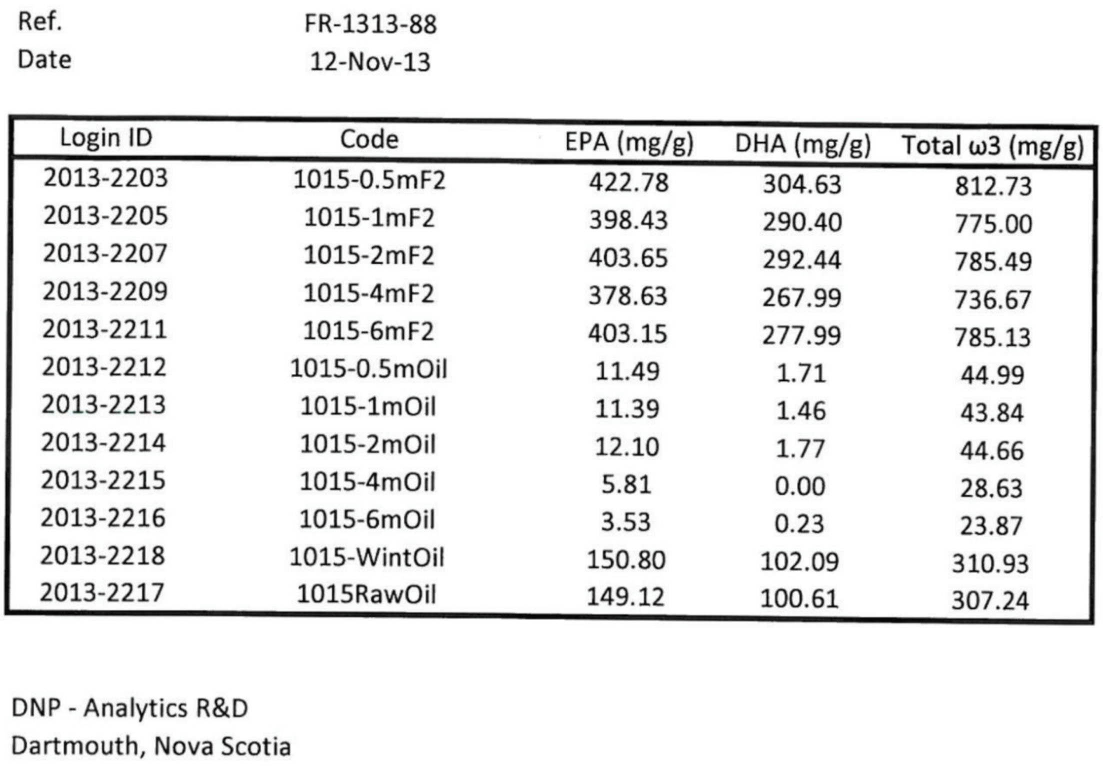
	EPA			DHA		
Distance (m)	EPA F1	EPA F2	EPA Fr-Oil	DHA F1	DHA F2	DHA Fr-Oil
0.5	18.2	47.3	1.4	2.1	35	0
1	17.2	45.8	1.4	2	34.4	0
2	20.9	45.9	1.5	2.4	34.1	0
4	9.8	45.7	0.7	1.2	33.3	0
6	12.4	46.4	0	1.3	32.9	0

*F1 – Fraction 1 from De -emulsification, F2 – Final fraction from hexane from Aqueous Phase, Fr –Oil – Sample from Oil Phase.

Omega 3 PUFA content in various fractions

A4. Gas Chromatography details of the sample:

Table A4 .1 –Gas Chromatography Analytical Details



Ref.	FR-1313-88
Date	12-Nov-13

Login ID	Code	EPA (mg/g)	DHA (mg/g)	Total ω3 (mg/g)
2013-2203	1015-0.5mF2	422.78	304.63	812.73
2013-2205	1015-1mF2	398.43	290.40	775.00
2013-2207	1015-2mF2	403.65	292.44	785.49
2013-2209	1015-4mF2	378.63	267.99	736.67
2013-2211	1015-6mF2	403.15	277.99	785.13
2013-2212	1015-0.5mOil	11.49	1.71	44.99
2013-2213	1015-1mOil	11.39	1.46	43.84
2013-2214	1015-2mOil	12.10	1.77	44.66
2013-2215	1015-4mOil	5.81	0.00	28.63
2013-2216	1015-6mOil	3.53	0.23	23.87
2013-2218	1015-WintOil	150.80	102.09	310.93
2013-2217	1015RawOil	149.12	100.61	307.24

DNP - Analytics R&D
Dartmouth, Nova Scotia

In order to estimate the concentration of EPA and DHA in fraction 1, the plot between area of GC chromatogram for fraction 2 and concentration of EPA and DHA in fraction 2 is performed and based on the equation from the graph, the quantity of EPA/DHA is calculated for fraction 1.

