DEVELOPMENT & CONSTRUCTION OF MINI-FLUIDIC FLOW REACTOR FOR SOLVENT EXTRACTION OF EPA/DHA FROM FISH OIL.

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Abstract

Process intensification for separation of Omega 3 PUFA from fish oil (FO) is necessary to replace conventional and complex processes. Miniaturization is a kind of process intensification for extraction process and the result of reduction of characteristic dimension in mini-channels offers a potential alternative for overcoming bottlenecks in heat & mass transfer and mixing. The performance of extraction in a slug flow based mini-fluidic flow reactor offers enhancement of interfacial area which results in enhanced mass transfer and offering large surface-to-volume ratio. This work explores the development and construction of mini-fluidics plug/slug flow reactor for extraction processes for Omega 3 PUFA. The various components of the flow reactor are described and commented deviated flow patterns in the experimental setup. The performance of extraction of Omega 3 PUFA using silver ions was within a mini-fluidic and practical extraction yields of EPA–Et, DHA-Et approaching 60 to 70 Wt.%. Additionally, Equilibrium was reached in less than 36 seconds in the mini-fluidic reactor at 10°C. 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 conventional technologies.

INTRODUCTION

The efficient extraction and concentration methods are required to produce concentrated pharmaceutical and food grade Omega 3 PUFA, 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.

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 [1] [2]. 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 [3] [4], who described Liquid–Liquid Extractions (LLE) 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.

Miniaturization 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 [5]. 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). Mixing and solvent inventory are key issues in the separation performance in extraction, with large reactor volumes requiring significant secondary storage capacity [6]. In this work, it was necessary to carry out LLE experiments using commercial trans-esterified fish oils and silver nitrate-based solvent solutions at mini scale and characterize the results.

I. MATERIALS AND METHODS

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. 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 (*Engraulisringens)* and Sardine (*Sardinopssagaxsagax*) on 29thJune 2013.

A. 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.(Kamio *et al.* (2010, 2011) 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.

B. Winterization of fish Oil

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.

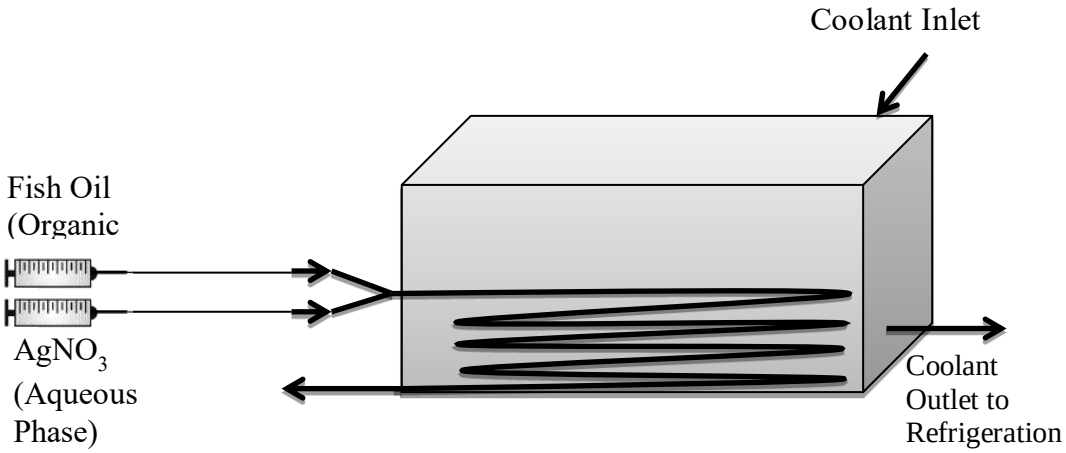
C. Development of Slug flow mini-fluidic reactor

The slug flow mini-fluidic flow 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.1, consisting of a 1/16th 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.

D. 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. (Fisher Scientific, 2014),

E. Figures and Tables



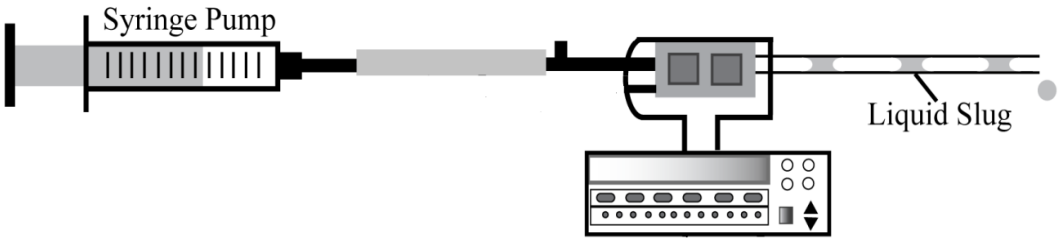


Fig.1. Slug flow mini-fluidic extraction experimental set up. The 1.58mm ID mini-fluidic channel is immersed in sub cooled water (10° C) in the bath which is circulated by submersible pump. In the second picture of the fig 3.1, the flow patterns produced also depends on the contactor (mixing system) 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.