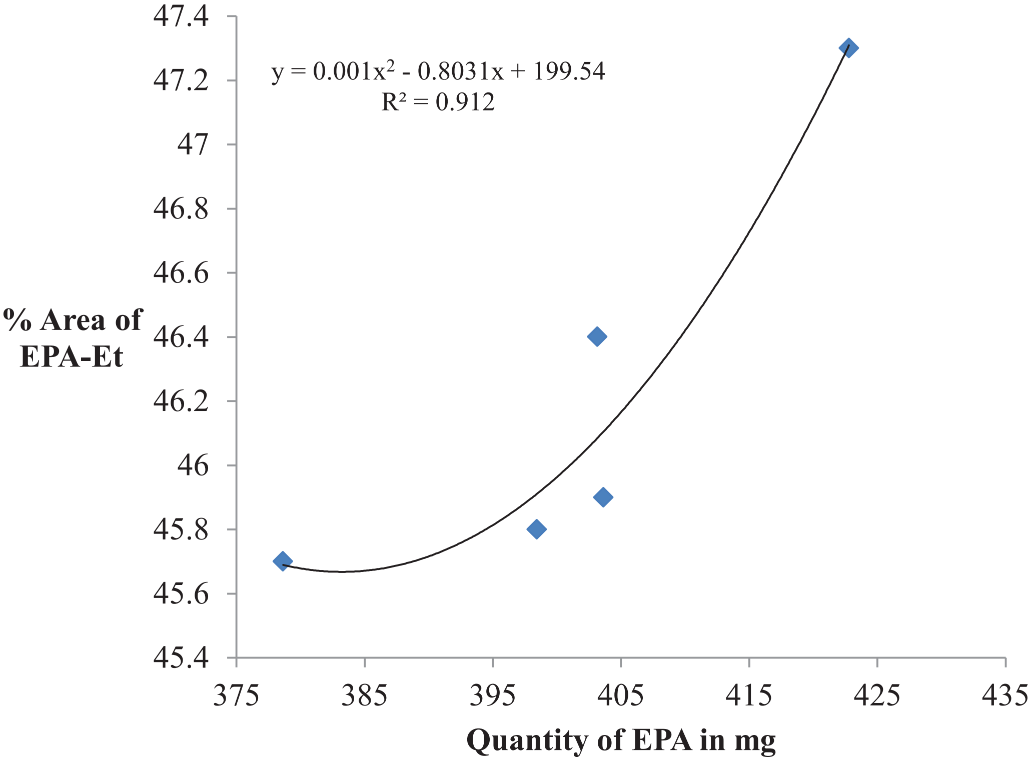


Figure A4.1. Plot between GC Area vs Concentration of EPA in Fraction 2

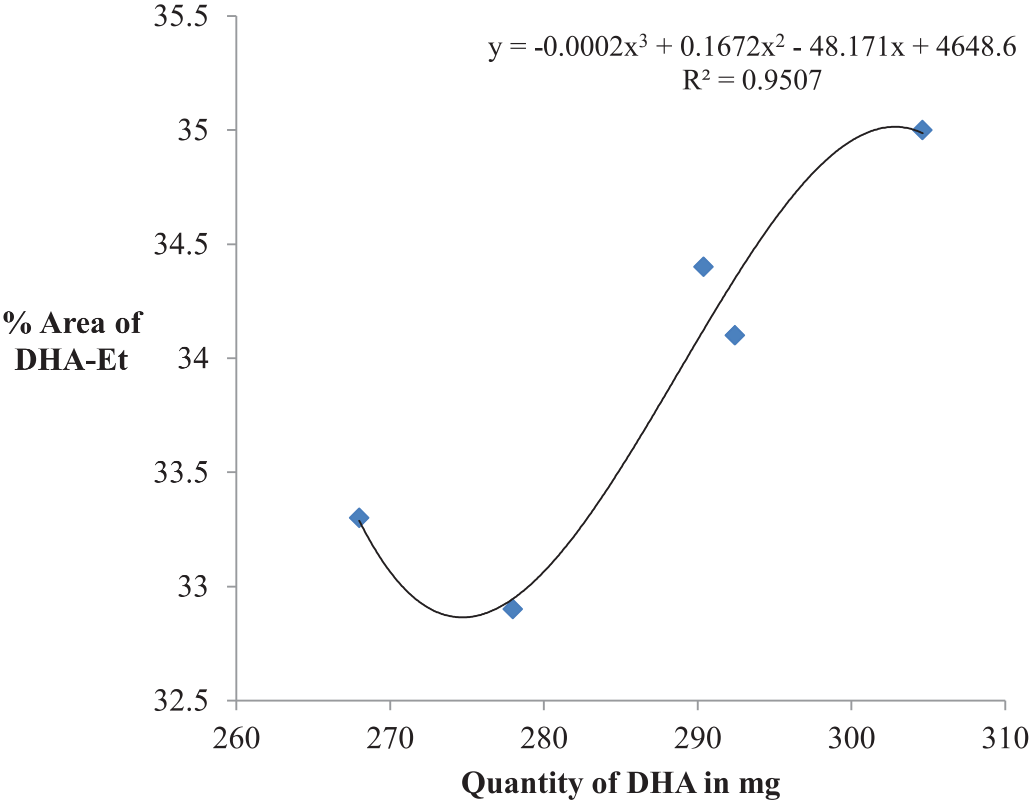


Figure A4.2. Plot between GC Area vs Concentration of DHA in Fraction 2

A5. Model Calculation:

The yield of Omega 3 PUFA in various fractions such oil layer, fraction 1 (De-emulsified layer) and fraction 2(De-complexed layer) from LLE are calculated for evaluation of performance of solvent extraction using the following method.

$$Yield_{EPA-Et} = \frac{EPA - Et \inf raction}{EPAinOil}$$

$$Yield_{DHA-Et} = \frac{DHA - Et \inf raction}{DHAinOil}$$

Similarly,

$$Total = \frac{TotalfromComplexed}{Complex + Emulsion + Oil}$$

$$Actual = \frac{AmountfromComplexed}{Omega3PUFAinInitial}$$

A6. Model Calculation in Flow pattern studies

To develop and analyze the flow transition in mini-channels, the various dimensionless numbers are calculated and mentioned here.

Bond Number for Fish Oil Water System

Physical Property	Value
Density of Fish Oil Ethyl esters	898 Kg/m^3
Density of Water	1000 Kg/m^3
Interfacial Tension of Fish Oil - Water	0.5mN/m
Δρ	102 Kg/m^3
Diameter of Channel	1.58 mm

$$Bo = \frac{\Delta\rho g d^2{}_H}{\sigma}$$

$$Bo = \frac{102 * 9.81 * (1.58/1000)^2}{2.584 * 1000}$$

$$Bo = 3.84\text{E} - 06$$

Weber Number for Fish Oil Water System

Velocity of Mixed Phase = 0.022679509 m/sec

IFT for Fish Oil Water System = 0.002584509 N/m

Density of Mixed Phase = 969.4 Kg/m^3

Viscosity of Mixed Phases = 0.0029747

$$We = \frac{d_H \rho u^2}{\sigma}$$

$$We = \frac{(1.58/1000) * 969.4 * 0.0227^2}{0.00258}$$

$$We = 0.3048$$

Capillary Number

$$Ca = \frac{\mu u}{\sigma}$$

$$Ca = \frac{0.0029747 * 0.0227}{0.002585}\ ,\ Ca = 0.0261$$

A7. Process Economics Details

The raw material cost and by product cost in silver recovery process has been taken from http://www.icis.com/chemicals/channel-info-chemicals-a-z/. However, The minimal cost is considered for process calculation.

Table A7.1. Raw material Price for production cost of Silver Recovery.