Components of Experimental Setup

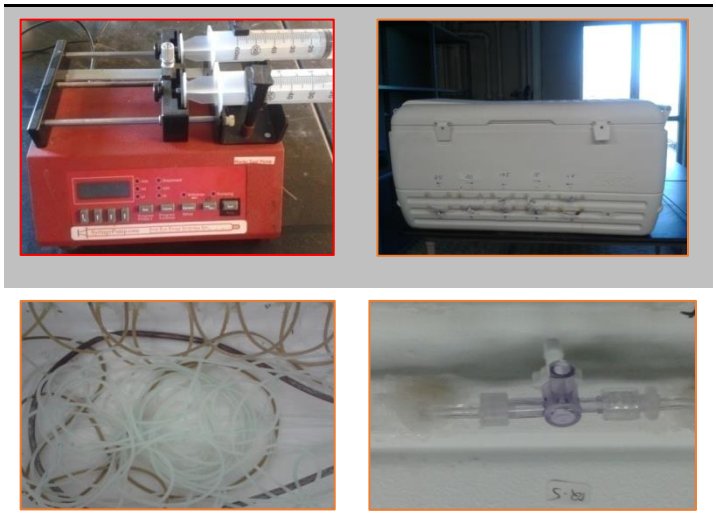


Fig .2. Dual syringe Pump from Longer Instruments used to control flow into the mini-fluidic system, Immersion vessel for cooling the mini-fluidic channels, Tygon mini-fluidic channel, Sample Port.

TABLE I. COMPOSITION ANALYSIS OF THE 18/12EE FISH OIL FEEDSTOCK.

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

TABLE II. WEIGHT PERCENT EPA/DHA/OMEGA 3 RECOVERED IN FRACTION 2, RELATIVE TO THE INITIAL FEED USED IN EACH OF THE EXPERIMENTS.

Mini-fluidic Reactor			
$T_{Residence}$ (mins)	EPA–Et Wt.%	DHA–Et Wt. %	Ω 3 Wt. %
0.6	64.2	68.5	59.9
1.2	79.3	85.7	74.9
2.4	78.6	84.4	74.2
4.8	60.0	63.0	56.7
7.3	79.3	81.2	75.1

F. Results of Extraction

The results are provided in Table 1 and 2. The variations in yield of EPA and DHA at 0.6 min and 4.8 min for the mini-fluidic system are seen a potential outliers in data. 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. The remaining values 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. In the case of the mini-fluidic system, an unanticipated flow pattern within the channels could have potentially affected the relative flow rates of the two species (Fish Oil and Silver nitrate solution) in question (and the contact times), creating additional uncertainty in the yield [7] [8].

G. **Deviation of Flow patterns**

A notable difference between this study and previous designed 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 3. In addition to that, the flow patterns will be affected the effects of tube wettability and fluid properties. At present, it is important to note the high Bond number present under these conditions with interfacial tension IFT of this particular system, as the use of commercial fish oils seems result in a sufficient reduction in IFT 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. 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 such as saturated and unsaturated fatty acids which reduce the interfacial tension at the interface between fish oil and silver nitrate, resulting in a stratified flow within the channels used in this study.

TABLE III –INFLUENCE OF VARIOUS FORCE AT "Y" JUNCTION

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

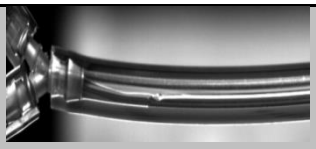
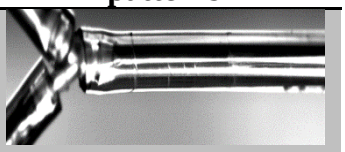
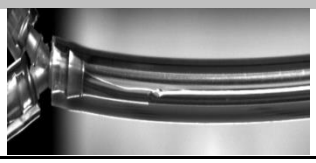
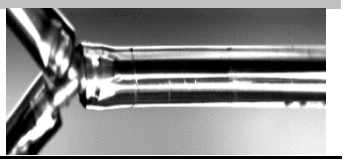
On Set of Stratification	Stratified Flow patterns
	
	

Fig .3 Stratification of Flow at Y Junction. Additionally, the properties of fish oil and silver nitrate solution would deviate the anticipated flow pattern.

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. Additionally, the relative area for mixed flow, stratified flow and slug flow in the extraction process are 290 m^2/m^3, 806.25 m^2/m^3 and 1520 m^2/m^3 respectively. From this information, the relative area for slug flow and stratified

flow is higher than the idealized stirred tank system and provided better contact area for mass transfer.

H. Recommendation

The investigation on LLE results for omega 3 PUFA 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 flow reactor system. The following recommendation was suggested as part of the investigation. Omega 3 PUFA recoveries after ~36 Seconds of contact time in the mini-fluidic system were similar to those obtained from a CSTR after 900 secs [7,8] approaching conservative estimates of nearly 80% based on actual mass collected after sample losses, and approaching 90% based on remaining Omega 3 PUFA in the residual oil fractions. Typical concentrations within the extract were Omega 3 content between 78 and 82 wt. %. 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. It is reported [7, 8] 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, the role of Bond number in flow pattern formation is commented and justified about stratification of flow in mini-fluidics experimental setup. 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 [7].

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. Additionally, 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. Based on these observations, there are a few recommendations to improve process design of LLE at mini-scale,

I. 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)

II. 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.

I. Conclusion

The extraction of Omega 3 PUFA using silver ions was compared within a mini-fluidic 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.

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