Raw material	Quantity US$
Sodium Hydroxide	420.00-850.00 per Ton
Hydrogen Peroxide	0.245-0.270 per lb.
Silver Oxide	800 -1600 Per Kg
Silver Nitrate	2000 Per Kg
Sodium Chloride	50 -200 per ton
Formaldehyde	2.92 $ per liter
Nitric Acid	215.00-225.00 /Ton
Sodium Nitrate	450-500 /Tons
Sodium formate	30 /Kg

A8. Exponents for Equipment Cost Vs Capacity

Table A8.1. Batch Process– Multiple CSTR in parallel:

Equipment	Unit Operation	Range	Purchased Cost in US$	Reference
Mixer (Jacket and Agitated)	Dissolution of Silver nitrate Solution	0.1-30 m^3	145600	www.matche.com
Mixer (Fish Oil Ethyl Esters)	Homogenization of Fish Oil ethyl esters	0.1-30 m^3	130100	www.matche.com
CSTR (3 Reactors in Parallel)	Chemical reaction based	20 m^3	653400	www.matche.com
Storage Tank and Settling tank	Collection of Organic and Aqueous layer	75 m^3	113500	
Mixer	De-emulsification	4 m^3	141300	www.matche.com
Gravity Settler	Removal of Organic layer and Aqueous Layer	0.1-30 m^3	429600	
Column (Separator)	Separation of Hexane rich layer	4 m^3	124700	www.matche.com
Mixer (4 quantity)	De–complexation	110 m^3	565200	
Gravity Settler (2 quantity)	Removal of Organic and aqueous layer	6 m^3	230900	
Column (Separator)	Recovery of Hexane from Organic Layer	8 m^3	124700	www.matche.com

Table A8.2. Continuous Process Using mini-fluidic contactor

Equipment	Unit Operation	Range	Purchased Cost in US$	Reference
Mixer (Jacket and Agitated)	Dissolution of Silver nitrate Solution	0.1-30 m^3	145600	www.matche.com
Mixer (Fish Oil Ethyl Esters)	Homogenization of Fish Oil ethyl esters	0.1-30 m^3	130100	
Mini-fluidic contacting systems[1]	Chemical reaction based	8 m^2	3100	www.matche.com
Mixer	De-emulsification	4 m^3	141300	www.matche.com
Gravity Settler	Removal of Organic layer and Aqueous Layer	0.1-30 m^3	429600	
Column (Separator)	Separation of Hexane rich layer	4 m^3	124700	www.matche.com
Mixer (4 quantity)	De–complexation	110 m^3	565200	
Gravity Settler (2 quantity)	Removal of Organic and aqueous layer	6 m^3	230900	
Column (Separator)	Recovery of Hexane from Organic Layer	8 m^3	124700	www.matche.com

1. The Double pipe heat exchanger is considered to be as mini-fluidic contacting systems and performing as plug flow reactor for LLE of omega 3 PUFA.

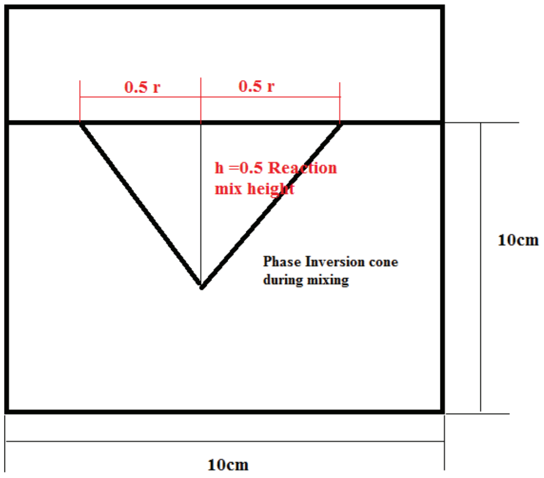
A9. Relative Area calculation for Stirred tank systems, mini-fluidic contacting system for Stratified flow pattern and slug flow pattern

A9.1. Stirred tank system:

The 250ml volume stirred tank system is used for liquid–liquid extraction of Omega 3 PUFA from fish oil. The diameter of Stirred tank is 10 cm and height 10 cm. The reaction mixture volume is 65 ml in the reactor.

Figure A9.1 – Idealized Stirred tank system for Liquid –Liquid Extraction system

Calculation of Relative Area:



During mixing, Phase inversion is formed, the conical shape is observed. The surface area of the cone is calculated.

Figure A9.2 – Area on conical shape during phase inversion

Calculation:

Surface area of Cone = $A_{cone} = 3.14r(r + \sqrt{h^2 + r^2})$

$$A = 0.0189m^2$$

Surface area of Circle = $A_{Circle} = 2 * 3.14r$

$$A_{Circle} = 2 * 3.14 * 0.05$$

$$A_{Circle} = 0.314m^2$$

Calculation of Contacting Area:

Total Contacting Area = A_{cone}

Contacting Area =0.0189 m^2

Relative Area for Stirred tank system = $A = \frac{Area}{Volume}$

$$A = \frac{0.0189m^2}{0.000065m^3}$$

$$A = 290\frac{m^2}{m^3}$$

A9.2. Relative Area for Stratified flow in mini-fluidic contacting system:

In mini-fluidic contacting system, stratified flow pattern was observed. So the area for stratified flow pattern in mini-fluidic channel is calculated based on the equivalent volume of stirred tank system.

$$\frac{A}{V} = \frac{DL}{\frac{3.14 * d^2 L}{4}}$$

$$\frac{A}{V} = \frac{4}{3.14 * D}$$

$$\frac{A}{V} = \frac{4}{3.14 * 0.00158m}$$

$$\frac{A}{V} = 806.25\frac{m^2}{m^3}$$

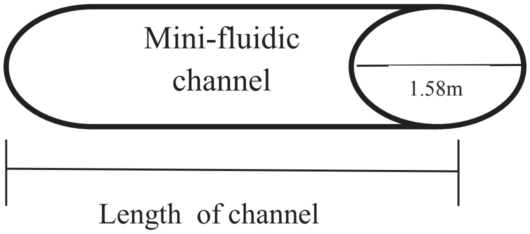


Fig A8.3. Mini-fluidic channel

A9.3. Relative Area for Slug flow in mini-fluidic contacting system:

The schematic diagram of idealized slug flow patterns which is anticipated to form this flow patterns in mini-fluidic contacting system is shown below. The area for slug flow is calculated by the following equation,

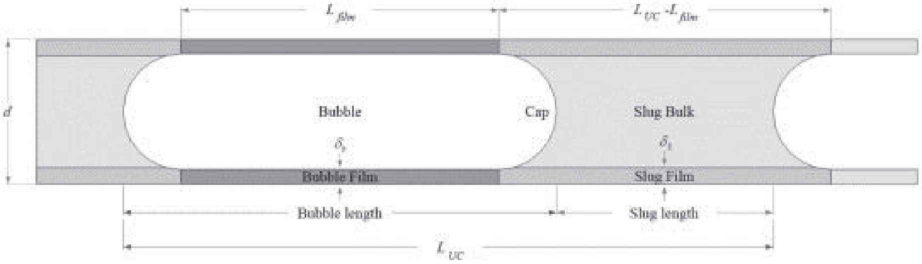


Fig A9.4 – Schematic of Idealized slug flow (A.A.Donaldson *et al,* 2011, Chemical Engineering Science).

The specific cap and film area per unit cell volume, a_{cap} and a_{film}, are determined based on the assumption of spherical caps for the leading and trailing bubble surfaces and a negligible film thickness relative to the bubble radius.

$$a_{cap} = \frac{A_{cap}}{V_{uc}} = \frac{3.14d^2}{0.25*3.14*d^2*L_{uc}} = \frac{4}{L_{uc}}$$

$$a_{film} = \frac{A_{film}}{V_{uc}} = \frac{3.14*dL_{film}}{0.25*3.14*d^2*L_{uc}} = \frac{4L_{film}}{d*L_{uc}}$$

$$a_{cap} = \frac{4}{L_{uc}} = \frac{4}{0.005} = 800\frac{m^2}{m^3}$$

$$a_{film} = \frac{4L_{film}}{d*L_{uc}} = \frac{4*0.003}{0.00158*0.005} = 1518.98\frac{m^2}{m^3}$$

From the above calculation, the relative area for slug flow and stratified flow is higher than the idealized stirred tank system.